JPPT | Clinical Investigation

Evaluation of Heparin Anti–Factor Xa Levels Following Antithrombin Supplementation in Pediatric Patients Supported With Extracorporeal Membrane Oxygenation

Sharon E. Gordon, PharmD; Travis S. Heath, PharmD; Ali B.V. McMichael, MD; Christoph P. Hornik, MD, PhD, MPH; and Caroline P. Ozment, MD

OBJECTIVE Thrombotic events are potential complications in patients receiving extracorporeal membrane oxygenation (ECMO) necessitating the use of systemic anticoagulation with heparin. Heparin works by potentiating the effects of antithrombin (AT), which may be deficient in critically ill patients and can be replaced. The clinical benefits and risks of AT replacement in children on ECMO remain incompletely understood.

METHODS This single-center, retrospective study reviewed 28 neonatal and pediatric patients supported on ECMO at a tertiary care hospital between April 1, 2013, and October 31, 2014, who received at least 1 dose of AT during their ECMO course. The primary outcome of the study was the change in anti–factor Xa levels after pooled human AT supplementation. Secondary outcomes included the percentage of anti–factor Xa levels within the therapeutic range surrounding AT administration; survival to decannulation; 30 days after cannulation and discharge; time to first circuit change; and incidence of bleeding and thrombotic events.

RESULTS A total of 78 doses of AT were administered during the study period. The mean increase in anti–factor Xa level following AT administration in patients without a ≥10% concurrent change in heparin was 0.075 ± 0.13 international units/mL. A greater percentage of anti–factor Xa levels were therapeutic for the 48 hours following AT administration (64.2% vs 38.6%). Survival and adverse events were similar to Extracorporeal Life Support Organization averages, with the exception of a higher incidence of intracranial hemorrhage.

CONCLUSIONS Patients experienced a small but significant increase in anti–factor Xa level and a greater percentage of therapeutic anti–factor Xa levels following AT supplementation.

ABBREVIATIONS ACT, activated clotting time; AT, antithrombin; ECMO, extracorporeal membrane oxygenation; ELSO, Extracorporeal Life Support Organization; GI, gastrointestinal; ICH, intracranial hemorrhage

KEYWORDS anticoagulation; anti–factor Xa; antithrombin; drug monitoring; ECMO; heparin; pediatric

J Pediatr Pharmacol Ther 2020;25(8):717–722

DOI: 10.5863/1551-6776-25.8.717

Introduction

Hemorrhagic and thrombotic events are among the most common complications in patients receiving extracorporeal membrane oxygenation (ECMO).[1] Inflammation due to underlying critical illness as well as blood contact with the foreign surface of the ECMO circuit results in activation of the coagulation system and consumption of both procoagulation and anticoagulation factors.[2] Systemic anticoagulation is necessary to mitigate consumption and help prevent inappropriate bleeding and clotting while on ECMO. A continuous infusion of unfractionated heparin is the standard of care at most institutions.[3] Heparin works by irreversibly binding the endogenous anticoagulant, antithrombin (AT), and potentiating its activity by up to 1000 times.[4] Antithrombin exerts its anticoagulant activity by inhibiting factors Xa and IIa as well as other serine proteases involved in the coagulation cascade.[4] Low levels of AT activity are common in ECMO patients because of the previously described consumption as well as dilution when the circuit prime to patient blood volume ratio is high and/or the circuit prime lacks fresh frozen plasma.[3] This deficiency is magnified in neonates because of the immaturity of the coagulation system. Neonatal levels of AT activity are only 30% to 40% of adult levels and do not reach adult levels until approximately 6 months of life.[5]

Decreased AT levels may lead to decreased heparin response and smaller changes in anti–factor Xa levels and activated clotting time (ACT) despite increases in heparin infusion rate.[3] The anti–factor Xa assay mea-

sures the ability of patient plasma to inhibit exogenous factor Xa via unfractionated heparin-AT complexes.[6] Heparin response can be evaluated using the heparin sensitivity index score, a measure of change in ACT adjusted for heparin bolus dose.[7] A retrospective cohort study of neonates and infants undergoing congenital cardiac surgery showed that heparin response was significantly associated with AT activity.[7] Patients with AT deficiency had a lower heparin sensitivity index score, suggesting a role for AT replacement in neonatal patients undergoing cardiopulmonary bypass.

