ORIGINAL CLINICAL REPORT

Novel Coagulation Test Detects Anticoagulation Resistance and Is Associated With Thrombotic Events in Pediatric Patients Requiring Extracorporeal Membrane Oxygenation

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OBJECTIVES: Bivalirudin, an IV direct thrombin inhibitor, and unfractionated heparin (UFH) are frequently used anticoagulants in the pediatric critical care setting. An accurate, specific, point-of-care test to quantify and detect anticoagulation resistance is not currently available. This study evaluates the ability of a rapid (< 10 min), micro-volume (< 50 uL) coagulation test to detect and quantify the anticoagulation effect of bivalirudin and UFH using a functional, clot time endpoint in pediatric critical care patients.

DESIGN: Single-site retrospective laboratory sample analysis and chart review.

SETTING: A 105-bed pediatric and cardiac ICUs delivering extracorporeal membrane oxygenation.

SUBJECTS: Forty-one citrated, frozen, biobanked plasma specimens comprising 21 with bivalirudin and 20 with UFH from 15 anticoagulated pediatric patients were analyzed. Thirteen patients were on extracorporeal membrane oxygenation, one had a submassive pulmonary embolism, and one was on a left ventricular assist device.

INTERVENTIONS: None.

MEASUREMENT AND MAIN RESULTS: A Clotting Time Score (CTS) was derived on each sample. The CTS detected patients that had developed a pathologic clotting event with 100% sensitivity and 82% specificity compared with prothrombin time with 25% sensitivity/76% specificity and activated partial thromboplastin time with 0% sensitivity/0% specificity. Additionally, the CTS detected subtherapeutic anticoagulation in response to UFH in patients that were clinically determined to be UFH resistant requiring alternative anticoagulation with bivalirudin.

CONCLUSIONS: The CTS appears to be a clinically valuable indicator of coagulation status in patients treated with either UFH or bivalirudin. Results outside of the therapeutic range due to inadequate dosing or anticoagulation resistance appeared to be associated with clot formation. CTS testing may reduce the risk of anticoagulation-related complications via the rapid identification of patients at high risk for pathologic thrombotic events.

KEY WORDS: anticoagulation; bivalirudin; ECMO; heparin; thrombosis; VAD

Target anticoagulation ranges for unfractionated heparin (UFH) or bivalirudin in the setting of extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD), and thrombosis have been published. However, since hemostasis and anticoagulant response can be variable among patients, target ranges based on routine coagulation testing may not apply universally. This is especially true for patients subjected to prolonged

KEY POINTS

- **Question:** This study evaluates the ability of the factor IIa Clotting Time Score (CTS) test to detect and quantify the anticoagulation effect of bivalirudin and unfractionated heparin (UFH) in pediatric patients receiving extracorporeal membrane oxygenation.

- **Findings:** In this retrospective clinical study utilizing biobanked samples, the CTS detected patients that developed a thrombotic event and subtherapeutic anticoagulation effect in response to UFH in patients that were UFH resistant.

- **Meanings:** The CTS appears to be a valuable indicator of coagulation status in patients treated with either UFH or bivalirudin and results outside of the therapeutic range due to inadequate dosing or anticoagulation resistance appeared to be associated with clot formation.

anticoagulation and with concurrent systemic inflammation, such as those receiving ECMO/VAD treatment and pediatric patients (< 1 yr) with immature hemostatic systems (1–4). Anticoagulation resistance (AR) is identified as a failure to achieve a specified anticoagulation effect despite the administration of an appropriate dose of anticoagulation. While frequently encountered, there is no clear definition of AR (5). Among neonatal ECMO patients, the Bleeding and Thrombosis During ECMO study reported a 60–77% bleeding event rate and a 32–44% thrombotic event (TE) rate (6). Titration of anticoagulation in pediatric patients remains challenging. Up to 26% of pediatric patients receiving ECMO develop resistance to UFH, increasing the risk of thrombosis and the need for alternate anticoagulation management with direct thrombin inhibitors, such as bivalirudin, or the administration of antithrombin (1, 2, 7–10). There is an immediate need for a test that accurately monitors both UFH and bivalirudin therapy that can detect AR early to avoid life-threatening adverse events.

Currently, UFH monitoring tests include: activated partial thromboplastin time (aPTT), anti-factor Xa (anti-Xa) chromogenic assay, activated clotting time (ACT), or viscoelastic testing (11–14). aPTT and/or anti-Xa are most commonly used to assess adequate UFH-related anticoagulation given that ACT is unreliable in the pediatric ECMO setting and viscoelastic testing is not broadly available (11–14). However, aPTT is effected by other factors such as presence of lupus anticoagulant, elevated C reactive protein, and increased factor VIII level (15–18). Anti-Xa assays use platelet-poor-plasma (PPP) and are not functional clot time-based tests as they do not account for thrombin, fibrinogen, and other blood cells, which contribute to coagulation. Additionally, chromogenic assays suffer from preanalytical variables including hemolysis, lipemia, and icterus, which are increasingly common in pediatric patients receiving ECMO (1, 19–21). Finally, multiple studies demonstrate a lack of concordance between the aPTT and anti-Xa assay, especially among pediatric ECMO populations (12, 22, 23). Bivalirudin is monitored by aPTT (with or without heparinase), diluted thrombin time (dTT), or ecarin chromogenic assay (ECA) (21–25). Similar to UFH monitoring, there is lack of agreement between these various laboratory assays and the effect and anticoagulation level of bivalirudin.

While aPTT, prothrombin time (PT), and ACT can be performed at the point-of-care (POC), dTT, ECA, and anti-Xa assays are specialty laboratory-based tests, have longer turnaround times, and require larger blood volumes (≥ 200 μL) due to the need to obtain both sample and waste volumes and the requirement for PPP. Due to the higher blood volume typically required, multiple test events increase the risk of iatrogenic anemia potentially requiring a blood transfusion in this highly vulnerable pediatric population (26, 27). Having an accurate, rapid, low-volume coagulation test to guide anticoagulation therapy for UFH and bivalirudin would be a significant clinically valuable innovation for coagulation management of pediatric patients receiving ECMO. In this study, we investigated if a novel, low volume, rapid, factor IIa (FIIa) Clotting Time Score (CTS) test can accurately measure the anticoagulation effects of UFH and bivalirudin, can detect AR and can predict the development of TEs (28, 29).

