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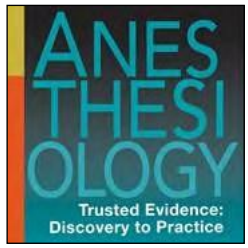

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Abstract

Background

Unfractionated heparin, administered during veno-arterial extracorporeal membrane oxygenation to prevent thromboembolic events, largely depend on plasma antithrombin for its antithrombotic effects. Decreased heparin responsiveness seems frequent on extracorporeal membrane oxygenation however its association with acquired antithrombin deficiency is poorly understood. Our objective was to describe longitudinal changes in plasma antithrombin levels during extracorporeal membrane oxygenation support and evaluate the association between antithrombin levels and heparin responsiveness. We hypothesized that extracorporeal membrane oxygenation support would be associated with acquired antithrombin deficiency and related decreased heparin responsiveness.

Methods

Adults receiving veno-arterial extracorporeal membrane oxygenation were prospectively included. All patients received continuous intravenous unfractionated heparin using a standardized protocol (target anti-Xa 0.3-0.5 $IU.mL^{-1}$). For each patient, arterial blood was withdrawn into citrate-containing tubes, at 11 time-points (from H0 up to day 7) . Anti-Xa (without dextran or antithrombin added) and antithrombin levels were measured. The primary outcome was antithrombin plasma level. In the absence of consensus, antithrombin deficiency was defined as a time-weighted average of antithrombin ≤70%. Data regarding clinical management and heparin dosage were collected.

Results

Fifty patients, including 42% post-cardiotomy, were included between April 2020 and May 2021, with a total of 447 samples. Median extracorporeal membrane oxygenation duration was 7 (interquartile range, 4-12) days. Median antithrombin level was 48 (37-60)% at H0. Antithrombin levels significantly increased throughout the follow-up. Time-weighted average of antithrombin levels was 63 (57-73)%, and was ≤70% in 32 (64%) of patients. Overall, 45 (90%) patients had at least one antithrombin value below 70%, and 35 (70%) below 50%. Antithrombin levels were not significantly associated with heparin responsiveness evaluated by anti-Xa assay or heparin dosage.

Conclusions

Veno-arterial extracorporeal membrane oxygenation support was associated with a moderate acquired antithrombin deficiency, mainly during the first 72 hours, that did not correlate with heparin responsiveness.

Introduction

Veno-arterial extracorporeal membrane oxygenation (VA-ECMO) is increasingly used for managing refractory circulatory failure[1]. Bleeding and thrombosis remain frequent and associated with high mortality[2,3]. They occur as a result of a complex interplay between the underlying critical illness, blood exposure to shear stress and non-biological surfaces, and antithrombotic strategies[2]. International guidelines recommend systemic anticoagulation with unfractionated heparin to prevent ECMO- and patient-related thromboembolic events, though the evidence for both thrombosis and bleeding is very weak[4,5]. The Extracorporeal Life Support Organization (ELSO) recommends a therapeutic heparin level monitored using aPTT or anti-Xa level (target 0.30 to 0.70 $IU.mL^{-1}$) whereas the International Society on Thrombosis and Haemostasis (ISTH) recommends an anti-Xa range of 0.30 to 0.50 $IU.mL^{-1}$ [5]. Current prospective data does not support the efficacy of low-anticoagulation strategies in reducing bleeding [4–8].

Unfractionated heparin requires binding to antithrombin (AT) to inhibit coagulation factors, especially factor Xa and thrombin. As a negatively charged glycosaminoglycan, it can bind to numerous proteins, cells and non-biological surfaces, reducing its anticoagulant activity, especially during acute inflammatory situations in intensive care unit (ICU) [9,10]. The management of unfractionated heparin is challenging due to its high inter-individual variability in anticoagulant response and to the risk of heparin-induced thrombocytopenia [2,3,9,11]. The biological heparin responsiveness, or anticoagulant effect, must be differentiated from the clinical response (thrombosis prevention). As of now, there are no routine tests that can reliably capture the full scope of the anticoagulant effect. For lack of better options, we rely on activated partial thromboplastin time (aPTT) or anti-Xa measurements. Decreased heparin responsiveness, also called heparin resistance, is an alteration in the heparin dose-response, often reported as the need

for high doses of heparin to achieve desired anticoagulation levels. However, there is no evidence supporting the numerous definitions found in the literature[12]. Decreased heparin responsiveness may be particularly frequent during ECMO [2,13].

Acquired AT deficiency is often arbitrarily defined as plasma AT level <70 to 80% or $U.dL^{-1}$, based on the definition of constitutional deficiency and the normal AT range in plasma (80 to 120% or $U.dL^{-1}$) [14]. It is frequently reported in ICU patients, especially in the setting of cirrhosis, sepsis or cardio-thoracic surgery [15,16]. Severe inherited AT deficiency is a major risk factor for thrombosis, associated with both clinical and laboratory decreased heparin responsiveness [17]. So far, evidence is lacking to support the association between acquired AT deficiency and decreased heparin responsiveness or thrombotic events. Moreover, the AT level required to achieve therapeutic anticoagulation is unclear, with limited data on severity thresholds and therapeutic targets.

Whereas acquired AT deficiency seems common during VV-ECMO support[3,18,19], data remain limited for VA-ECMO[19–21]. One small prospective randomized trial evaluated antithrombin supplementation during VV-ECMO, without any beneficial effect reported [5,18]. Despite the absence of evidence regarding efficacy, safety and cost, AT monitoring and supplementation is routinely performed during VV and VA-ECMO [3,22,23].

This study aimed to evaluate antithrombin plasma levels, analyze heparin responsiveness, and to evaluate the association between AT levels and heparin responsiveness during VA-ECMO support. We hypothesized that VA-ECMO support is associated with acquired AT deficiency and related alterations in heparin responsiveness.