The effects of AT supplementation on anticoagulation assays in the pediatric ECMO population remain unclear. Studies have shown variable changes in anti–factor Xa levels and ACT following AT administration in neonatal and pediatric ECMO patients.[8,9] Antithrombin dosing and administration in ECMO patients are not standardized across institutions, and the 2014 Extracorporeal Life Support Organization (ELSO) *Anticoagulation Guideline* does not provide specific recommendations for AT supplementation. The guideline states that AT replacement may be considered if low AT activity levels are confirmed; however, the guideline does not define low.[10] Finally, the amount of AT deemed to be sufficient is likely variable depending on the maturity of the patient's hemostatic system.

Because of the variability of anticoagulation in pediatric ECMO patients and AT administration practices, we sought to examine AT supplementation in the ECMO population at our institution. The purpose of our study was to determine the change in anti–factor Xa levels using an assay that does not require the addition of exogenous AT following pooled human AT supplementation in neonatal and pediatric patients supported on ECMO. We hypothesized that AT supplementation would significantly increase anti–factor Xa levels for neonatal and pediatric patients on ECMO.

Materials and Methods

A single-center, retrospective chart review was performed on neonatal and pediatric patients supported with ECMO between April 1, 2013, and October 31, 2014, at Duke University Hospital in Durham, NC. Patients were included in the study if they were 0 days to less than 18 years of age and on ECMO support for at least 48 hours during the predetermined study period and received AT supplementation at least once while on ECMO support. Patients were excluded from the study if they did not have at least 1 documented anti–factor Xa level before and after AT administration. Demographic and clinical data were collected from the patients' electronic medical records. All patients received unfractionated heparin according to the institution's neonatal and pediatric ECMO protocol. Patients were given a heparin bolus of 50 units/kg (max 5000 units) at the time of ECMO cannulation and were initiated on a continuous heparin infusion of 25 units/kg/hr if they weighed ≤25 kg or 15 units/kg/hr if they weighed >25 kg. The heparin infusion was titrated every 6 hours as needed to maintain an anti–factor Xa level of 0.4 to 0.7 units/mL and a PTT of 60 to 80 seconds. In the case of discrepancy between anti–factor Xa levels and PTT, fresh frozen plasma was administered if deemed clinically appropriate. Pooled human AT (Thrombate III, Grifols Therapeutics Inc, Research Triangle Park, NC) was administered for functional AT activity levels of less than 60% and/or signs of heparin resistance at the discretion of the provider. The dose of AT was calculated by subtracting measured AT from desired AT, multiplying by patient weight in kilograms, and dividing by a factor of 1.4. This is the manufacturer recommended dose for congenital AT deficiency.[11] Doses of AT were administered over 15 to 20 minutes. Standard practice was to continue heparin at the current rate of infusion at the time of AT administration; however, changes to heparin infusion rate were made at the provider's discretion. Blood products were administered to target a platelet count of greater than 100,000/μL, fibrinogen greater than 150 mg/dL, and hematocrit >35%.

Anti–factor Xa levels were collected every 6 hours for the first 24 hours of ECMO support and at least every 12 hours for subsequent days. The anti–factor Xa assay employed by our institution (HemosIL Liquid Anti-Xa, Instrumentation Laboratory, Bedford, MA) uses a Factor Xa reagent mixture that does not add exogenous AT to the sample.[12] All anti–factor Xa levels collected during the ECMO course were recorded for each patient. Calculation of the change in anti–factor Xa level after AT supplementation was performed using the closest documented anti–factor Xa level prior to the administration of AT and the closest documented anti–factor Xa level following AT administration. Patients were grouped into those with and those without a concurrent change of ≥10% in heparin infusion rate at the time of AT administration. A ≥10% change was chosen because of standard recommendations for heparin dose adjustment which advise for a 10% change in infusion rate when anti–factor Xa levels are outside of the therapeutic range.[13] In addition, the percentage of anti–factor Xa levels within the therapeutic range of 0.4 to 0.7 units/mL 48 hours before and after AT administration was calculated.