MATERIALS AND METHODS

Sample Collection

We performed a single-center, retrospective pilot study evaluating the CTS using citrated, frozen, biobanked

serial samples from 15 pediatric patients from the Texas Children's Hospital and Baylor College of Medicine (BCM), Houston, TX. A total of 41 samples were available to test comprising 21 with bivalirudin and 20 with UFH. Various specimens were available from 13 patients receiving ECMO, one on a left VAD, and one with a submassive pulmonary embolism (**Supplementary Table 1**, http://links.lww.com/CCX/B67).

All samples and patient information were collected, deidentified, and handled according to The Institutional Review Board (IRB) for Human Subject Research for BCM and Affiliated Hospitals (Protocols: H45528, UTILITY OF POC COAGULATION DIAGNOSTIC DEVICE FOR USE IN PATIENTS UNDERGOING EXTRACORPOREAL LIFE SUPPORT, approved July 5, 2019; H40470, BLEEDING AND THROMBOTIC COMPLICATIONS IN A PEDIATRIC AND ADULT POPULATION, approved January 1, 2021). Informed consent was obtained, as detailed in the IRB protocols used in this study. Appropriate procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975. Details on sample handling

and processing are described in the **Supplementary Information** (http://links.lww.com/CCX/B67). Retrospective chart review for each day of sample collection identified: clinical outcome, bleeding/clotting events, anticoagulant type and dose, and concurrent clinician-ordered coagulation test results. "Bleeding" and "thrombotic events" definitions and details of concurrent coagulation testing are included in the Supplementary Information (http://links.lww.com/CCX/B67).

Clotting Time Score Investigation

A CTS, a unitless value, was calculated for control and patient samples as previously described (30). Briefly, coagulation curves for UFH and bivalirudin were derived based on clotting times (**Fig. 1**, *Ai* and *Bi*). Minimum sample volume in this study was 50 µL of PPP per patient. This testing volume does not include any discard sample that was collected on each patient in order to perform the clinician-ordered laboratory-based assays as listed in Supplementary Table 1 (http://links.lww.com/CCX/B67). The CTS was then calculated and plotted against the known drug concentration in the control sample to obtain a best-fit line. This
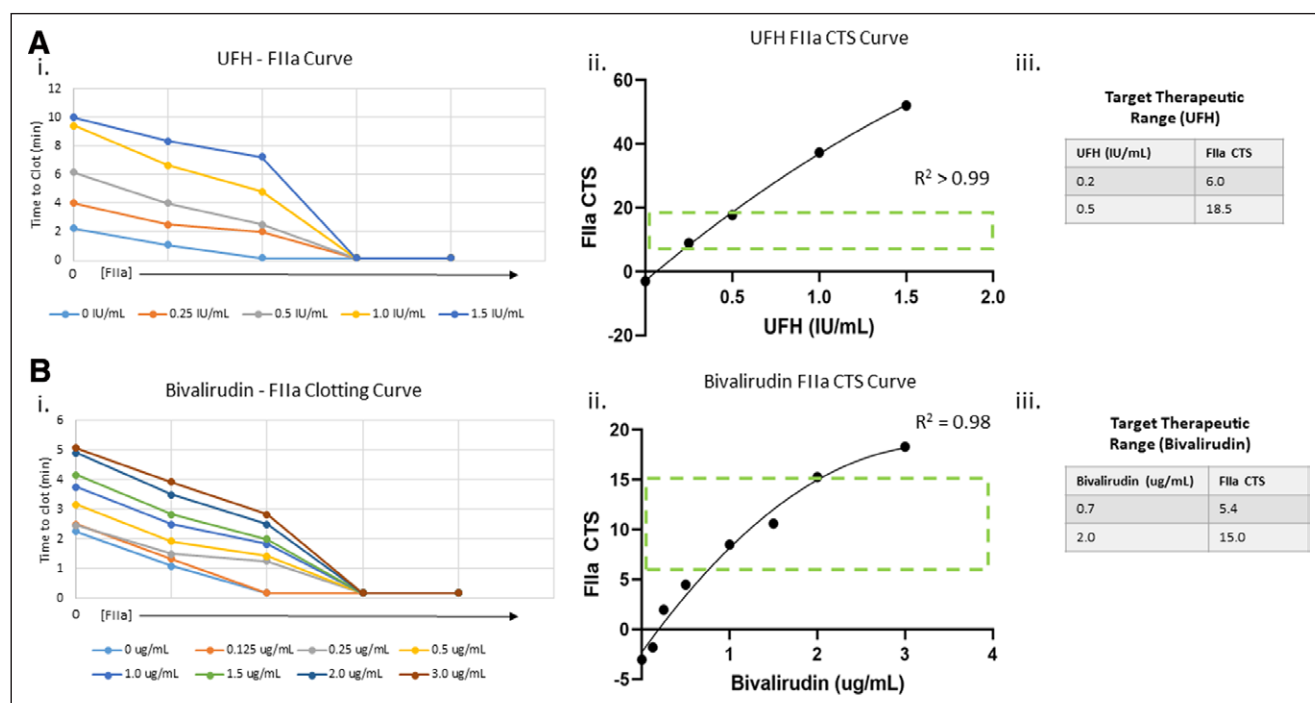
Figure 1. Clotting Time Score (CTS) generation and reference ranges. Clotting times in response to varying concentrations of factor IIa (FIIa) using plasma samples with known concentrations of unfractionated heparin (UFH) or bivalirudin were plotted (**Ai** and **Bi**). Using the clotting time, the FIIa CTS was calculated and plotted against the anticoagulant concentration. **Aii** and **Bii**, A best-fit curve was calculated and was then used to back-calculate the expected CTS for the desired concentration of each anticoagulant to derive a target "therapeutic" range. This is represented as the "*green zone*" in (**Aii** and **Bii**) and is also represented numerically in the tables (**Aiii** and **Biii**).

allowed the target therapeutic range of the drug, provided as a drug concentration, to provide an expected CTS range (**Fig. 1**, *Aii–iii* and *Bii–iii*). The target therapeutic range is delineated by a green dashed line incorporating a "Green Zone." A detailed description of sample handling and CTS calculation is included in the Supplementary Information (http://links.lww.com/CCX/B67).