Methods

Study Design

The ATECMO study (evaluation of AntiThrombin deficiency during ECMO support; NCT04133844) was designed as a prospective observational study. It was conducted in a 1500-bed tertiary university hospital (University Hospital of Rennes, France), comprising a cardiothoracic surgery department and three adult ICUs. The study was ethically approved (Ethics Committee CPP OUEST VI, France; 2019-A011440-57, October 1st, 2019) with procedures followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants or relatives (deferred emergency consent).

Participants

Patients older than 18 years supported by VA-ECMO (first ECMO run) were screened for inclusion. The exclusion criteria were: moribund patient with expected life expectancy of less than 24h, pregnancy, ECMO for periprocedural hemodynamic support, acute arterial (except for myocardial infarction) or venous thrombosis, inherited bleeding disorder, contraindication for unfractionated heparin (history of heparin-induced thrombocytopenia or unfractionated heparin allergy) and known inherited AT deficiency.

ECMO management

Indications for VA-ECMO therapy included medical and surgical causes of refractory cardiogenic shock in patients for whom satisfactory systemic perfusion could not be achieved despite optimal intravascular volume status, high-dose inotropic medication and/or other support. In case of post-cardiotomy ECMO, heparin was fully reversed using protamine using Hepcon HMS Plus™ (Medtronic, Paris, France). The standard protocol for VA implantation in our institution has been

previously published[24]. The ECMO program was jointly run by cardiothoracic surgeons and anesthesiologist-intensivists. Whenever possible, peripheral access through femoral vessels was used. Three different centrifugal pumps were used during the study: Rotaflow® (Getinge, Gothenberg, Sweden), Cardiohelp® (Getinge, Gothenberg, Sweden) and Medos® (Xenios, Heilbron, Germany).

Anticoagulation management

Anticoagulation was initiated as soon as possible after ECMO cannulation, in the absence of overt bleeding or severe thrombocytopenia (platelet count <50 $x10^9$/L). In the absence of high-quality clinical data to support any ECMO anticoagulation protocol, we developed a pragmatic approach at our center in 2019. This protocol, adapted from Raschke's work, involves the use of therapeutic doses of unfractionated heparin (UFH) with weight-based boluses and continuous intravenous infusion in situations without overt bleeding (Supplementary Digital Content 1 for unfractionated heparin dosage protocol, https://links.lww.com/ALN/D450) [25]. We opted for antiXa monitoring, which is readily available as a routine test in our laboratory, targeting a range of 0.30 to 0.50 IU/mL. This approach, therefore, aligns with the Extracorporeal Life Support Organization (ELSO) and International Society on Thrombosis and Haemostasis (ISTH) recommendations that were published later [4,5].

Antithrombin supplementation was not routinely performed in our center. The decision to measure AT level and to administer antithrombin supplementation (human antithrombin) was at the discretion of the physician, in case of marked AT deficiency associated with anti-Xa level <0.30 $IU.mL^{-1}$ despite unfractionated heparin dosage > 35 $IU.kg^{-1}.h^{-1}$. Heparin-induced thrombocytopenia (HIT) was suspected and confirmed according to published guidelines[26].

Heparin was discontinued in case of suspected HIT with positive anti-PF4/heparin antibodies and switched to argatroban.

Study outcomes

The primary outcome was AT plasma level, measured from ECMO initiation and up to 7 days (eleven time points: H0, H2, H6, H12, H24 and daily from D2 to D7 or ECMO decannulation). In the absence of consensus, AT deficiency on ECMO was arbitrarily defined as a time-weighted average of AT $\leq$ 70% (corresponding to the therapeutic target for recombinant AT in the summary of product characteristics). For each time-point, AT deficiency was categorized as follows: normal values above 70%, moderate deficiency between 50 and 70% and severe deficiency below 50%.

The secondary outcomes of our study included unfractionated heparin dosage and anti-Xa levels at each dosage adjustment, time spent in the therapeutic anti-Xa range, and the number of heparin interruptions. These outcomes were selected to best characterize heparin responsiveness, which is a dynamic and complex concept requiring the integration of unfractionated heparin dosage and its effect, measured by anti-Xa in this case.

Furthermore, in an exploratory manner we developed a Heparin Responsiveness Index, defined for each anti-Xa measurement as the ratio of unfractionated heparin dosage (IU/kg/h) to the anti-Xa level (IU/mL). This index facilitates a concurrent and dynamic assessment of both heparin dosage and its corresponding anti-Xa with each adjustment in dosage. It was developed by adapting the approach of the Heparin Sensitivity Index, a concept used for heparin responsiveness during cardiopulmonary bypass [27,28].

Additional variables were collected including demographic characteristics, cannulation related variables (place of cannulation, type of ECMO, cannulation site), use of AT supplementation, thrombotic and bleeding events, length of ECMO support, and 30-day mortality. No systematic

screening was performed for bleeding and thrombotic complications. Bleeding events were defined as intracranial bleeding or bleeding requiring at least a decrease in hemoglobin levels >2g.dL^{-1} or the need for at least two packed red blood cells transfusion in 24 hours including upper or lower gastrointestinal hemorrhage, peripheral cannulation site bleeding, retroperitoneal bleeding and pulmonary hemorrhage. Thrombotic events included: ischemic stroke, deep vein thrombosis, pulmonary embolism acute mesenteric ischemia, acute limb ischemia, macroscopic clotting of circuit and/or membrane without need to change the circuit or the oxygenator, oxygenator failure requiring change due to clot formation, acute circuit clotting requiring change. To minimize potential information biases, data were collected prospectively, and both hemorrhagic and thrombotic events were independently qualified by two separate investigators who were blinded to the biological results.