Secondary end points included survival to decannulation; 30 days after cannulation to discharge; time to first circuit change; and incidence of bleeding and thrombotic events. The ECMO circuits were changed per provider discretion when clinically indicated. We defined bleeding as intracranial hemorrhage (ICH), GI hemorrhage, and surgical site bleed, and thrombosis as thrombus in the ECMO circuit, bridge, central line, or a systemic thrombus. These events were recorded from progress notes or imaging results located in the patient's electronic medical record.

Categoric variables were presented as frequencies

Table 1. Patient Demographics

Indication for Extracorporeal Membrane Oxygenation	n (%)
Neonatal	
Congenital diaphragmatic hernia	4 (14.3)
Meconium aspiration syndrome	3 (10.7)
Persistent pulmonary hypertension of the newborn	7 (25)
Sepsis	1 (3.6)
Respiratory	
Acute respiratory distress syndrome	1 (3.6)
Acute respiratory failure (non-ARDS)	2 (7.1)
Other	1 (3.6)
Cardiac	
Congenital defect	5 (17.8)
Cardiogenic shock	1 (3.6)
Cardiomyopathy	1 (3.6)
Myocarditis	2 (7.1)

ARDS, acute respiratory distress syndrome

and percentages. Continuous variables were summarized using means and standard deviations, with the exception of patient age, which was presented as median and range. A 1-sample *t* test was used to assess change in anti–factor Xa level following AT supplementation. The mean percentages of anti–factor Xa levels within the therapeutic range for the 48 hours before and after the administration of AT were compared using a paired *t* test. All statistical analyses were performed using Stata SE 15.1 (StataCorp LLC, College Station, TX). A $p < 0.05$ without adjustment for multiple comparison was considered statistically significant.

Results

A total of 20 neonatal patients and 8 pediatric patients were supported on ECMO and received at least 1 dose of AT while on ECMO during the study period. The median patient age was 3.5 days (range, 0–2 years) with indications for ECMO support as outlined in Table 1. A total of 78 doses of AT (mean dose, 51.9 units/kg; range, 10.1–189.7 units/kg) were administered, with each dose treated as an independent event. Specific indication for AT administration was not recorded in the electronic medical record. One dose of AT did not have a measured anti–factor Xa level prior to administration and was excluded from the analysis. The time to first anti–factor Xa level following AT administration varied between 2 and 5 hours. The mean increase in anti–factor Xa level following AT administration was 0.08 ± 0.11 units/mL (95% CI, 0.05–0.1; $p < 0.01$). When examining only those patients without ≥10% concurrent change in heparin (n = 67 doses), the mean increase in anti–factor Xa level following AT administration was 0.075 ± 0.013 units/mL (95% CI, 0.05–0.1; $p = 0.08$; Table 2). The mean anti–factor Xa levels prior to and after AT administration in this group were 0.35 ± 0.09 and 0.42 ± 0.12 international units/mL, respectively. Of measured anti–factor Xa levels, 38.6% were within the therapeutic range for the 48 hours prior to AT administration and 64.2% were within it for the 48 hours following AT administration ($p = 0.03$).

Secondary outcome data are shown in Table 3. A total of 24 patients (85.7%) survived to decannulation, 20 patients (71.4%) survived to 30 days after cannulation, and 18 patients (64.3%) survived to discharge. A total of 9 patients (32.1%) experienced a thrombotic event. There were 11 hemorrhagic events, including 7 instances of ICH (25%), 2 GI bleeds (7.1%), and 2 surgical site bleeds (7.1%). One ICH event and 3 thrombotic events occurred before any doses of AT were given. The average time to first circuit change was 142.1 ± 71.8 hours.

