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Research Article

Thrombin Generation Assay for Evaluation of Thromboprophylaxis in Neurointensive Care

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Abstract

Background: Pharmacologic thromboprophylaxis with low molecular weight heparin (LMWH) increases the risk of intracranial rebleeding if started too early in patients with traumatic brain injuries. The risk of thromboembolic complications increases progressively to 20% after ten days even with mechanical thromboprophylaxis measures such as calf compressions and graded elastic stockings.

Aim: The aim of this study was to evaluate when it is safe to administer LMWH in neurointensive care unit patients using consecutive routine and advanced coagulation testing. A secondary aim was to evaluate if the thrombin generation could better monitor the LMWH peak and trough effects than standard anti-factor Xa (Anti-fXa).

Method: This study was performed in the Neurointensive care unit at Skane University Hospital in Lund. The six included patients received mechanical thromboprophylaxis in the initial phase of their hospitalization. The doctor in charge of each patient decided when to start LMWH thromboprophylaxis. Arterial blood samples were taken repetitively before and after the start of LMWH. Blood was collected in citrated tubes for direct analysis of whole blood thromboelastometry. Plasma vials were stored at -85°C until the activated partial thromboplastin time, prothrombin time, fibrinogen, Anti-fXa and thrombin generation could be analyzed.

Results: The thrombin generation area under curve (AUC) correlated well with Anti-fXa during LMWH treatment ($r = -0.9011$, $p = 0.0071$). Thromboelastometry clotting time ($p = 0.0399$) and fibrinogen ($p = 0.0213$) showed significant increases compared to the reference ranges for the samples taken before the start of LMWH treatment.

Conclusion: The thrombin generation AUC correlated well with Anti-fXa levels during LMWH treatment, but we were unable to determine if thrombin generation was superior to Anti-fXa for monitoring LMWH peak and trough effects. To investigate whether thrombin generation is better than other analyses to better define the optimal time to start LMWH treatment, a larger sample size and a more detailed sampling schedule over a prolonged period is needed.

Introduction

Patients with intracerebral hemorrhages generally have an altered coagulation profile and often have excessive coagulation even after the bleeding has stopped. While in neurointensive care, these patients are often unconscious or sedated and lie still for long periods, potentially causing blood stasis in the deep veins [1]. Intracerebral hemorrhages are an indirect evidence of injured endothelial cells in the brain; some traumatic brain injury (TBI) patients have damaged endothelium in other parts of the body as well. The injured areas cause inflammation, increasing the concentration of acute phase reactants, making the blood even more hypercoagulable. All these factors are included in Virchow's triad, a theory that explains the pathogenesis of venous thrombosis.

Venous thromboembolism (VTE) is estimated to occur in 20% of patients with TBI, so guidelines recommend the use of mechanical and pharmacologic thromboprophylaxis [1,2]. Advanced age, excess weight and the severity of the TBI all increase the risk of VTE. In the early treatment phase after a TBI, there is a danger that the patient's intracerebral hemorrhage (ICH) might expand. Pharmacologic thromboprophylaxis with LMWH increases that risk. There is no clear evidence indicating when it is safe to initiate LMWH treatment. Most studies suggest starting LMWH between 24 to 72 hours after the trauma while monitoring the patient's progress with consecutive control computed tomography to avoid initiating LMWH in patients with an expanding ICH [2-4].

The risks and benefits of LMWH were evaluated by Dudley et al. in a retrospective study of 287 TBI patients. In that study, patients received LMWH treatment at 48 to 72 hours' post-trauma after two consecutive computed tomography scans showed no sign of intracranial hemorrhage expansion. There was a low incidence of VTE (7.3%), and only one patient (0.4%) had a symptomatic expansion of a pre-existing ICH [4]. A large observational cohort study of 2468 TBI patients compared early (< 72 h) and late (> 72 h) initiation of LMWH. The patients who received early prophylaxis had lower incidences of both deep venous thrombosis (DVT) and pulmonary embolism (PE) without any increased risk of neurosurgical intervention or death [5]. Two other studies have corroborated these results [6,7]. A small randomized, double-blinded pilot study found that patients with minor TBIs who received LMWH after a stable control computed tomography within 24 hours post trauma (34 patients) had ICH progression rates similar to the placebo group (28 patients) [8]. Norwood et al. found similar results in a prospective, single-cohort observational study including 150 patients [9]. Interestingly, Kurtoglu et al. found no significant differences in mortality or the incidence of DVT or PE in a small prospective, randomized, controlled trial on brain and spinal trauma patients who were treated with either intermittent graded pneumatic compression devices (60 patients) or LMWH

(60 patients). LMWH was administrated after a stable control computed tomography within 24 hours post trauma. There was only one exacerbation of an epidural hematoma in each group [10].