Statistical Analysis

Both Excel (Microsoft, Redmond, WA) and GraphPad Prism (GraphPad Software, La Jolla, CA) were used for basic descriptive and comparative statistics. Student t test was used to compare between groups with statistical significance defined as p value of less than 0.05. Best-fit line functions were used to evaluate the relationship between two variables and calculate an R^2 variable. Receiver operating characteristic (ROC) was calculated for the FIIa CTS group using GraphPad, with an ROC greater than 0.90 considered very good. The target therapeutic range of the FIIa CTS was between 5.4 and 18.5, which encompasses both the bivalirudin and UFH target ranges (Fig. 1, *Aiii* and *Biii*). Reference ranges for the traditional coagulation tests are listed in the Supplementary Information (http://links.lww.com/CCX/B67).

RESULTS

A total of 41 samples from 15 patients were evaluated in this study. There were eight major TEs clinically identified on the days of sample collection (Supplementary Table 1, http://links.lww.com/CCX/B67). Upon stratification of the patient samples into no TE, "No Event," and TE, "Thrombosis," there was a clear association with the CTS, where TEs tended to occur when the CTS was below the target therapeutic range (**Fig. 2**). There was a significant difference in both the CTS and aPTT between patients with a TE versus no TE ($p < 0.001$ and $p = 0.02$) as compared with the PT ($p = 0.47$). Although it is known that UFH does not prolong PT at the traditional target therapeutic rang (0.2–0.4 U/mL UFH), we included patients receiving both UFH and bivalirudin to demonstrate the robustness of the FIIa CTS in evaluating coagulation response to both these anticoagulants using one single test. Upon applying the target therapeutic range (Fig. 1), the CTS had 100% sensitivity and 82% specificity along with a 100% negative predictive value (NPV) and 57% positive predictive value (PPV) in the association with a TE as compared with the PT and aPTT, which had much lower sensitivity/specificity and NPV/PPV, respectively (20%/29% and 67%/29% for PT and 0%/0% and 83%/0% for aPTT)
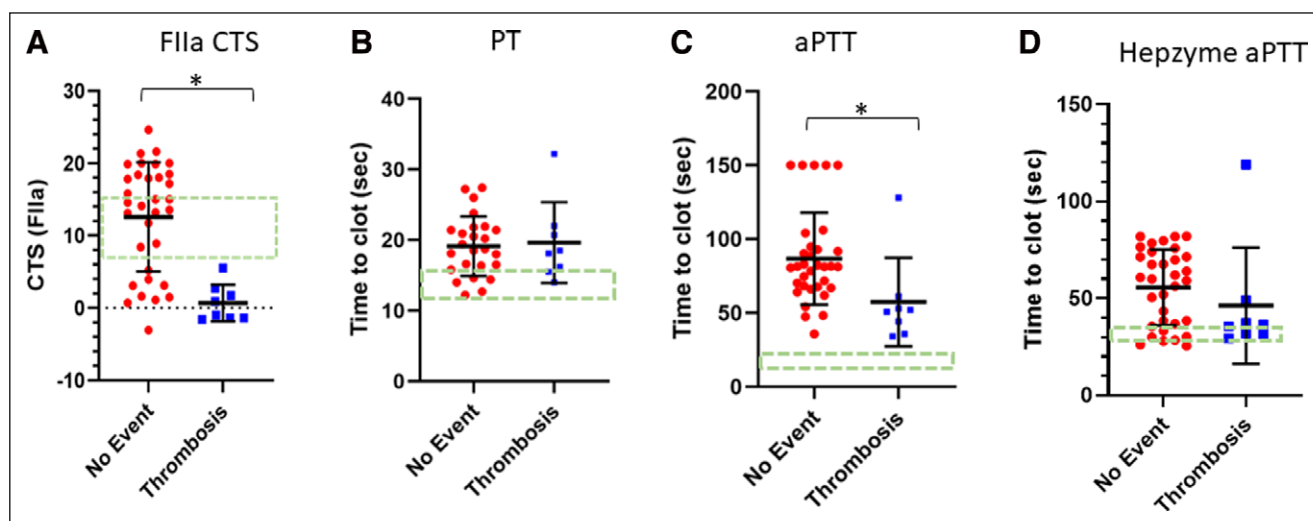
Figure 2. Comparison of the Clotting Time Score (CTS) and routine coagulation tests with respect to thrombotic events. Scatter plots of coagulation test results for patients with a thrombotic event (*red circles*) compared with patients without a thrombotic event (*blue squares*) on the day of blood sample collection. The *green dashed line* represents the target therapeutic range in (**A**) and the normal range in (**B–D**). **A–D**, Both the CTS and the activated partial thromboplastin time (aPTT) are sensitive to the development of a thrombotic event ($p < 0.001$ and $p = 0.02$, respectively), whereas the prothrombin time (PT) ($p = 0.47$) and hepzyme-aPTT (and $p = 0.28$) were not. Student t test was performed to evaluate for statistical significance with a $p < 0.05$ being considered significant and indicated by an *. FIIa = factor IIa.

(**Fig. 2A–C**). aPTT with Hepzyme exhibited limited correlation with the development of a TE ($p = 0.28$; 38%/82% and 84%/33%) (**Fig. 2D**).

A ROC curve was generated to evaluate the FIIa CTS' relationship with the development of TEs in patients receiving bivalirudin or UFH, which resulted in a very good ROC of 0.91 (SE, 0.05; 95% CI, 0.81–0.99; $p < 0.001$) (**Fig. 3A**). All patients treated with UFH on the day when a TE was diagnosed (patients 6, 8, 11, and 12) had an anti-Xa level of less than 0.2 IU/mL, indicating subtherapeutic levels or effects of UFH (Supplementary Table 1, http://links.lww.com/CCX/B67). However, not all of these patients had aPTT values below the target range (**Fig. 3Ciii**). Because the goal of this study was to evaluate the CTS as a "universal" anticoagulant monitoring tool, the anti-Xa data was not included in Figures 2 and 3, as this test was only performed on patients on UFH and not on patients on bivalirudin. Unlike the anti-Xa assay, the

PT and aPTT are generally employed for monitoring of anticoagulation and to assess hemostasis and were, therefore, frequently ordered by clinicians for patients receiving ECMO irrespective of the type of anticoagulant used (Supplementary Table 1, http://links.lww.com/CCX/B67).