Discussion

In this study, AT supplementation in neonatal and pediatric ECMO patients resulted in a significant increase in anti–factor Xa levels of 0.08 ± 0.11 units/mL and an increased percentage of therapeutic anti–factor Xa levels during the 48 hours following AT supplementation. Although some studies have seen a similar increase in anti–factor Xa level with AT administration, others have seen variable effects on anti–factor Xa levels and ACT.[8,9] A study by Niebler et al[8] observed no significant change in ACT with AT supplementation at an average dose of 138 ± 72 units/kg in patients younger than 1 year. Although this study examined ACT as a primary outcome, clinical evidence supports superior correlation between heparin dose and anti–factor Xa levels compared with ACT in pediatric patients supported on

Table 2. Descriptive Analysis of Change in Anti–Factor Xa Levels After Antithrombin (AT) Supplementation

	Pre-AT Anti–Factor Xa Assay, mean ± SD*	Post-AT Anti–Factor Xa Assay, mean ± SD*	Change in Anti–Factor Xa Assay, mean ± SD (95% CI)
All AT doses (n = 78)	0.34 ± 0.09	0.42 ± 0.13	0.08 ± 0.11 (0.05, 0.1)
< 10% change in heparin infusion (n = 67)	0.35 ± 0.09	0.42 ± 0.12	0.075 ± 0.13 0.05, 0.1)
≥10% increase in heparin infusion by (n = 10)	0.3 ± 0.1	0.37 ± 0.13	0.07 ± 0.11 (−0.01, 0.14)
≥10% decrease in heparin infusion (n = 1)	0.21	0.67	0.46

* Pre-AT anti–factor Xa assay was the closest measured assay prior to AT administration, and post-AT anti–factor Xa assay was the closest measured assay after AT administration for each administered dose of AT.

ECMO.[14,15] A retrospective chart review by Ryerson et al[9] examined changes in anti–factor Xa level with AT treatment at a mean dose of 222 units/kg. In a subgroup analysis of the study, patients younger than 12 months experienced a significant increase in average anti–factor Xa level from 0.22 units/mL to 0.43 units/mL, even in the presence of a protocol-required, concurrent 50% decrease in heparin drip rate.[9] The results of our study similarly found an increase in anti–factor Xa level following AT administration but to a lesser extent, with a mean change of 0.08 units/mL.

Our study observed a significant increase in the percentage of anti–factor Xa levels within our therapeutic range following AT administration (64.2% vs 38.6%). Maintaining therapeutic levels is thought to be important in preventing clot formation in the ECMO circuit to potentially prolong circuit life and to prevent systemic clots. Irby et al[16] found that lower anti–factor Xa levels in pediatric ECMO patients were associated with an increased risk of circuit change, suggesting the importance of maintaining therapeutic levels. Our analysis was limited to the 96 hours surrounding each AT administration, so it is unclear how long the benefit of increased anti–factor Xa levels within the therapeutic range is conferred beyond the 48 hours following AT supplementation. The published half-life of Thrombate III is approximately 2.5 days with concurrent heparin administration and may be shorter in patients on ECMO because of the potential for consumption, suggesting that the greatest benefit of supplementation would be seen within the first few days following administration.[11] Future studies examining changes in the elimination half-life of pooled human AT in ECMO patients may be warranted.

When discussing the use of the anti–factor Xa assay, it is important to clarify whether or not exogenous AT is added to the specific assay employed by the institution. The addition of exogenous AT to the anti–factor Xa assay is intended to correct for conditions causing low endogenous levels of AT.[6] When exogenous AT is added to the test sample, however, the assay provides information on specific heparin dose efficiency but does not provide information on AT level or heparin dose effectiveness for specific patients.[6] The anti–factor Xa assay used in our study does not add exogenous AT to the test sample, and therefore, may provide a more accurate representation of *in vivo* heparin activity in patients with lower levels of AT.

Although a previous study showed a correlation between increased heparin administration and improved survival, the mortality benefit of therapeutic anti–factor Xa levels remains uncertain.[17] One study examining the effects of anticoagulation in another hypercoagulable state, pregnancy, found an association between anti–factor Xa levels and parameters of the thrombin generation assay, including area under the curve, peak height, and time to peak.[17] If higher anti–factor Xa levels are associated with decreased thrombin formation, then there is a potential for decreased formation of clots while one is supported on ECMO. A study examining routine administration of AT in neonates on ECMO observed higher serum anti–factor Xa levels and lower rates of mechanical clots within the ECMO circuit; however, there was no resultant extension of ECMO circuit life.[18] Although similar studies examining surrogate end points continue to emerge, there remains a lack of data on correlation between anti–factor Xa levels and survival outcomes.