Byrne et al. found LMWH to be superior to heparin in preventing PE in patients after major trauma. Their propensity-matched analysis included 153,474 patients. The matched results were 1.4% PE in patients receiving LMWH and 2.4% PE in the group that received heparin [11]. Minshall et al. compared the safety and efficacy of heparin with the LMWH enoxaparin and found that patients who received LMWH after TBI had fewer complications. However, in that study, the patients receiving heparin had more severe TBIs, suggesting that heparin might be favored by the physicians treating those patients, perhaps due to better monitoring capabilities, a shorter half-life and the option of reversal with protamine [3]. Dengler et al. retrospectively evaluated heparin and LMWH in TBI patients and found no difference in DVT or hemorrhage expansion using intracranial pressure monitoring devices. In that study, heparin was not reserved to the more severe TBI cases [12].

In addition to the risks discussed above, brain trauma patients can also develop an early acute coagulopathy of trauma shock (aCoTS) [13] or a later disseminated intravascular coagulation defect (DIC). DIC usually occurs 6 to 72 hours' post-trauma [14]. Both aCoTS and DIC are strong predictors of a poor outcome after TBI.

Patients with spontaneous subarachnoid hemorrhages are also at thromboembolic risk. Mechanical thromboprophylaxis is initially used in this group as LMWH treatment is often delayed due to increased intracranial bleeding following surgical aneurysm occlusion. The European stroke organization recommends LMWH treatment 12 hours after surgical aneurysm occlusion, immediately after coiling, or if DVT prophylaxis is indicated [15].

At the neurointensive care unit (NICU) in Lund, mechanical calf compression and compression stockings are initially used in TBI patients. However, no clear routine exists regarding when to start LMWH. Intracranial pressure monitoring with or without intraventricular drainage of hemorrhagic cerebrospinal fluid (CSF) also affects when LMWH treatment is started, often after catheter withdrawal. A recent retrospective study of 155 patients, however, indicated that both standard heparin and LMWH were safe to administer while using active invasive monitoring devices [12].

There are several types of LMWH. They all inhibit the common coagulation pathway by indirectly inhibiting factor Xa (fXa) and directly inhibiting factor IIa (fIIa) to varying extents as described by each type's Anti-fXa/Anti-fIIa ratio [16]. The most commonly used LMWH for thromboprophylaxis in our NICU is enoxaparin. Enoxaparin is primarily eliminated through the kidneys, making renal insufficiency an important factor for potential accumulation and thereby increased bleeding risk. Body

weight (BW) is also known to affect the enoxaparin dose response [17], with low BW posing a risk of overdose and high BW leading to potentially insufficient thromboprophylaxis under the standard dose regimes. Individual differences in bleeding risk and dose response to LMWH further complicate this issue. Enoxaparin is generally administered by subcutaneous injection once a day, and exhibits peaks and troughs during treatment.

In this study, we measured the following routine plasma coagulation parameters from frozen plasma vials: activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen. In addition, the frozen plasma vials were analyzed for Anti-fXa and thrombin generation. Overall hemostatic function was tested using citrated whole blood Rotational Thromboelastometry (ROTEM).

The APTT and PT tests are routine tests in most hospitals, but they mostly represent the intrinsic (APTT) and extrinsic (PT) initiation of the coagulation cascade that comes before the common pathway. The thrombin generation assay (TGA) provides information about the total thrombin generation potential of the analyzed blood and reflects the thrombin generated in both the amplification and propagation phases of the common pathway. Theoretically, TGA should be more useful than the routine plasma coagulation assays in assessing a patient's coagulative status [18]. Fibrinogen is an acute phase reactant that can indicate hypercoagulation, but it only reflects one of the coagulation factors in the coagulation cascade. ROTEM describes the characteristics of the fibrin clot and has shown potential for use in monitoring a patient's coagulative status. A strong clot structure and a dense fibrin network indicate hypercoagulation and increased risk for VTE [19].

The current gold standard for monitoring LMWH treatment measures the Anti-fXa activity in a patient's plasma, however this method neglects its Anti-fIIa effect. TGA reflects the LMWH inhibition of both fXa and fIIa, potentially providing a better analysis that describes the full effect of the LMWH [16].