Blood sample collection and laboratory analyses

Routine care unfractionated heparin anticoagulation monitoring was performed by physicians using anti-Xa levels using a reagent without added AT or dextran (STA-Liquid-anti-Xa®, Stago, France). In order to avoid influencing clinical practices and to maintain the rigorous methodological standards of a cohort study, additional biological assessments required by the study, particularly antithrombin measurements, were conducted in a blinded and centralized manner and processed in batches. Quantitative measurements of antithrombin, based on the inhibition of thrombin (STA-Stachrom ATIII®, Stago, France), and of anti-Xa levels (STA-Liquid-anti-Xa®, Stago, France; without added AT or dextran) were performed on a STA-R Max coagulometer (Stago, France) at each prespecified time-point (up to 11 time points). Blood samples were drawn at each time point from pre-existing arterial line and collected into vacuum tubes (Vacutainer®, Becton Dickinson, USA) containing citrate (0.109M) or EDTA (complete blood

count). Plasma was obtained from citrated blood after centrifugation at 2000 g for 10 min, within 1h following withdrawal. After a second centrifugation, plasma samples were stored frozen at −80°C before thawing. In case of suspected HIT, anti-PF4/heparin antibodies were assessed using Asserachrom® HIPA – IgG (Stago, France).

Statistical analysis

In the absence of published data regarding the temporal evolution of AT deficiency during VA-ECMO support and time-weighted average, calculating the sample size was not possible. An adhoc sample size of 50 participants was chosen and provided us with 95% confidence and a margin of error of 5 points to estimate mean AT level, considering the standard deviation previously reported [20]. Given the nature of the invasive treatments administered to these patients, and the fact that our study was conducted in a tertiary referral center, we expected no loss to follow-up. Although the methodology used for this study does not allow for multivariable analyses to assess causality and the direct effects of antithrombin deficiency, we have attempted to minimize major confounding factors in our descriptive and correlational approach. Therefore, patients receiving AT supplementation were censored for all analyses at the time of first administration. Patient with suspected HIT were censored for laboratory analyses at the time argatroban was initiated and were excluded from thrombosis analysis if HIT was confirmed. Patient characteristics were expressed as number (percentage) for categorical variables and median with interquartile range for continuous variables. As the samples were not collected at a constant rate (time between sample collection ranging between 2 and 24h), we calculated a time-weighted average of plasma AT levels using linear interpolation between each sampling points. We did not use any imputation methods for addressing missing data. When an antithrombin measurement was missing at a specific time point, we calculated the time-weighted average using linear interpolation based on the data from

the nearest available time points before and after the missing entry. Pairwise comparisons of AT levels at each sampling points with baseline (H0) were calculated using a mixed model for repeated measures with Dunnett correction for multiple comparisons. Correlations between AT levels and anti-Xa levels, unfractionated heparin dosage and Heparin Responsiveness Index were assessed using Spearman's rank-order correlation. For comparison between patients with or without AT deficiency, bleeding or thrombosis, a $\chi 2$ test or a Fisher's exact test were used for categorical variables and a Kruskal-Wallis test for continuous variables. All tests used a two-tailed hypothesis. Statistical significance was achieved for $P < 0.05$. Statistical analyses were performed with R 4.2.1.

Results

Study population

Between April 2020 to May 2021, of 85 patients supported by VA-ECMO, fifty were included in the study, with a total of 447 blood samples collected (Figure 1). Main characteristics of included patients are reported in Table 1. Most patients were men (80%) and the median age was 60 years (IQR; 52 - 68). Patients had severe condition upon admission with a Simplified Acute Physiology Score II score of 51 (37-76) and a Survival after Veno-Arterial ECMO score of -4 (-7 to -1). No patient was affected with nephrotic syndrome or liver failure before hospitalization. Only one patient had cirrhosis. Twenty-one patients (42%) received ECMO for post-cardiotomy low cardiac output syndrome. The primary non-postcardiotomy indications included acute coronary syndrome (22%) and cardiac arrest (28%). Two patients (4%) had their plasma AT levels measured by the clinician as part of the routine anticoagulation protocol (see Supplementary Digital Content 1, https://links.lww.com/ALN/D450) and subsequently received AT supplementation. These patients were censored for all analyses at the time of first administration (respectively on day 3 and day 5). Heparin-induced thrombocytopenia was suspected in four (8%) of patients, who were censored for

laboratory analyses at the time of argatroban initiation; and diagnosed for two (4%) patients, who were excluded from thrombosis analysis. Evolution of platelet count and fibrinogen levels during ECMO support are reported in Supplementary Table S1 (Supplementary Digital Content 2, https://links.lww.com/ALN/D451). Median duration of ECMO support was 7 (4 - 12) days, with a median ICU length of stay of 16 (10 – 23) days. Mortality while on ECMO therapy was 32%. In-hospital mortality was 48 % at day 28.

Changes in antithrombin plasma levels during VA-ECMO support

The changes in AT plasma levels during the first seven days of ECMO support are depicted in Figure 2, Figure 3 and supplemental digital Figure S1 (https://links.lww.com/ALN/D451). The median AT level at H0 was 51 (39 – 65) %. Median AT levels significantly increased after ECMO implantation as early as H6, with 55 (44 – 70) % (p=0.044 compared to H0) and rose gradually to reach nearly normal median levels around day 4 of ECMO support, to 71 (56 – 86) % (p=< 0.001 compared to H0). This temporal trend remained consistent after excluding the 24 patients who received fresh frozen plasma transfusions during the first seven days of ECMO (Supplementary Digital Content 2 - Figure S2, https://links.lww.com/ALN/D451). Overall, 45 (90%) patients had at least one AT value below 70%, 35 (70%) below 50%, 8 (16%) below 30% and 2 (4%) below 20%. Time-weighted average of AT levels was 63 (57-73) %, and was ≤ 70% in 32 (64%) of patients. Overall, patients spent 71 (41-100) % of ECMO-time with AT levels under 70% and 11 (0-32) % under 50% (Supplementary Digital Content 2 - Table S2, https://links.lww.com/ALN/D451). Patients with a time-weighted average of AT levels ≤ 70% were more frequently in post-cardiac arrest condition and had a more severe ICU presentation with lower SAPS II score, pH, fibrinogen levels and platelet count, and higher aspartate aminotransferase levels (Table 1). Postcardiotomy ECMO was associated with lower AT levels at

ECMO initiation with 45 (34 – 55) % compared to non-postcardiotomy ECMO with 58 (44 – 73) %, though time-weighted average of AT levels was similar (Supplementary Digital Content 2 - Table S2, https://links.lww.com/ALN/D451).