The rate of survival to hospital discharge in our study (64.3%) was comparable to the average rate of survival to hospital discharge reported in the ELSO database of 45% to 68% in neonatal patients supported on ECMO between 2009 and 2015.[1] The incidence of ICH in our study (25%) was higher than the ELSO average of 11% in neonatal patients on ECMO between 2009 and 2015, which may have been due to the underlying disease states of our population or small sample size.[1] It is unclear the extent to which AT supplementation may have contributed to the rate of ICH in our study given the lack of a comparative cohort. A larger retrospective study of neonatal ECMO patients found a rate of ICH similar to that of our study population, with rates of 27.6% in patients receiving AT supplementation and 21.7% in control patients.[18] The study examined a cohort of 162 neonates who received AT (125 units/kg, up to 500 units) at ECMO initiation and 12 hours after cannulation compared with similar historical controls, and they found no difference overall in hemorrhagic

Table 3. Descriptive Analysis of Survival and Adverse Event Rates

Outcome	Result
Survival, n (%)	
Survival to decannulation	24 (85.7)
Survival to 30 days after decannulation	20 (71.4)
Survival to discharge	18 (64.3)
Adverse events, n (%)	
Thrombus	9 (32.1)
Hemorrhage	
Intracranial hemorrhage	7 (25)
Gastrointestinal bleed	2 (7.1)
Surgical site bleed	2 (7.1)

complications and fewer thrombotic complications in the AT cohort.[18] Retrospective studies in the general pediatric ECMO population, however, have seen variable effects of AT supplementation on hemorrhagic and thrombotic event rates.[19,20] Given the variability in complication rates, health care providers should continue to use caution when prescribing AT, and further investigation into the benefits and risks of therapy may be warranted.

It is important to note key distinctive features of the coagulation system when examining the rates of hemorrhagic and thrombotic complications in the neonatal ECMO population. The serum concentration of contact and vitamin K–dependent coagulation factors in neonates is about 50% of adult values.[5,21] Neonates also have a reduced ability to generate thrombin and impaired platelet function.[21] Although these changes would be expected to predispose neonates to hemorrhagic complications, increased activity of von Willebrand factor and decreased levels of coagulation inhibitors like AT help to balance bleeding risk.[5,21] The immaturity of both the coagulation and fibrinolytic pathways at the time of birth makes neonates particularly vulnerable to both hemorrhagic and thrombotic complications. Most of the patients in the current study were members of this vulnerable population; thus, secondary outcomes may not be generalizable to the pediatric ECMO population as a whole.

There are additional limitations to this study. The retrospective nature of the study prevented control of potential confounding factors that may have affected study outcomes. Although our institution follows a protocol for anticoagulation in neonatal and pediatric patients on ECMO support, the administration of AT and ECMO circuit changes is left to the discretion of the medical team. Anti–factor Xa levels were not collected at the same time relative to AT administration in all patients. Standardization of pre-AT and post-AT anti–factor Xa levels may increase the validity of measured changes in anti–factor Xa assay attributed to AT administration alone. The generalizability of the results of this study to all neonatal and pediatric ECMO centers is limited by variability in target AT activity levels, specific anti–factor Xa assay employed, dosing strategies for exogenous AT, and anticoagulation monitoring among institutions.

Conclusion

In summary, study patients experienced a small increase in anti–factor Xa level and a greater percentage of therapeutic anti–factor Xa levels following AT supplementation. Survival and rates of adverse bleeding and thrombotic events were similar to ELSO averages. The paucity of evidence for the efficacy of AT supplementation on clinical outcomes, paired with the potential risks, support continued caution in the use of AT supplementation in neonatal and pediatric patients supported on ECMO.

ARTICLE INFORMATION

Affiliations Department of Pharmacy (SEG), Children's Hospital Colorado, Aurora, CO, Department of Pharmacy (TSH), Duke University Hospital, Durham, NC, Department of Pediatrics (ABVM), University of Texas Southwestern Medical Center, Dallas, TX, Duke Clinical Research Institute (CPH), Durham, NC, Department of Pediatrics (CPO), Duke University Hospital, Durham, NC

Correspondence Sharon E. Gordon, PharmD; sharon.gordon@childrenscolorado.org

Disclosure The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Ethical Approval and Informed Consent The study was approved by the Institutional Review Board at Duke University Hospital, which waived the need for informed consent.