Clinical Value Significance

Patients in the NICU who have suffered from traumatic or spontaneous intracranial hemorrhage are generally subjected to delayed LMWH thromboprophylaxis treatment due to the risk of re-bleeding. There is a strong correlation between the delay in initiation of LMWH thromboprophylaxis and the frequency of VTEs. Finding better methods to determine the optimal time to initiate treatment will potentially prevent VTEs without increasing the risk of cerebral rebleeding.

Aim/Objective

The aim of this study was to evaluate when it is safe to start pharmacologic thromboprophylaxis in neurointensive care patients

using consecutive routine and advanced coagulation testing. A secondary aim was to evaluate whether TGA better monitors the LMWH peak and through effects than standard Anti-fXa.

Hypothesis

The use of TGA could better define the optimal time to start LMWH thromboprophylaxis treatment than routine coagulation tests and ROTEM in neurointensive care patients and could better monitor the LMWH peak and through effects.

Material and Methods

This study was performed in the NICU at Skane University Hospital in Lund. Approval was obtained from the Regional Ethical Review Board (Lund, Protocol DNR 2017/636) and signed consent was provided by the patient or a relative. Patients were sampled from September 25 through November 1 2017. The sampling period started as soon as possible after all necessary preparations had been completed. The preparations included: learning the workflow of the different professions at the NICU, creating sample protocols, practicing the study procedure, and talking to the NICU staff at meetings to provide general information about the study and to establish a means of communication. The sampling period for this master thesis stopped earlier than planned due to restricted laboratory and staff resources at the Coagulation Laboratory, Dept. of Clinical Chemistry, Division of Laboratory Medicine, Skane University Hospital, Malmö, Sweden during November and December, limiting our ability to have the frozen plasma samples analyzed.

Patients

The following inclusion criteria were used for this study: patients aged 18 or older who had suffered a hemorrhagic TBI, non-traumatic subarachnoid bleeding or intracranial hemorrhage and who were admitted to the NICU in Lund. The exclusion criteria included: patients with a known coagulation disorder, patients with ongoing anticoagulation medication and patients with a short expected stay at the NICU. Patients who were unable to communicate in Swedish or English or who had no relatives present were also excluded.

Thromboprophylaxis

All patients included in the study received mechanical thromboprophylaxis in their initial treatment phase with Kendall SCDTM Express Sleeves (Covidien, USA) and graded elastic compression stockings (T.E.D., Covidien, USA). The doctor in charge of the patient decided when to start pharmacologic (LMWH) thromboprophylaxis with subcutaneous enoxaparin (Clexane®, Sanofi-aventis, Gentilly, France).

Sampling

Arterial blood samples were drawn from an indwelling

radial arterial catheter with continuous flushing and a sampling membrane, eliminating the need to dispose of blood samples. Alternatively, venous blood samples were retrieved from central or peripheral venous catheters with good back-flow after disposing of the first blood (in patients without an arterial catheter).

The following sampling schedule was used. First sample: within 24 hours of arrival at the NICU. Second sample: the third day at the NICU. Third sample: the sixth day at the NICU. Fourth sample: the start of LMWH treatment, the time of which differed between patients. Fifth sample: two hours after the first LMWH injection to note the maximum effect. Sixth sample: four hours after the first LMWH injection to note the decay of the maximum effect. Seventh sample: day one after the first LMWH injection (12 to 24 hours after the first injection). Blood was collected in 4.5 mL 0.109 M citrated tubes (CTAD: Citrate + Theophylline + adenosine and Dipyridamole, BD Vacutainer Systems, Becton-Dickinson and Company, UK) for laboratory plasma and whole blood ROTEM analyses.

ROTEM

Thromboelastometry (ROTEM, TEM International GmbH, Munich, Germany) was used to measure clot formation and clot elasticity. ROTEM has a fixed sample cup with a pin that is suspended in the blood sample. After the addition of 20 μ L of 0.2 M CaCl_2 (StartTEM) to 300 μ L of blood, coagulation was initiated by tissue factor alone (ExTEM). The pin oscillates, and its movement is registered in the coagulating sample, giving rise to a curve. Several variables were obtained from the ROTEM EXTEM curve: (1) Clotting Time (CT), the time it takes for the clot to start to form after the reagent has been added, with a reference range of 38-79 s and a Coefficient of Variation (CV) < 6%; (2) Clot Formation Time (CFT), the time it takes for a clot to reach a firmness of 2 mm once the reagent has been added, with a reference range of 34-159 s and CV < 16%; (3) alpha angle (AA), the angle of the tangent of the curve at clot firmness 20 mm, with a reference range of 63-83° and CV < 1%; and (4) the Maximum Clot Firmness (MCF) with a reference range of 50-72 mm and CV < 4%. All samples were analyzed shortly after sampling, within 20 minutes of blood collection, while being kept at 37°C in a heating block in the NICU laboratory [20].