Heparin responsiveness during VA-ECMO support

Heparin was initiated early after ECMO initiation, with a median 0 (0 – 10.8) hours. Most patients received sub-therapeutic unfractionated heparin dose during the first 24 hours with a median of 152 (113-242) $IU.kg^{-1}.day^{-1}$, corresponding to low anti-Xa target levels due to high bleeding risk in post-cardiotomy setting (Supplementary Digital Content 2 - Table S1 and S2, https://links.lww.com/ALN/D451). Heparin dosage was stable between day 1 and day 7 (301 to 403 $IU.kg^{-1}.day^{-1}$). The time to reach a first anti-Xa level $\geq$ 0.3 $IU.mL^{-1}$ was 13 (5 - 31) hours (Table 1). The time spent within anti-Xa target 0.30 to 0.50 $IU.mL^{-1}$ was only 38 (15-56) % of total ECMO time, due to frequent reductions of anti-Xa target related to overt bleeding events (Table 2) and to a median of 1 (1-2) unfractionated heparin interruption per patient. As exploratory analysis, the Heparin Responsiveness Index (ratio of unfractionated heparin dosage to the anti-Xa level at each timepoint) exhibited high variability between patients and time points, without displaying a distinct pattern during the course of ECMO support (Supplementary Digital Content 2 – Figure S3, https://links.lww.com/ALN/D451). The use of unfractionated heparin bolus in our protocol was not associated with heparin overdose, with 0 (0-4)% of ECMO time above anti-Xa > 0.70 $IU.mL^{-1}$ (Table 2).

Relationship between heparin responsiveness and antithrombin levels

Patients with a time-weighted average of AT levels $\leq$ 70% did not demonstrate decreased heparin responsiveness, as evidence by a short time to achieve a first anti-Xa activity >0.30 $IU.mL^{-1}$ with 12 (5-38) hours as compared to patients with time-weighted average $\leq$ 70%with 15 (4-28) hours

(Table 2; p=0.990). In the same way, the percentage of ECMO time spent in the 0.30-0.50 anti-Xa range was not significantly different according to time-weighted average of AT levels, with 39 (13-50)% and 32 (23-60)% for time-weighted average ≤ 70% and > 70%, respectively (Table 2). However, patients with a time-weighted average of AT levels > 70% spent significantly more of their ECMO time above an anti-Xa level of 0.50 $IU.mL^{-1}$, with 10 (5-19)% of ECMO time (Table 2; p=0.042). The relationship between heparin responsiveness and AT levels during the first seven days of ECMO is depicted in Figure 4. Antithrombin levels were not correlated to anti-Xa activity (spearman rho r=0.03; p=0.660) and demonstrated a weak correlation with unfractionated heparin dosage (r=0.26; p=0.009). Further, the Heparin Responsiveness Index was not correlated to antithrombin levels (r=0.04; p=0.570).

Bleeding and thrombotic complications

Overall, 36 (72%) patients suffered from 70 bleeding events – irrespective of severity - during total ECMO support, with cannulation site being the most frequent bleeding type (20%). Intracranial hemorrhage accounted for 4.6 % of total events (three events - three patients). Post-cardiotomy ECMO was associated with a high bleeding incidence of 90% (Supplementary Digital Content 2 - Table S2, https://links.lww.com/ALN/D451). Bleeding patients had a higher time-weighted average of AT levels compared to non-bleeding patients with 69 (59-79)% and 57 (46-62)%, respectively (Supplementary Digital Content 2 - Table S3, https://links.lww.com/ALN/D451; p=0.004). Despite a higher number of unfractionated heparin interruption, the time spent within anti-Xa target 0.30 to 0.50 $IU.mL^{-1}$ was non different between bleeding and non-bleeding patients (Supplemental Table S3, https://links.lww.com/ALN/D451). The number of red blood cell transfusions was not significantly different based on the time-weighted average of AT levels, with a median of 3 (2-8) units versus 5 (3-10) units (p=0.328),

respectively for the groups with a time-weighted average ≤70% and >70%. The same results were observed for fresh frozen plasma, with 1 (0-4) units versus 0 (0-6) units (p=0.939), and for platelet concentrates with 0 (0-2) units in both groups (p=0.690). Twenty-three patients (48%, n=48 patients without HIT) experienced at least one thrombotic event (0.8 thrombotic events per patient). Device-related thrombosis, including macroscopic clotting of circuit and/or membrane, oxygenator failure and acute circuit thrombosis, accounted for most thrombotic events. Ischemic stroke was diagnosed in five patients (seven events). No relationship between thrombotic events and either AT or anti-Xa levels was found (Supplementary Digital Content 2 - Table S4, https://links.lww.com/ALN/D451).

Discussion

Our study reports extensive data regarding AT levels and heparin responsiveness during the first seven days of VA-ECMO support. The main findings were as follows. First, moderate acquired AT deficiency was frequent and significantly improved over the course of ECMO support, particularly after the initial 72 hours following VA-ECMO initiation. Second, the use of a weight-based heparin protocol with boluses and continuous infusion was associated with a rapid achievement of the target anti-Xa levels following the initiation of heparin. Third, AT levels were not correlated with heparin responsiveness, as assessed by the concurrent measurement of heparin dosage and its effect on anti-Xa levels at each time point.