Test results were evaluated in the context of bleeding events (i.e., for the correlation with the presence of bleeding events). In this batch of samples, there was no major bleeding event; however, other minor bleeding events were noted, including hematuria and oozing/bleeding from the nasal cannula or catheter insertion site and some patients were flagged as potentially in disseminated intravascular coagulation (DIC), with thrombocytopenia and elevated D-dimer levels (31). Upon further stratification of the patients with "No Event," "Thrombosis," and "Bleed/DIC/Oozing," there was no significant trend noted in the CTS score (**Fig. 3B**).
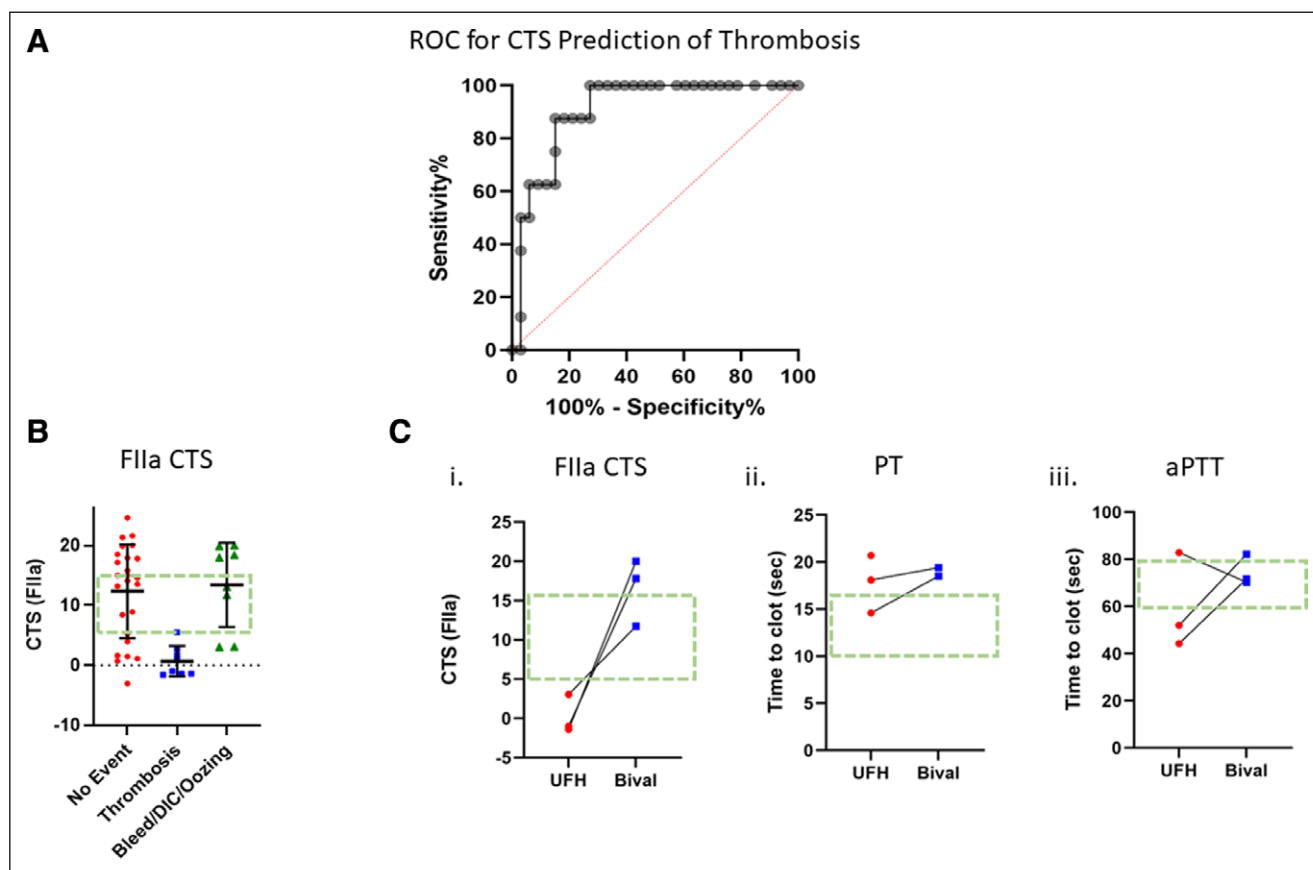

Figure 3. Evaluation of the Clotting Time Score (CTS). **A**, The receiver operating characteristic (ROC) graph is shown for the correlation between the factor IIa (FIIa) CTS and the development of a thrombotic event. **B**, Scatter plot comparing the FIIa CTS between patients with no event (*red circles*), a thrombotic event (*blue squares*) or a reported minor bleeding/oozing or disseminated intravascular coagulation event (*green triangles*). **C**, Scatter plots from three patients that were clinically diagnosed with unfractionated heparin-resistance (*red circles*) and were subsequent switched to treatment with bivalirudin (*blue squares*). The *green dashed lines* represent the target therapeutic ranges for FIIa CTS and activated partial thromboplastin time (aPTT) and represent the expected normal range for prothrombin time (PT).

During the study period, we had samples from three patients during both UFH treatment and the subsequent switch to bivalirudin upon the identification of AR to UFH (patients 2, 8, and 12) (Supplementary Table 1, http://links.lww.com/CCX/B67). The determination of the presence of AR to UFH was guided by the lack of a consistent anticoagulant effect at a UFH dose of 40 U/kg/hr as indicated by the development of a TE in addition to a low Anti-Xa test result and, in some cases, an inappropriately low aPTT. The anti-Xa level for all three UFH-resistant patients included in this figure was less than 0.2 IU/mL (Supplementary Table 1, http://links.lww.com/CCX/B67). For these patients, samples from the day the AR was detected and from the first 1–3 days that bivalirudin was initiated were available for evaluation. The FIIa CTS was able to successfully detect the subtherapeutic effect of UFH on the day that AR was detected (**Fig. 3Ci**), unlike the PT and aPTT (**Fig. 3Cii–iii**), and the subsequent increase in anticoagulation effect upon the switch to bivalirudin, as represented by an increase in the CTS level to target or high effect, indicating an improved anticoagulation response.