Centrifugation and Freezing

The blood samples intended for laboratory plasma analysis were centrifuged within 20 minutes after sampling at 200 g and 20°C to obtain the plasma. The plasma was then pipetted into four separate Eppendorf Safe-Lock tubes in 0.5 ml plasma vials and stored at -86°C until the analyses could be performed.

Plasma Analyses

Plasma analyses were performed at the Coagulation

Laboratory, Dept. of Clinical Chemistry, Division of Laboratory Medicine, Skane University Hospital, Malmö, Sweden on a BCS-XP automated analyzer (Siemens, Marburg, Germany). APTT was measured using Actin FSL reagent (Siemens, Marburg, Germany), reference interval: 26–33 s, CV < 4%. The Owren PT assay was calibrated using International Normalized Ratio (INR) calibrators certified by the Swedish external quality assessment organization (Equalis, Uppsala, Sweden). PT-INR was performed using a combined thromboplastin reagent (Owren PT, Medirox, Sweden), reference range: < 1.2, CV < 5%. Fibrinogen was measured with a photometric assay, Dade Thrombin, (Siemens), reference range: 2-4 g/L, CV < 6%. Anti-fXa activity was quantified with Coamatic Heparin (Chromogenic, Instrumentation Laboratories, Bedford, USA), CV < 8%. Peak range with LMWH thromboprophylaxis dose; 0.2-0.5 kIU/L.

Thrombin generation (endogenous thrombin potential) was measured on a Ceveron Alpha instrument using the TGA reagent B (RB) (containing 2 pM human tissue factor) and tissue-factor rich TGA reagent C high (RCH) (containing 5 pM human tissue factor) to obtain the variables of lag time, thrombin peak and area under the curve (AUC) for both reagents. The Coagulation Laboratory, Dept. of Clinical Chemistry, Division of Laboratory Medicine, Skane University Hospital, Malmö has defined reference intervals for the different TGA parameters using the RB and RCH reagents: RB lag time: 5.2-11.3 min, RB thrombin peak: 36–372 nmol/L, RB AUC: 1538-2652 nmol/L; RCH lag time: 2.2-3.4 min, RCH thrombin peak: 6-459 nmol/L, RCH AUC: 1195-2568 nmol/L.

Clinical Data Collection

The registered parameters were: (1) patient diagnosis (traumatic brain injury, subarachnoid hemorrhage or intracranial bleeding); (2) time from admission to start of enoxaparin treatment; (3) type of surgery or coiling; (4) radiological diagnosis of intracranial bleeding; and (5) intraventricular drainage of hemorrhagic CSF.

Statistical Analysis

Excel was used to store the acquired data. GraphPad Prism (GraphPad Software, La Jolla, CA) was used for the statistical analyses, tables and diagrams of the acquired data. The Spearman's rank correlation test was used to evaluate correlations, using two tailed p-values with statistical significance set at $p < 0.05$. The Kruskal-Wallis test was used to determine if the change between sampling points was significant, with significance set at $p < 0.05$.

Results

Six patients were included during the study sampling period. The study group included four males and two females, with a median age of 65.5 (range 58-74) and a median weight of 95.5 kg (range 63-115). All patients had creatinine levels < 83 μ mol/L

(range 39-83) (Table 1). None of the patients developed DVT or PE. The N-values for the sample points varied from 2-6. Fully completed sampling schedules, including initiation of LMWH treatment (samples 1-7), was attained in one ICH patient (a 61-year-old male, 92 kg) and one TBI patient (a 58-year-old male, 115 kg) (Appendix). None of the patients received LMWH treatment before the third sample (day six at the NICU).

Patient	Sex	Age	Weight (Kg)	Creatinine (μmol/L)
1	M	70	99	79
2	M	61	92	74
3	M	74	63	62
4	M	58	115	57
5	F	70	66	39

6	F	61	102	83
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Table 1: Demographic data of all patients included in the study. Patient number, sex male (M) or female (F), age, weigh and plasma creatinine at admission.

Thrombin Generation

None of the TGA parameters showed any significant change between sample 1 and sample 4 (before the initiation of LMWH treatment). The thrombin peak and AUC values dipped post LMWH injection (samples 5 and 6) and increased between the samples taken four hours post LMWH injection (sample 6) and the samples taken the next day (sample 7) for both the TBI and the ICH patients. The lag time (RCH reagent) medians for all patients deviated from the normal reference range; all other medians were within the normal reference ranges (Figure.1).