We found slightly higher AT levels than previously reported during adult VA-ECMO support[19–21]. This might be explained by the lower frequency of post-cardiac arrest ECMO, associated in our cohort with a lower time-weighted average of AT levels. Conversely, AT levels were lower compared to published data on venovenous-ECMO [3,18,19]. Finally, the progressive increase in AT levels over time on VA-ECMO was consistent with previously reported results by Mazzeffi et al.

[20] but not with those reported by Cartwright et al. [19]. Our study was not designed to address the mechanisms underlying this correction in AT levels. However, we can hypothesize that it may be due to a decrease in consumption with the resolution of shock, as well as an increase in production accompanying the correction of any potential hepatic failure associated with the initial shock.

While data showed that heparin responsiveness during cardiopulmonary bypass wasn't always dependent on AT levels [29,30], interventional studies have demonstrated an increase in activated coagulation time following AT supplementation, though without benefit on clinical outcomes [31–33] . Following these findings, the use of AT supplementation has increased in both adult and pediatric ECMO to enhance anticoagulation [3,9,22,31,34]. However, data on unfractionated heparin management during cardiopulmonary bypass cannot be easily extrapolated to ECMO support due to its prolonged duration, associated organ failures and the delicate thrombosis/bleeding balance. Indeed, only one small trial has assessed AT supplementation in VV-ECMO without positive outcomes, and no evidence exists for VA-ECMO[18]. Moreover, the exact AT plasma level needed for effective heparin anticoagulation remains unclear, but some suggest 50% to 60% of AT might sustain the heparin response measured with an anti-Xa [35].

Our study showed no correlation between heparin responsiveness, as monitored using an anti-Xa assay without added AT or dextran, and AT levels. Due to high variability in the response to unfractionated heparin, using either unfractionated heparin dosage or anti-Xa alone does not accurately assess heparin responsiveness. As exploratory analysis, we evaluated a Heparin Responsiveness Index, defined for each anti-Xa measurement as the ratio of unfractionated heparin dosage (IU/kg/h) to the anti-Xa level (IU/mL). Compared to previously developed index for cardiopulmonary bypass (Heparin Sensitivity Index)[10,27,28], this index facilitates a concurrent and dynamic assessment of both heparin dosage and its corresponding anti-Xa with each adjustment

in dosage. This study was not designed to validate this index against clinical outcomes, which will require future prospective validation. Overall, our results do not advocate for AT supplementation aiming at either increasing anti-Xa levels and/or decreasing unfractionated heparin dosage during VA-ECMO support.

Decreased heparin responsiveness, also referred to as heparin resistance, is well-documented during cardiopulmonary bypass with unfractionated heparin monitored using activated coagulation time, but its frequency during ECMO is debated [9,11,34]. This inconsistency likely arises from the lack of an established heparin responsiveness definition and the high heterogeneity in unfractionated heparin dosing and monitoring practices across centers[3]. In our study, the use of a weight-based dosing protocol, which includes a bolus at the initiation of unfractionated heparin, a high starting dosage (18 IU/kg/h), and boluses with dose increments, allowed us to quickly achieve therapeutic anti-Xa targets of ≥ 0.30. However, despite targeting an anti-Xa level of 0.30 to 0.50 $IU.mL^{-1}$ using the weight-based nomogram, time within the therapeutic range was limited because of frequent bleeding events necessitating unfractionated heparin dose adjustments or halts. This highlights once again the challenge of managing ECMO patients with high bleeding and thrombosis risk.

Beyond heparin responsiveness, AT supplementation has been considered to mitigate systemic inflammation and organ damage [36]. Previous studies on its effects in severe sepsis found no benefits or bleeding risks [37]. Similarly, a recent placebo-controlled trial of preoperative AT supplementation in cardiac surgery failed to demonstrate an improvement in adverse postoperative outcomes[38]. In our study, the time-weighted average of AT levels was higher in bleeding patients aligning with reports linking elevated AT levels to increased postoperative bleeding after cardiac surgery and during ECMO [33,39,40]. However, our study was not designed to assess the direct link

between AT levels and bleeding or thrombotic events and it was not feasible to account for the various confounding factors and competitive risks necessary for causal inference. Future research should evaluate the efficacy and safety of AT supplementation during ECMO support.

Our study has several strengths. First, it provides a longitudinal analysis of both AT levels and laboratory heparin responsiveness measured by anti-Xa, extending up to seven days following the initiation of VA-ECMO. Second, we used a multidimensional approach to heparin responsiveness, which combines unfractionated heparin dosage and anti-Xa levels at each dosage adjustment, and time spent in the therapeutic anti-Xa range. Third, anticoagulation was consistently managed through a weight-based protocol including boluses at initiation and with each dosage increase. Finally, our approach to anticoagulation on ECMO is in full agreement with the guidelines issued later by the Extracorporeal Life Support Organization (ELSO) and the International Society on Thrombosis and Haemostasis (ISTH) [4,5].

Our study has several limitations. First, this study evaluates the impact of AT levels on the response to heparin as measured by anti-Xa without the addition of AT or dextran. Consequently, its generalizability to other heparin dosage protocols or monitoring methods cannot be guaranteed. Second, this study was not designed to address the choice of monitoring between aPTT and anti-Xa. Third, the moderate AT deficit observed does not allow us to draw conclusions for very severe deficit situations of $\leq 20\%$, which were scarcely represented in our cohort. Fourth, the small sample size precluded us from drawing conclusions regarding the impact of AT levels on thrombosis and bleeding events. Fifth, although we excluded patients with known inherited AT deficiency, we did not test for Heparin Binding Site AT deficiency. However, given the rarity of type II AT deficiencies, it is highly unlikely that any of our patients had such deficiency. Finally, the Heparin

Responsiveness Index was introduced as an exploratory tool and lacks prospective validation against clinical outcomes.