The routinely clinician-ordered coagulation tests were evaluated to explore whether there were any correlations with TEs or the CTS (**Supplementary Fig. 1**, http://links.lww.com/CCX/B67). The level of antithrombin activity (evaluated in patients on UFH) in this patient population did not correlate as expected with the development of a TE and did not appear to be correlated to the CTS (32). The CTS was moderately correlated to the anti-Xa activity level (33).

Finally, a thorough evaluation of PT and aPTT, as it relates to bivalirudin and UFH administration as well as FIIa CTS, was conducted. While there was no significant difference in aPTT between patients on bivalirudin compared with UFH ($p = 0.78$), there was a significant increase in PT for patients on bivalirudin as compared with UFH ($p < 0.001$) (**Fig. 4A**). This is expected, as PT is known to be relatively sensitive to bivalirudin and insensitive to UFH at the traditional therapeutic range, whereas aPTT is known to be
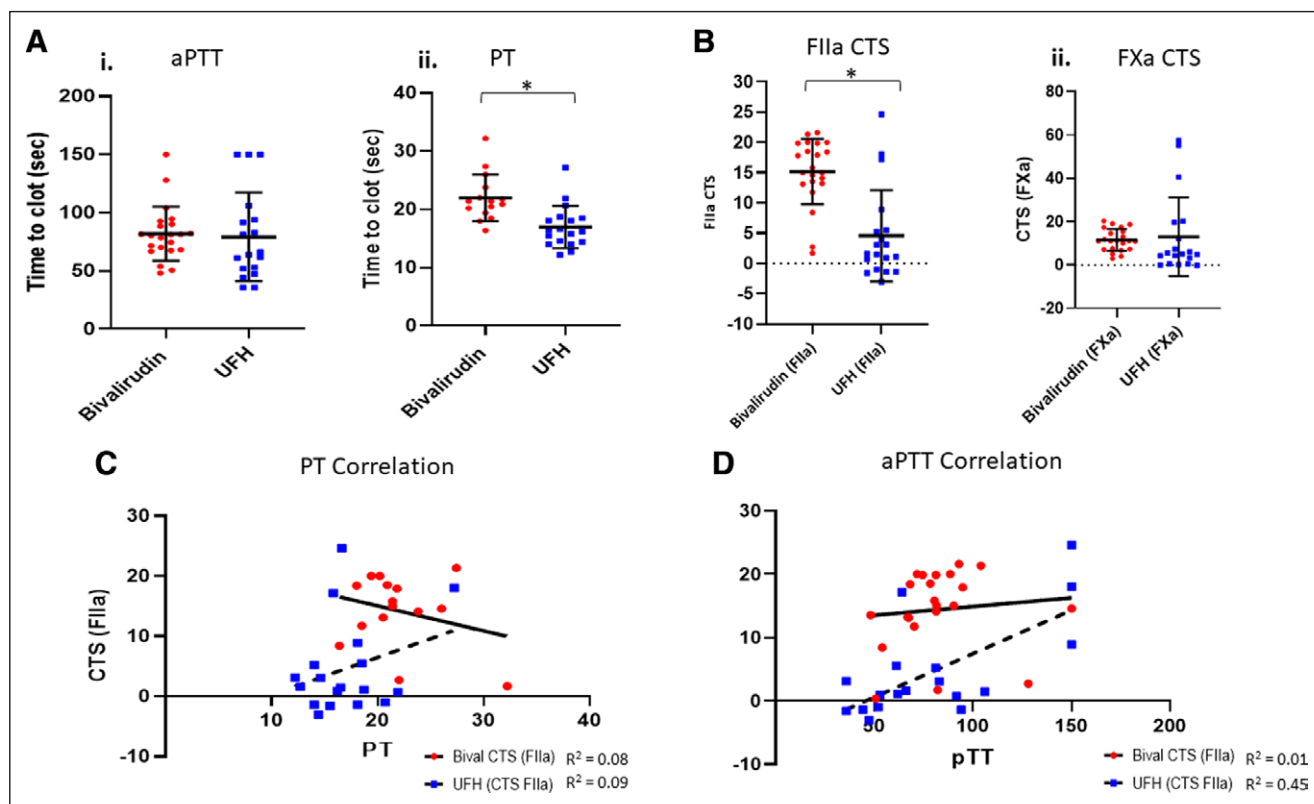
Figure 4. Coagulation test results for unfractionated heparin (UFH) and bivalirudin. **A**, Compares the activated partial thromboplastin time (aPTT) (**i**) and prothrombin time (PT) (**ii**) values for patients on both bivalirudin (*red circles*) and UFH (*blue squares*). **B**, Compares the factor IIa (FIIa) Clotting Time Score (CTS) (**i**) and the factor Xa (FXa) CTS (**ii**) for patients on both bivalirudin (*red circles*) and UFH (*blue squares*). The FIIa CTS demonstrated a statistically significant difference in the effect of anticoagulation between the patients treated with UFH as compared with bivalirudin. Plot (**C** and **D**) evaluates whether there is a correlation between the FIIa CTS and the PT (**C**) and aPTT (**D**) for both bivalirudin (*red circles*) and UFH (*blue squares*). *Trend line* for bivalirudin is *solid* and the one for UFH is *dashed*. Student *t* test was performed to evaluate for statistical significance with a $p < 0.05$ being considered significant and indicated by an *.

sensitive to both UFH and bivalirudin (34, 35). A comparison between the FIIa and FXa CTS demonstrated that the FIIa CTS was more sensitive to the thrombin inhibition secondary to bivalirudin (**Fig. 4B**). As demonstrated in **Figure 4**, **C** and **D**, there was no correlation between PT and FIIa CTS for bivalirudin and UFH, there was no correlation between aPTT and FIIa CTS for bivalirudin, and there was a mild-moderate correlation between aPTT and UFH.