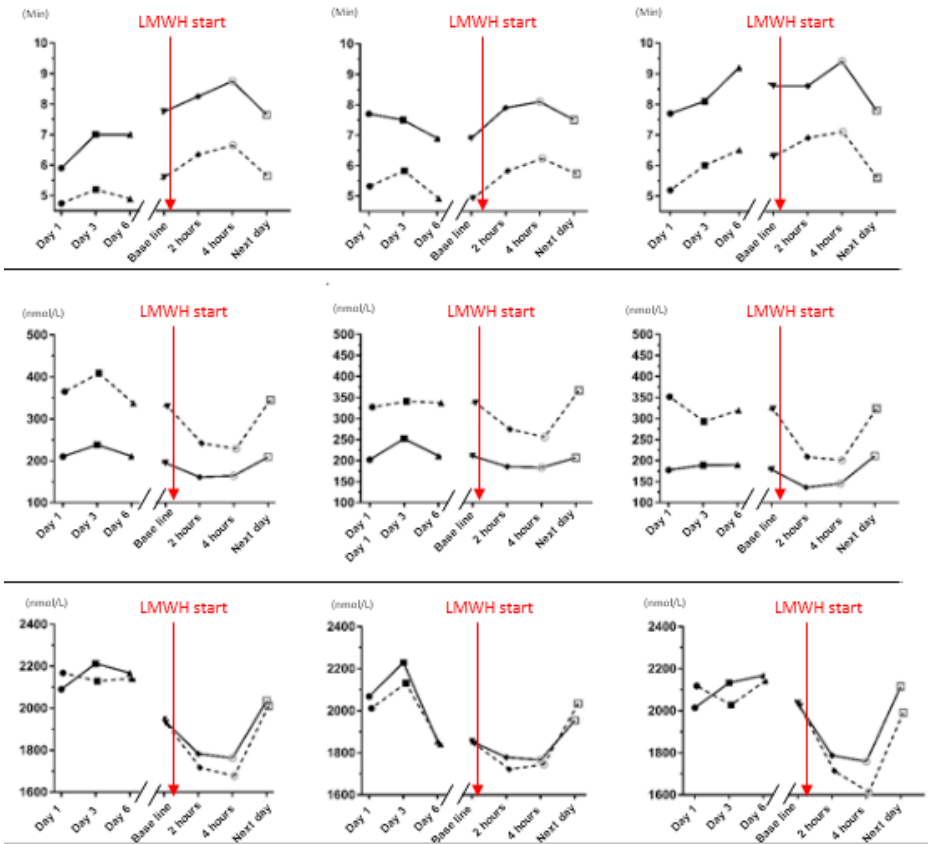


Figure 1: Thrombin generation lag time (Y-axis: Minutes (Min)), Thrombin peak (Y-axis: Nano mole per litre (nmol/L)) and Area under the curve (AUC) (Y-axis: nmol/L) values for groups: All patients (median), one traumatic Brain Injury (TBI) patient and one Intra cranial haemorrhage (ICH) patient. Full line represents reagent B (RB), dotted line represents reagent C high (RCH) in all graphs. Left X-axis: Time from admission, Day 1: within 24 hours from admission to NICU, n=6. Day 3: n=5. Day 6: n=3. Right X-axis: Time in relation to first low molecular weight heparin (LMWH) injection, Base line: Immediately before first LMWH injection, n=2. Two hours: Two hours after first LMWH injection, n=2. Four hours: Four hours after first LMWH injection, n=2. Next day: 12-24h after first LMWH injection, n=2. None of the patients received LMWH treatment before day six. Min-max ranges are stated in appendix.

Anti-fXa

The medians for all the sample points were zero except for sample points 5-7 in the two patients receiving LMWH. The results showed an increase of Anti-fXa inhibition at 2 h (sample 5) and 4 h (sample 6) post LMWH injection (Figure.2).

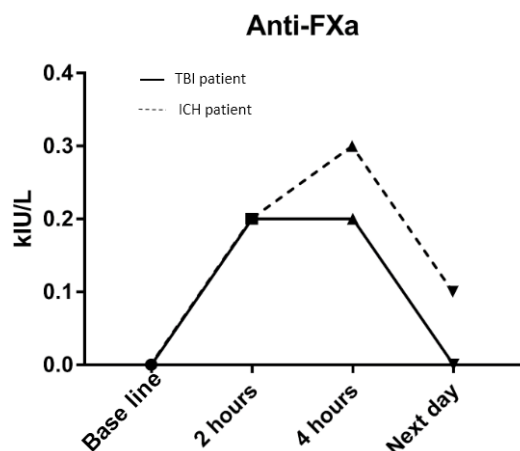


Figure 2: Anti-fXa measurements from two patients, one after traumatic brain injury (TBI) and one after intracranial haemorrhage (ICH). X-axis: Baseline: Immediately before first low molecular weight heparin (LMWH) injection. 2 hours: Two hours after first LMWH injection. 4 hours: Four hours after first LMWH injection. Next day: 12-24h after the first LMWH injection. Y-axis: Thousand international units per litre (kIU/L).