Conclusions

In this single-center prospective cohort study including 50 patients, VA-ECMO support was associated with decreased antithrombin levels that significantly improved over the course of ECMO support, particularly after the initial 72 hours following VA-ECMO initiation. These antithrombin levels did not correlate with heparin responsiveness, as monitored using an anti-Xa level without addition of dextran or AT, as part of a weight-based heparin protocol. Our study does not support the routine measurement of antithrombin levels and its supplementation to enhance heparin responsiveness during VA-ECMO support. This highlights the need for prospective interventional studies on anticoagulation during ECMO and challenges the concept of decreased heparin responsiveness.

Supplemental Digital Content

Supplemental Digital Content 1: Unfractionated heparin dosage protocol during VA-ECMO support, https://links.lww.com/ALN/D450

Supplemental Digital Content 2: Supplementary Tables and Figures, https://links.lww.com/ALN/D451

Details of authors' contributions

AM: Conceived the study, elaborated the analysis plan, obtained funding, contributed to the investigation and data collection, performed the statistical analysis, analyzed and interpreted the data, wrote the first draft of the manuscript, designed the tables and figures, revised the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work

MB: Contributed to the investigation and data collection, analyzed and interpreted the data, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

JO: Contributed to the investigation and data collection, analyzed and interpreted the data, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

AP: Contributed to the investigation and data collection, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

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YL: Contributed to the investigation and data collection, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

RG: Performed the statistical analysis, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

EF: Contributed to the investigation and data collection, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

TL: Analyzed and interpreted the data, provided critical revisions of the manuscript , approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work.

NN: Conceived the study, elaborated the analysis plan, contributed to the investigation, analysed and interpreted the data, provided critical revisions of the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work

IGT: Conceived the study, elaborated the analysis plan, obtained funding, contributed to the investigation and data collection, analyzed and interpreted the data, wrote the first draft of the manuscript, designed the tables and figures, revised the manuscript, approved the final work and agree to be accountable for all aspects of the research and for the accuracy and integrity of the work

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Figure legends

Figure 1: Flow chart of the study

VA-ECMO: veno-arterial extracorporeal membrane oxygenation, HIT: heparin-induced thrombocytopenia, AT: antithrombin.

Figure 2: Antithrombin plasma levels changes during the first seven days of VA-ECMO support

P values for multiple comparisons with baseline H0 AT levels: *P<0.05; **P<0.01; ***P<0.001

Figure 3: Antithrombin plasma levels changes according to the extent of antithrombin deficiency

For each time-point (H0 to H24 and day 2 to day 7), discrete AT levels were defined as follows: normal values above 70%, moderate deficiency between 50 and 70% and severe deficiency below 50%. Number of patients are shown under each time points. AT: antithrombin.

Figure 4: Relationship between antithrombin and heparin responsiveness

(A) Relationship between antithrombin, anti-Xa levels and heparin dosage (B) Relationship between antithrombin levels and Heparin Responsiveness Index. Heparin Responsiveness Index defined as unfractionated heparin dosage ($IU.kg^{-1}.h^{-1}$) / anti-Xa level ($IU.mL^{-1}$). Values at H0, H2, and H6 were removed to avoid any residual effect from possible unfractionated heparin administration before the start of the study.

Table 1: Baseline patient, hematological and surgery characteristics

Characteristics	All patients (N=50)	Time-weighted average of AT levels ≤70% (N=32)	Time-weighted average of AT levels >70% (N=18)	p-value
Age (year)	62 (54-70)	63 (55-70)	60 (53-66)	0.240
Female sex	10 (20)	7 (22)	3 (17)	0.730
Body mass index (kg.m^{-2})	25.0 (22.5-28.4)	25.3 (21.8-29.4)	24.9 (22.9-26.2)	0.934
Comorbidities				
Hypertension	19 (38)	10 (31)	9 (50)	0.190
Diabetes	8 (16)	5 (16)	3 (17)	>0.999
Chronic respiratory disease	1 (2)	1 (3)	0 (0)	>0.999
Chronic heart failure	10 (20)	8 (25)	2 (11)	0.295
Chronic kidney disease	1 (2)	0 (0)	1 (6)	0.360
Active solid cancer	4 (8)	4 (12)	0 (0)	0.283
Active smoking	11 (22)	5 (16)	6 (33)	0.172
Chronic alcoholism	6 (12)	3 (9)	3 (17)	0.654
Thromboembolic disease	4 (8)	3 (9)	1 (6)	>0.999
Liver failure	0 (0)	0 (0)	0 (0)	-
Nephrotic syndrome	0 (0)	0 (0)	0 (0)	-
ECMO indication				
Post-cardiotomy	21 (42)	15 (47)	6 (33)	0.352
Myocarditis	3 (6)	1 (3)	2 (11)	0.291
Acute coronary syndrome	11 (22)	8 (25)	3 (17)	0.724
Pulmonary embolism[a]	2 (4)	2 (6)	0 (0)	0.530
Cardiac arrest	14 (28)	13 (41)	1 (6)	0.008
Others	12 (24)	4 (12)	8 (44)	0.017
Other cardiac assistance				
Intra-aortic balloon pump	12 (24)	5 (16)	7 (39)	0.123
Impella	2 (4)	1 (3)	1 (6)	0.999
Simplified Acute Physiology Score II score	51 (37 76)	59 (46-80)	36 (29-55)	0.003
Survival after Veno-Arterial ECMO score	-4 (-7 - 1)	-4(-9 - 3)	-2 (-4 - 0)	0.070
Norepinephrine at H0	39 (78)	23 (72)	16 (89)	0.287
Dobutamine at H0	38 (76)	22 (69)	16 (89)	0.170
Adrenaline at H0	18 (36)	14 (44)	4 (22)	0.128
Baseline laboratory parameters (H0)				
Antithrombin level (%)	51 (40-65)	47 (36-56)	60 (43-78)	0.035
Creatinine (µmol.L^{-1})	99 (77-141)	101 (80-164)	94 (75-131)	0.795
pH	7.34 (7.21-7.44)	7.25 (7.06-7.42)	7.41 (7.30-7.46)	0.018
Lactate (mmol.L^{-1})	5.5 (2.5-9.1)	5.9 (2.9-11.4)	3.0 (2.0-8.0)	0.103
Hemoglobin (g.dL^{-1})	11.2 (8.7-13.7)	10.6 (8.8-12.8)	12.4 (9.1-14.1)	0.352
Platelet count (10^{9}.L^{-1})	164 (96-220)	126 (89-210)	195 (163-250)	0.036
Fibrinogen (g.L^{-1})	2.6 (2.1-3.6)	2.3 (1.9-3.0)	3.7 (2.7-4.2)	0.004
Aspartate aminotransferase (IU.L^{-1})	356 (82-584)	500 (333-1510)	127 (43-306)	0.004
Bilirubin (µmol.L^{-1})	15 (10-22)	12 (10-22)	17 (12-22)	0.220