Acknowledgment We would like to acknowledge Tracy Spears for her contribution to the initial data analysis.

Accepted June 9, 2020

REFERENCES

1. Thiagarajan RR, Barbaro RP, Rycus PT, et al. Extracorporeal Life Support Organization registry international report 2016. *ASAIO J.* 2017;63(1):60–67.
2. Saini A, Spinella P. Management of anticoagulation and hemostasis for pediatric extracorporeal membrane oxygenation. *J Lab Clin Med.* 2014;34(3):655–673.

3. Bembea MM, Schwartz, Shah N, et al. Anticoagulation monitoring during pediatric extracorporeal membrane oxygenation. *ASAIO J.* 2013;59(1):63–68.
4. Griffith M.J. Kinetics of the heparin-enhanced antithrombin III/thrombin reaction: evidence for a template model for the mechanism of action of heparin. *J Biol Chem.* 1982;257(13):7360–7365.
5. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the full term infant. *Blood.* 1987;70(1):165–172.
6. Gehrie E, Laposata M. Test of the month: the chromogenic antifactor Xa assay. *Am J Hematol.* 2012;87(2):194–196.
7. Chin VM, Holland ML, Parker MM, et al. Antithrombin activity and heparin response in neonates and infants undergoing congenital cardiac surgery: a retrospective cohort study. *Can J Anaesth.* 2016;63(1):38–45.
8. Niebler RA, Christensen M, Berens R, et al. Antithrombin replacement during extracorporeal membrane oxygenation. *Artif Organs.* 2011;35(11):1024–1028.
9. Ryerson LM, Bruce AK, Lequier L, et al. Administration of AT concentrate in infants and children on extracorporeal life support improves anticoagulation efficacy. *ASAIO J.* 2014;60(5):559–563.
10. Extracorporeal Life Support Organization (ELSO). Anticoagulation guideline. Accessed August 14, 2017. https://www.elso.org/Portals/0/Files/elsoanticoagulationguideline8-2014-table-contents.pdf
11. Thrombate III (antithrombin III [human]) [prescribing information]. Research Triangle Park, NC: Grifols Therapeutics Inc; February 2016.
12. HemosIL [package insert]. Bedford, MA: Instrumentation Laboratory Company; 2017.
13. Heparin sodium. Lexicomp Online, Pediatric & Neonatal Lexi-Drugs. Wolters Kluwer Clinical Drug Information Inc. Accessed August 14, 2017.
14. Liveris A, Bello RA, Friedmann P, 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*. 2014;15(2):e72–e79.
15. Delmas C, Jacquemin A, Vardon-Bounes F, et al. Anticoagulation monitoring under ECMO support: a comparative study between the activated clotting time and the anti-Xa activity assay. *J Intensive Care Med.* 2020;35(7):679–686. doi:10.1177/0885066618776937
16. Irby K, Swearingen C, Byrnes J, et al. Unfractionated heparin activity measured by anti–factor Xa levels is associated with the need for extracorporeal membrane oxygenation circuit/membrane oxygenator change: a retrospective pediatric study. *Pediatr Crit Care Med.* 2014;15(4):e175–e182.
17. Chowdary P, Despoina A, Riddell A, et al. Thrombin generation assay identifies individual variability responses to low molecular weight heparin in pregnancy: implications for anticoagulant monitoring. *Br J Haematol.* 2015;168(5):719–727.
18. Stansfield BK, Wise L, Ham PB III, et al. Outcomes following routine antithrombin III replacement during neonatal extracorporeal membrane oxygenation. *J Pediatr Surg*. 2017;52(4):609–613.
19. Todd Tzanetos DR, Myers J, Wells T, et al. The use of recombinant antithrombin III in pediatric and neonatal ECMO patients. *ASAIO J.* 2017;63(1):93–98.
20. Wong TE, Nguyen T, Shah SS, et al. Antithrombin concentrate use in pediatric extracorporeal membrane oxygenation: a multicenter cohort study. *Pediatr Crit Care Med.* 2016;17(12):1170–1178.
21. Kenet G, Cohen O, Bajorat T, Nowak-Gottl U. Insights into neonatal thrombosis. *Thromb Res*. 2019;181(suppl 1):S33–S36.