Rotem Extem

The CFT and MCF parameters indicated a stronger coagulation, but all values were within the normal reference ranges after the initial sample point until the initiation of LMWH treatment: CFT (57.6% decrease, $p = 0.0017$, samples 1-4) and MCF (54.2% increase, $p = 0.0031$, samples 1-4). Only CT deviated from its normal reference range (24.5% increase, $p = 0.0399$, samples 1-4) (Figure 3).

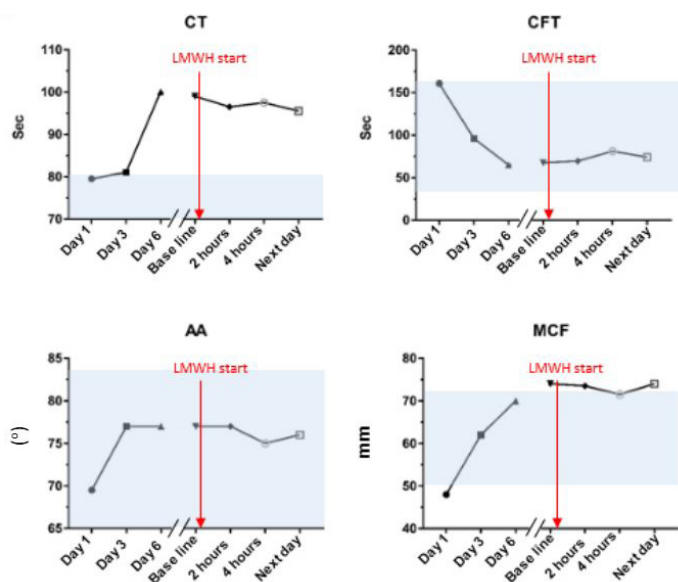


Figure 3: Rotational thromboelastometry parameters: Clotting time (CT) (Y-axis: Seconds (Sec)), Clot formation time (CFT) (Y-axis: Sec), Alpha angle (AA) (Y-axis: Degrees (°)) and Maximum clot firmness (MCF) (Y-axis: millimetre (mm)) median values for all patients. Left X-axis: Time from admission. Day 1: within 24 hours from admission to NICU, $n=6$. Day 3: $n=5$. Day 6: $n=3$. Right X-axis: Time in relation to first low molecular weight heparin (LMWH) injection: Base line: Immediately before first LMWH injection, $n=2$. Two hours: Two hours after first LMWH injection, $n=2$. Four hours: Four hours after first LMWH injection, $n=2$. Next day: 12-24h after first LMWH injection, $n=2$. None of the patients received LMWH treatment before day six. Min-max ranges are stated in the appendix. Normal reference ranges are toned.

Standard Coagulation Tests

The fibrinogen levels were above the reference range from the start and increased from samples 1 to 4 (62% increase, $p = 0.0213$), but the APTT and PT-INR values stayed within their reference ranges for all samples without any significant change (Figure 4).