DISCUSSION

Anticoagulation management can be complex in critically ill patients. Specifically, pediatric patients on ECMO are at an especially high risk of developing coagulopathies due to primary or secondary mechanisms. Unlike adults, a pediatric patient (< 1 yr) has an inherently immature hemostatic system with varying concentrations and activities of coagulation factors changing with age (3, 4). Superimpose secondary inflammation and/or the presence of mechanical circulation (ECMO, left ventricular assist device [LVAD]) and the risk increases coagulation-related complications (36). In this study, we evaluated a novel approach to evaluating coagulation status in these patients using a rapid, micro-volume coagulation test, with a CTS read-out. We evaluated the CTS for its ability to detect whether pediatric patients on UFH or bivalirudin were functionally anticoagulated, or were anticoagulation resistant (AR), and for its potential association with the development of TEs. Results showed that CTS's indicating subtherapeutic effects of UFH and bivalirudin were associated with TEs, while the aPTT, which was somewhat sensitive to the development of a TE, was inferior to the CTS. Importantly, the CTS provided unique information and did not correlate with the PT or aPTT for patients on either bivalirudin or UFH.

The development of AR to UFH is a well-known complication among patients requiring ECMO (1, 12). Currently, the standard of care for the diagnosis of AR to UFH requires multiple tests to demonstrate the presence of attenuated increase in aPTT, decreased levels of anti-Xa activity, low antithrombin activity levels, or some combination thereof (12, 23). In this study population, a single test, the CTS was able to detect anticoagulant resistance (AR) to UFH, even in the face of prolonged PT and aPTT values and, changing such

a patient's anticoagulation therapy to a direct thrombin inhibitor, bivalirudin, was associated with an increased CTS and achieved the target CTS therapeutic range. The assessment that the CTS provides novel, clinically useful information is supported by CTS' high sensitivity to effect of anticoagulation with UFH and bivalirudin and its' lack of correlation with the PT and aPTT. The CTS was developed to detect and quantify the inhibition of specific coagulation factors, in this case, FIIa, whereas the PT and aPTT are general functional tests for the intrinsic and extrinsic pathway. This is likely also why the FIIa CTS is more specific and sensitive to FIIa-based anticoagulation effects, including via direct thrombin inhibitions, such as bivalirudin, or via indirect thrombin inhibition, such as UFH.

Currently, specific tests are used for specific anticoagulants including PT, aPTT, anti-Xa, and dTT and therefore must be used in combination for complex cases. This complicates clinical processes with varying turnaround times availability and need for expert interpretation and may delay critical decision-making, compromising patient care and outcomes (12, 23, and 35). In this study, we have demonstrated that the CTS test can be used to monitor multiple classes of anticoagulants, both UFH and bivalirudin. Additionally, while the anti-Xa assay was able to identify AR to UFH in this study population, this test is not a "functional assay"; it does not measure the development of a clot, it instead tests the residual activity level of factor Xa via the addition of a chromogenic substance (39, 40). This testing approach misses the role of the downstream portion of the coagulation pathway, including endogenous thrombin and fibrinogen, which is frequently aberrant during severe inflammation (such as in patients receiving ECMO), which could also be one of the reasons for the frequent finding of discordant anti-Xa and aPTT, which is a clot-based test, results when monitoring UFH (21). The CTS, on the other hand, is a functional, clot-based test, potentially providing more clinically relevant information. As a single, rapid, functional test, the CTS potentially enables improved clinical process on these issues.

Finally, the CTS could potentially be useful as a complementary test for patients with other coagulopathies, such as with the development of APA (such as patient 6), where the aPTT was prolonged due to the presence of the Antiphospholipid Antibody (APA) and not just the presence of UFH (37, 38). Whereas the aPTT

uses phospholipids as a coagulation activator, the FIIa CTS assay does not; the FIIa CTS test is therefore not increased in the presence of APA but is specific for the presence of FIIa inhibition.

Limitations in this study should be noted. First, this is a single-center, retrospective study with limitations on sample and patient availability. This includes a lack of control or standardization of patient demographics, ECMO/LVAD circuitry, as well as comorbidities and polypharmacy. Small patient number and sample size subsets, while allowing initial evaluation of the CTS in this pediatric population, preclude definitive assessment and support proceeding with a larger patient study. Second, the lack of dTT data on the patients treated with bivalirudin with data only available for one patient as there was not enough PPP to perform the dTT on all patients. Third, few patients had a major bleeding event. While the association between the CTS and the development of a TE was significant, the detection of over-anticoagulation is also critical and not addressed sufficiently by our data. Finally, the coagulation studies were performed on PPP, which lacks essential whole blood components such as leukocytes, erythrocytes, and platelets contributing to clot formation (8, 42, 43). The new POC CTS platform design (which will be used for future studies) uses 50–100 uL of whole blood/test with a turnaround time for a CTS score of less than 10 minutes (**Fig. 5A**). As such the CTS could potentially be used as a sole bedside anticoagulation test for both heparins and direct thrombin inhibitors (DTIs).