Results are presented as n(%) or median (IQR). AT: antithrombin; ECMO: extracorporeal membrane oxygenation; SAPS II score: Simplified Acute Physiology Score; SAVE score: Survival After Venoarterial ECMO score
[a] cardiac arrest secondary to pulmonary emnbolism

Table 2: Heparin responsiveness according to time-weighted average of AT levels during VA-ECMO support

Characteristics	All patients (N=50)	Time-weighted average of AT levels ≤70% (N=32)	Time-weighted average of AT levels >70% (N=18)	P-value
Time to reach first anti-Xa ≥0.30 IU.mL^{-1} (hours)[a]	13 (5-31)	12 (5-38)	15 (4-28)	0.990
% of ECMO time spent in anti-Xa range[a]				
Anti-Xa <0.30 IU.mL^{-1}	50 (31-74)	55 (34-78)	34 (29-64)	0.148
Anti-Xa [0.30 – 0.50] IU.mL^{-1}	38 (16-55)	39 (13-50)	32 (23-60)	0.326
Anti-Xa >0.50 IU.mL^{-1}	6 (2-16)	5 (0-13)	10 (5-19)	0.042
Anti-Xa >0.70 IU.mL^{-1}	0 (0-4)	0 (0-4)	0 (0-4)	0.596
Number of unfractionated heparin interruptions	1 (1-2)	1 (0-2)	1 (1-2)	0.226

Results are presented as median (25th - 75th).
AT: antithrombin, ECMO: extracorporeal membrane oxygenation
[a] calculated based on all anti-Xa performed as routine care for UFH anticoagulation monitoring during ECMO support.