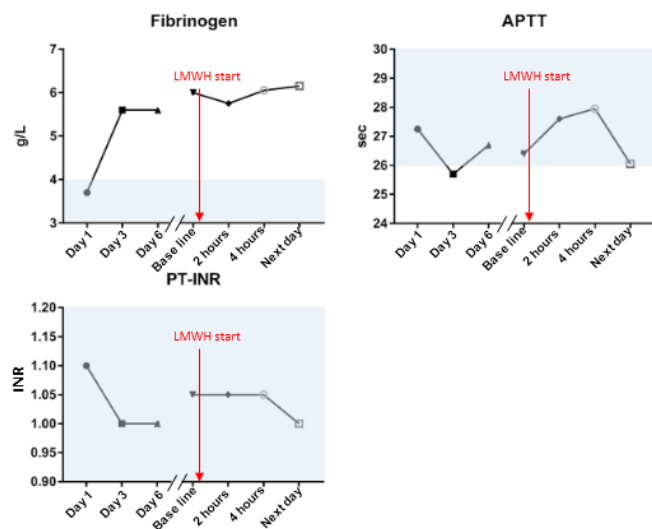


Figure 4: Fibrinogen (Y-axis: Grams per litre (g/L)), Activated Partial Thromboplastin Time (APTT) (Y-axis: Seconds (sec)) and prothrombin time international normalized ratio (INR) (Y-axis: PT-INR median values for all patients). Left X-axis: Time from admission. Day 1: within 24 hours from admission to NICU, n=6. Day 3: n=5. Day 6: n=3. Right X-axis: Time in relation to first Low Molecular Weight Heparin (LMWH) injection: Base line: Immediately before first LMWH injection, n=2. Two hours: Two hours after first LMWH injection, n=2. Four hours: Four hours after first LMWH injection, n=2. Next day: 12-24h after first LMWH injection, n=2. None of the patients received LMWH treatment before day six. Min-max ranges are stated in appendix.

TGA and Anti-fXa Correlation

The thrombin peak values and AUC using the RCH reagent showed the best correlation with Anti-fXa in the sample series ranging from just before the first LWMH injection (sample 4) to the day after (sample 7) ($r = -0.9011$, $p = 0.0071$). AUC using the RB reagent also correlated with Anti-fXa in this sample series ($r = -0.801$, $p = 0.0321$). No significant correlation was found between Anti-fXa and the thrombin peak using the RB reagent or with lag time using either reagent in this sample series (Figure 5).

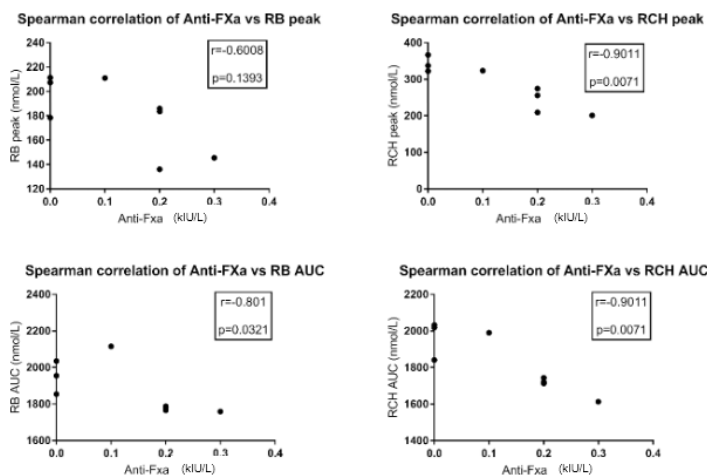


Figure 5: Correlations between Thrombin generation (TGA) parameters and Anti-FXa with RB/RCH reagents.

TGA and ROTEM correlation

The TGA lag time and ROTEM clotting time correlated significantly, albeit with a weak correlation coefficient using RB and RCH reagents; RB ($r = 0.5391$, $p = 0.0096$) and RCH ($r = 0.497$, $p = 0.0186$). No other correlations between TGA and ROTEM were significant.

Discussion

Both the TGA thrombin peak and AUC parameters correlated well with Anti-fXa during LMWH treatment, indicating that both could be used to monitor LMWH treatment. However, due to the small sample size of the present study, too few samples having been taken during LMWH treatment, and no included patients suffering from DVT or PE, we were unable to determine which analysis was better.

All ROTEM parameters except for CT moved toward increased coagulation from sample 1, even though they varied in significance within their normal reference values. Fibrinogen levels

increased above the reference range toward hypercoagulation. No TGA parameters changed significantly from admission until the start of LMWH treatment (samples 1-4), likely due to the sample size being too small to show a general change of coagulation in this population. However unlikely, this could also mean that our hypothesis that TGA could better define the optimal LMWH start time in these patients was false. To further investigate this issue, a larger sample size with more sample points over a prolonged period are needed.

It is worth noting that even though TGA and Anti-fXa correlated well in this study, LMWH inhibits both fXa and fIIa. These effects are expected to be reflected in the TGA (thrombin peak and AUC) values, theoretically providing a more accurate display of LMWH activity [16].

Fibrinogen was the only studied plasma parameter where the median values for all patients indicated a general progression of hypercoagulability over time. All other medians from the plasma analyses that we studied were within or very close to normal reference ranges. One explanation for these findings is that fibrinogen is an acute phase reactant, and all the included patients had at least suffered from an ICH. Some of the TBI patients had more substantial injuries that would have triggered elevated fibrinogen [21].