CONCLUSIONS

This is the first study to evaluate the CTS, a novel coagulation test, in a high-risk pediatric patient population undergoing mechanical circulatory interventions. The results, while reported in a small series, support
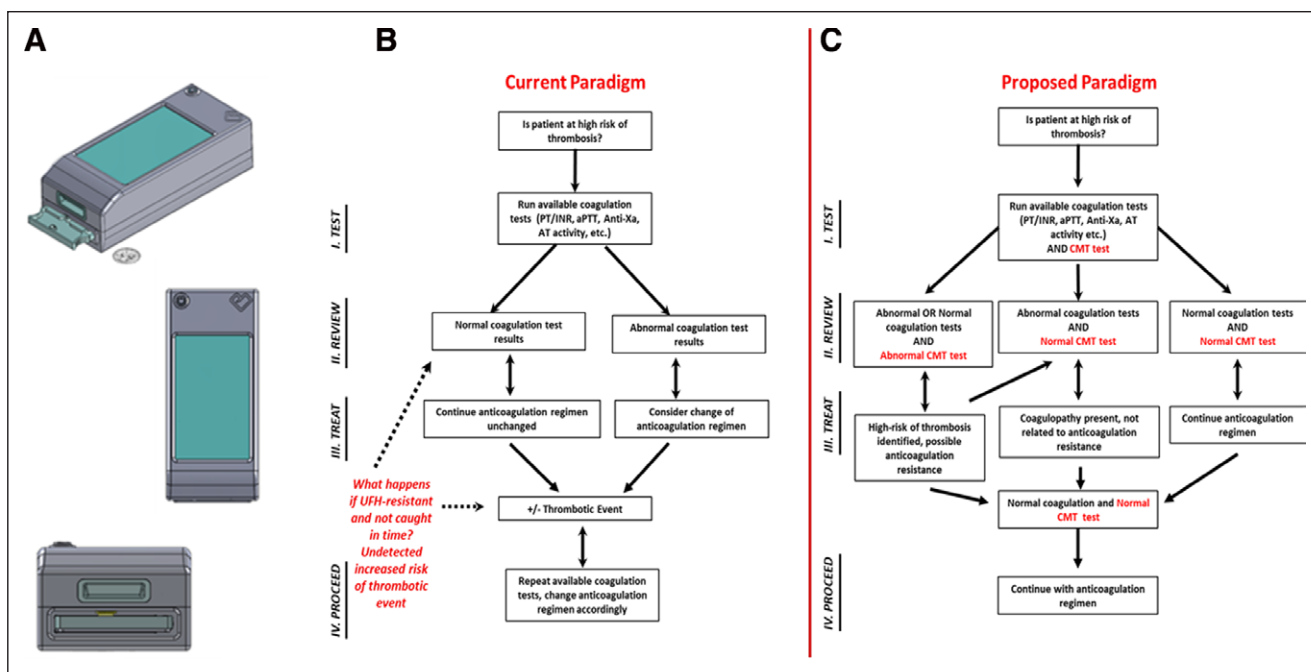
Figure 5. Proposal of potential future paradigm for the clinical use of the factor IIa (FIIa) Clotting Time Score (CTS). **A**, Representative image of an investigational use only point-of-care automated testing system. **B**, Current coagulation testing paradigm. **C**, Potential future coagulation testing paradigm. The current paradigm (**B**) includes clinical determination of high-risk status for thrombosis, for example, extracorporeal membrane oxygenation and includes routine coagulation tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], anti-factor Xa [anti-Xa], etc.). Expected changes in coagulation test results support continuation of current anticoagulation treatment and testing is repeated at specific time intervals. Unexpected results, such as an aPTT below target level, can lead to ordering specialty tests such as anti-Xa or AT levels. Some patients with expected results may have undetected anticoagulation resistance, which may manifest as a thrombotic event. Anticoagulation resistance detection leads to either an increase in anticoagulation dosing or a change in the anticoagulant. In the potential paradigm (**C**), the CTS is performed in conjunction with routine coagulation tests. In this example (FIIa CTS), if the CTS indicates subtherapeutic anticoagulation effects, regardless of normal/expected routine coagulation test results, the patient can be clinically reassessed and specialty tests, such as anti-Xa or AT levels, ordered to assess anticoagulation status to avoid potential thrombotic events. AT = antithrombin, CMT = Coagulo Medical Technologies, INR = International Normalized Ratio, UFH = unfractionated heparin.

the CTS' potential utility in monitoring the effect of anticoagulation using UFH or bivalirudin and also demonstrate an association between the CTS and the development of a TEs. In this study, as compared with the current multitest type standard of practice, we demonstrate that a single test, the CTS, may be used for the monitoring both UFH and bivalirudin, and, in the future, could potentially be used as a rapid, bedside test to alert clinicians as to the development of AR and the subsequent response to alternative anticoagulation treatment (**Fig. 5, *A*** and ***B***). Further prospective studies are warranted, including the next generation of the fully automated POC testing unit, which will use whole blood at the bedside.

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REFERENCES

1. Chlebowski MM, Baltagi S, Carlson M, et al: Clinical controversies in anticoagulation monitoring and antithrombin supplementation for ECMO. *Crit Care* 2020; 24:19

2. Raffini L: Anticoagulation with VADs and ECMO: Walking the tightrope. *Hematology Am Soc Hematol Educ Program* 2017; 2017:674–680

3. Achey MA, Nag UP, Robinson VL, et al: The developing balance of thrombosis and hemorrhage in pediatric surgery: Clinical implications of age-related changes in hemostasis. *Clin Appl Thromb Hemost* 2020; 26:1076029620929092

4. Andrew M, Paes B, Milner R, et al: Development of the human coagulation system in the full-term infant. *Blood* 1987; 70:165–172

5. Levy JH, Connors JM: Heparin resistance – clinical perspectives and management strategies. *NEJM* 2021; 385:826–832

6. Dalton HJ, Reeder R, Garcia-Filion P, et al: Factors associated with bleeding and thrombosis in children receiving extracorporeal membrane oxygenation. *Am J Respir Crit Care Med* 2017; 196:762–771

7. Kato C, Oakes M, Kim M, et al: Anticoagulation strategies in extracorporeal circulatory devices in adult populations. *Eur J Haematol* 2021; 106:19–31

8. Cashen K, Meert K, Dalton H: Anticoagulation in neonatal ECMO: An enigma despite a lot of effort! *Front Pediatr* 2019; 7:366

9. Drop JGF, Wildschut ED, Gunput STG, et al: Challenges in maintaining the hemostatic balance in children undergoing extracorporeal membrane oxygenation: A systematic literature review. *Front Pediatr* 2020; 8:612467

10. Thomas J, Kostousov V, Teruya J: Bleeding and thrombotic complications in the use of extracorporeal membrane oxygenation. *Semin Thromb Hemost* 2018; 44:20–29

11. Khaja WA, Bilen O, Lukner RB, et al: Evaluation of heparin assay for coagulation management in newborns undergoing ECMO. *Am J Clin Pathol* 2010; 134:950–954

12. Nguyen TP, Phan XT, Huynh DQ, et al: Monitoring unfractionated heparin in adult patients undergoing extracorporeal membrane oxygenation (ECMO): ACT, APTT, or ANTI-XA? *Crit Care Res Pract* 2021; 2021:5579936

13. Baird CW, Zurakowski D, Robinson B, et al: Anticoagulation and pediatric extracorporeal membrane oxygenation: Impact of activated clotting time and heparin dose on survival. *Ann Thorac Surg* 2007; 83:912–919; discussion 919–920

14. Chu DC, Abu-Samra AG, Baird GL, et al: Quantitative measurement of heparin in comparison with conventional anticoagulation monitoring and the risk of thrombotic events in adults on extracorporeal membrane oxygenation. *Intensive Care Med* 2015; 41:369–370

15. Van Cott EM, Villalba JA: High frequency of false-positive results of aPTT-based lupus anticoagulant tests in patients receiving argotraban. *Am H Hematol* 2019; 94:E166–E169