Figure 1

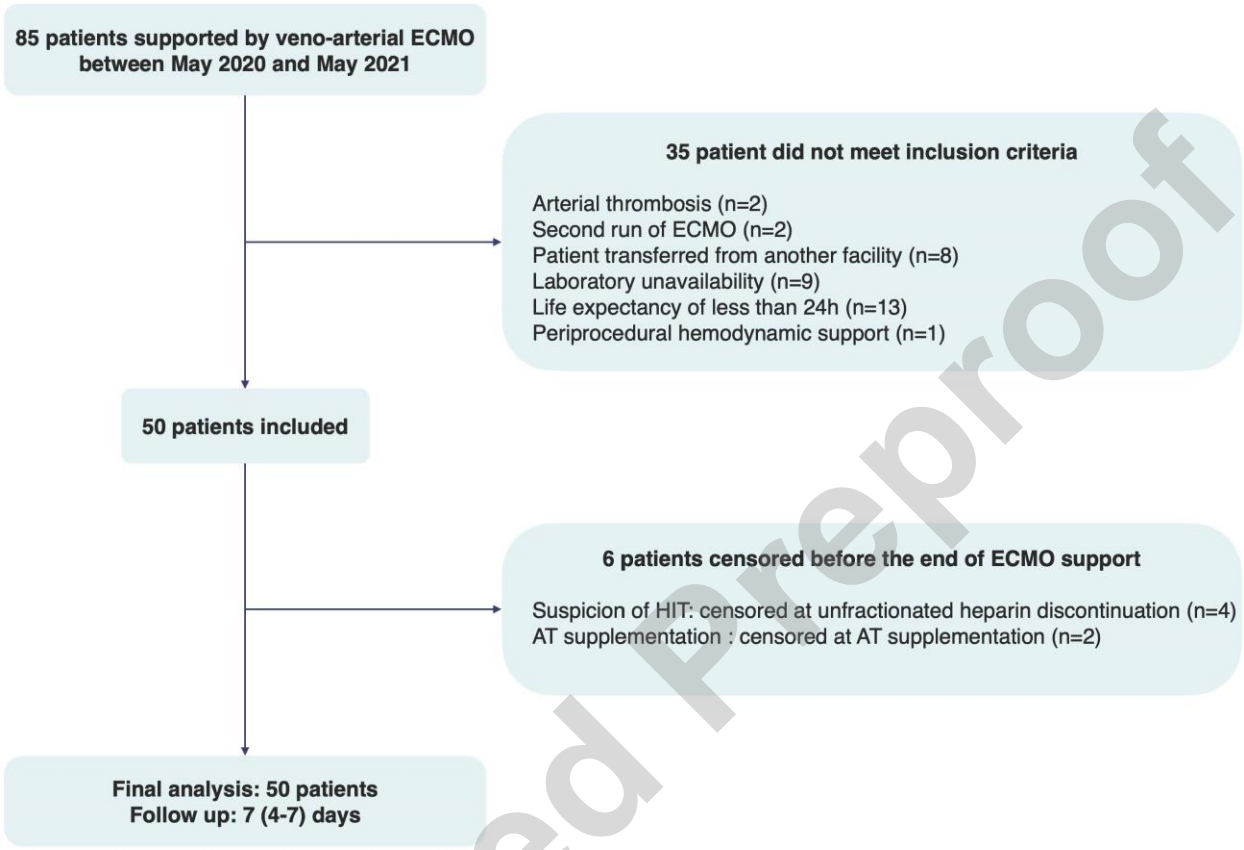

Figure 2

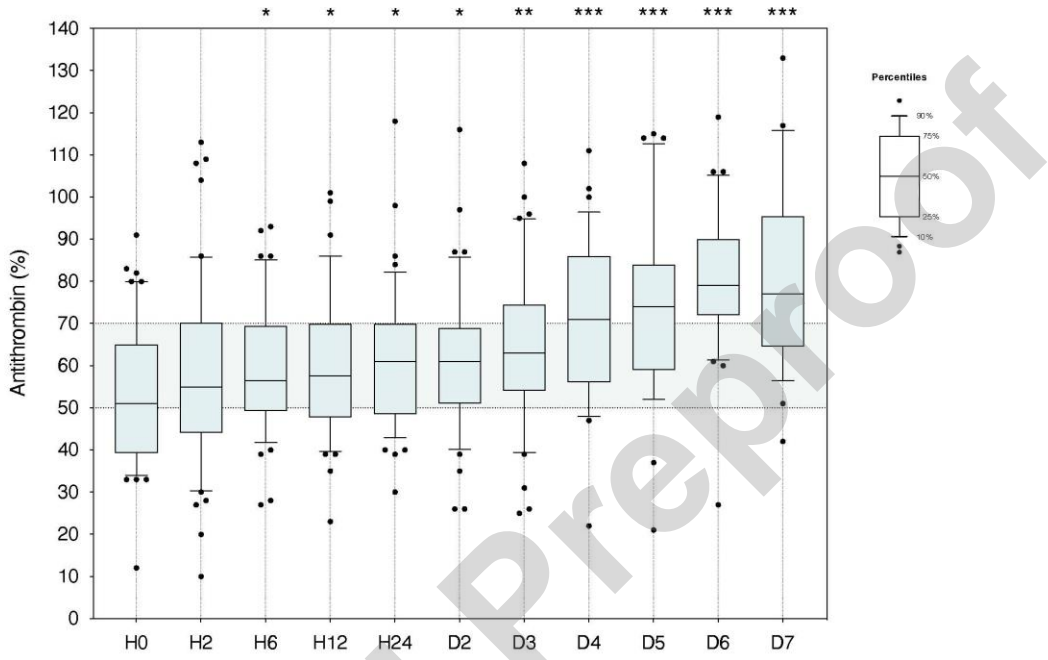

Figure 3

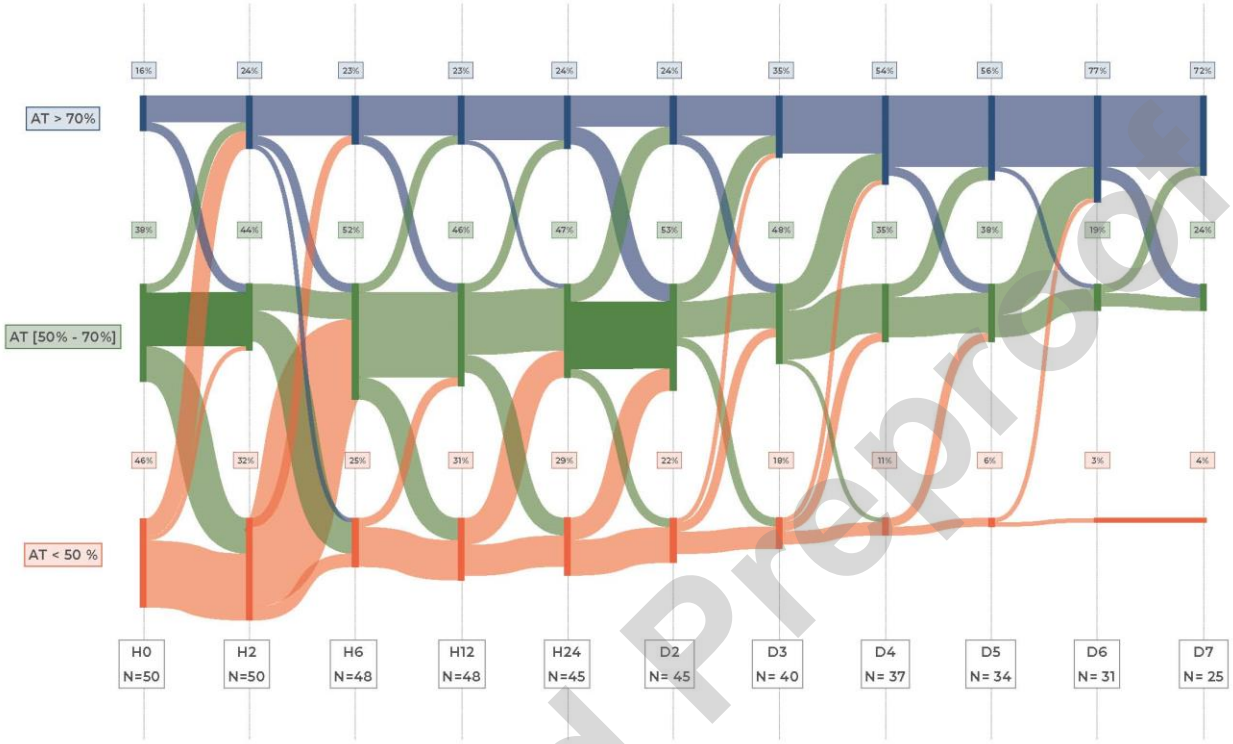

Figure 4