Most of the ROTEM parameters and the APTT and PT-INR plasma analyses moved toward the lower range of normal values, indicating hypercoagulability, from the first sample until the last sample before the initiation of LMWH treatment (sample 4). These results corroborate findings in other studies of TBI patients [22,23]. It has been suggested that the pathophysiology behind the hypercoagulability demonstrated by ROTEM (in addition to the fibrinogen acute phase response) involves platelet hyperactivity and extracellular vesicles [24]. The increased use of ROTEM-CT in the initial treatment phase could help to demonstrate the protein C hypothesis of acute coagulopathy of trauma [25], balancing the other signs of hypercoagulability as defined by Grainger et al [19].

A recent trauma study reported that although TGA parameters indicated hypercoagulable states, they did not identify patients with DVT or PE [26]. This was also true for viscoelastic tests [23]. Hincker et al. identified a preoperative hypercoagulable ROTEM both with thromboplastin reagent InTEM and tissue factor reagent ExTEM activated profiles (CFT, AA and MCF) in 10 out of 333 non-cardiac surgery patients who developed postoperative DVTs even after LMWH or heparin thromboprophylaxis. There was no indication of this hypercoagulation in the APTT, PT-INR or platelet count analyses [27].

In TBI patients and other trauma patients, alcohol can induce a hypocoagulable thromboelastographic (TEG, an alternative viscoelastic system) profile in the initial trauma setting, explaining

the DVT-reducing effect of alcohol [28]. None of our patients had alcohol intake at the first sampling point, but ROTEM can also detect this alcohol induced coagulopathy [29].

Subarachnoid hemorrhage patients also develop thromboses and are hypercoagulable [30]. The mechanisms underlying their conditions involve an imbalance in tissue factor, tissue factor pathway inhibitor (TFPI) and activated protein C (APC), differing from that of TBI patients. TEG has been reported to identify DVT patients who have a shorter r time (a variable that corresponds to the ROTEM CT) [30]. In our patients, CT was prolonged out of its normal range (as discussed above), but we had no incidences of DVT or PE in our small sample size. It will be interesting to see if we can corroborate this finding, e.g., a short ROTEM-CT in DVT or PE patients, during the extension of this study.

A much larger sample size is needed to prove our hypotheses that TGA is superior to the standard coagulation tests, that ROTEM is preferable for monitoring hypo- and hypercoagulability, and that TGA is more suitable than Anti-fXa for monitoring the peak and through effects of LMWH. The present study will continue until 40 TBI cases with full sampling schedules have been included. Hopefully, this will provide information from at least eight patients with DVT or PE, so that we can analyze the predictability, sensitivity and specificity of TGA and ROTEM for such complications.

A current problem with TGA is the lack of general reference values [18,31]. The values differ between laboratories, making comparisons difficult. However, if future studies can prove that TGA is superior to the routine coagulation analyses (that rely on ROTEM and Anti-fXa for monitoring hypercoagulation and the LMWH dose response), TGA will likely become more standardized. A whole blood point of care TGA is under development that could transform it from a research assay to a global test. This development could also remove the time-consuming centrifugation and plasma pipetting steps [32].

The biggest limitations of the present study were the small sample size and the short sampling schedule. During the shorter than anticipated sampling period, the NICU at Skane University Hospital in Lund treated relatively few patients of interest to this study, resulting in only one included TBI patient and one included ICH patient who stayed through all the sample points. The other patients never started LMWH treatment due to a range of factors, including: high ICP with the possible need for immediate craniotomy, swift recovery, mobility without the need for LMWH thromboprophylaxis, transfer to another hospital, and death. Another difficulty in including patients for this study was that most of the NICU patients of interest were unconscious or sedated, making it hard to obtain consent if no relatives were present.

A sample schedule with a longer sampling period would have captured coagulation changes beyond the first six days. The

study would also have benefited from more sampling points during LMWH treatment to provide more detail on the relationship between TGA and Anti-fXa when monitoring the peak and trough effects of LMWH.

The limited number of patients in this pilot study greatly affected the power of the study, making any results indicative rather than conclusive. With a larger study group, it might be possible to use the TGA parameters to observe changes in hyper- or hypocoagulation and to study how these factors change over time.

Conclusion

The TGA parameters AUC and thrombin peak correlated well with Anti-fXa activity during LMWH treatment. We were not able to determine if TGA was superior to Anti-fXa in monitoring the LMWH peak and trough effects. To investigate if TGA is better than other analyses in monitoring coagulative changes over time in NICU patients and to explore whether TGA could better define the optimal time to start LMWH treatment, a larger sample size and a more detailed sampling schedule over a prolonged period are needed.

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