16. Liu J, Li F, Shu K, et al: The analysis of false prolongation of the activated partial thromboplastin time (activator: silica): Interference of C-reactive protein. *J Clin Lab Anal* 2018; 32:e22571

17. Kostousov V, Devaraj S, Bruzdoski K, et al: C-reactive protein-induced activated partial thromboplastin time prolongation in heparinized samples is attenuated by elevated factor VIII. *Int J Lab Hematol* 2021; 43:139–142

18. Cheng T, Mathews KA, Abrams-Ogg AC, et al: Relationship between assays of inflammation and coagulation: A novel interpretation of the canine activated clotting time. *Can J Vet Res* 2009; 73:97–102

19. Khan J, Chandler WL: Interference in the anti-Xa heparin activity assay due to hemolysis and icterus during pediatric extracorporeal life support. *Artif Organs* 2019; 43:880–887

20. Kostousov V, Nguyen K, Hundalani SG, et al: The influence of free hemoglobin and bilirubin on heparin monitoring by activated partial thromboplastin time and anti-Xa assay. *Arch Pathol Lab Med* 2014; 138:1503–1506

21. Vera-Aguilera J, Yousef H, Beltran-Melgarejo D, et al: Clinical scenarios for discordant anti-Xa. *Adv Hematol* 2016; 2016:4054806

22. Byun JH, Jang IS, Kim JW, et al: Establishing the heparin therapeutic range using aPTT and anti-Xa measurements for monitoring unfractionated heparin therapy. *Blood Res* 2016; 51:171–174

23. Extracorporeal Life Support Organization: Anticoagulation Task Force: ELSO Anticoagulation Guideline. 2014. Available at: https://www.elso.org/portals/0/files/elsoanticoagulation-guideline8-2014-table-contents.pdf

24. Sanfilippo F, Asmussen S, Maybauer DM, et al: Bivalirudin for alternative anticoagulation in extracorporeal membrane oxygenation: A systematic review. *J Intensive Care Med* 2017; 32:312–319

25. Ryerson LM, Lequier LL: Anticoagulation management and monitoring during pediatric extracorporeal life support: A review of current issues. *Front Pediatr* 2016; 4:67

26. Counsilman CE, Heeger LE, Tan R, et al: Iatrogenic blood loss in extreme preterm infants due to frequent laboratory tests and procedures. *J Matern Fetal Neonatal Med* 2021; 34:2660–2665

27. Jakacka N, Snarski E, Mekuria S: Prevention of iatrogenic anemia in critical and neonatal care. *Adv Clin Exp Med* 2016; 25:191–197

28. Frydman GH, Berger B, Kostousov V, et al: Quantitation of bivalirudin effect using microliter scale sample microfluidic assay in pediatric ECMO population [abstract]. *Res Pract Thromb Haemost* 2020; 4 (Suppl 1). Available at: https://abstracts.isth.org/abstract/quantitation-of-bivalirudin-effect-using-microliter-scale-sample-microfluidic-assay-in-a-pediatric-ecmo-population/. Accessed October 19, 2022

29. Frydman GH, Berger BM, Kostousov V, et al: Novel, point-of-care coagulation test detects anticoagulation resistance predictive of thrombotic events in pediatric ECMO patients [abstract]. *Res Pract Thromb Haemost* 2021; 5 (Suppl 2). Available at: https://abstracts.isth.org/abstract/novel-point-of-care-coagulation-test-detects-anticoagulation-resistance-predictive-of-thrombotic-events-in-pediatric-ecmo-patients/. Accessed October 17, 2022

30. Frydman GH, Ellett F, Van Cott EM, et al: A new test for the detection of direct oral anticoagulants (rivaroxaban and apixaban) in the emergency room setting. *Crit Care Explor* 2019; 1:e0024

31. Wada H, Takahashi H, Uchiyama T, et al: The approval of revised diagnostic criteria for DIC from the Japanese Society on Thrombosis and Hemostasis. *Thromb J* 2017; 15:17

32. Frydman GH, Rosovsky RP, Lee J, et al: Novel, low-volume, point-of-care coagulation test successfully detects anticoagulation resistance in hospitalized patients with COVID-19. AHA Conference, Boston, MA. November 2021. Abstract number: 12966

33. Gerotziafas GT, Petropoulou AD, Verdy E, et al: Effect of the anti-factor Xa and anti-factor IIa activities of low-molecular-weight heparins upon the phases of thrombin generation. *J Thromb Haemost* 2007; 5:955–962

34. Lind SE, Boyle ME, Fisher S, et al: Comparison of the aPTT with alternative tests for monitoring direct thrombin inhibitors in patient samples. *Am J Clin Pathol* 2014; 141:665–674

35. Colucci G, Nagler M, Klaus N, et al: Practical guidelines for argotraban and bivalirudin in patients with heparin-induced thrombocytopenia. *J Transl Sci* 2015; 1:37–42

36. Olson SR, Murphree CR, Zonies D, et al: Thrombosis and bleeding in extracorporeal membrane oxygenation (ECMO) without anticoagulation: A systematic review. *ASAIO J* 2021; 67:290–296

37. Ortel TL: Antiphospholipid syndrome: Laboratory testing and diagnostic strategies. *Am J Hematol* 2012; 87(Suppl 1):S75–S81

38. Mehta TP, Smythe MA, Mattson JC: Strategies for managing heparin therapy in patients with antiphospholipid antibody syndrome. *Pharmacotherapy* 2011; 31:1221–1231

39. Newall F: Anti-factor Xa (anti-Xa) assay. *Methods Mol Biol* 2013; 992:265–272

40. Ignjatovic V, Summerhayes R, Gan A, et al: Monitoring unfractionated heparin (UFH) therapy: Which anti-factor Xa assay is appropriate? *Thromb Res* 2007; 120:347–351

41. Coenraad Hemker H, Al Dieri R, Beguin S: Heparins: A shift of paradigm. *Front Med (Lausanne)* 2019; 6:254

42. Cartwright B, Bruce HM, Kershaw G, et al: Hemostasis, coagulation and thrombin in venoarterial and venovenous extracorporeal membrane oxygenation: The HECTIC study. *Sci Rep* 2021; 11:7975

43. Doyle AJ, Hunt BJ: Current understanding of how extracorporeal membrane oxygenators activate haemostasis and other blood components. *Front Med (Lausanne)* 2018; 5:352