Bioactivity of enoxaparin in critically ill patients with normal renal function

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Venous thromboembolism is a frequent complication in critically ill patients that has a negative impact on patient outcomes.
- Critically ill patients have significantly lower plasma anti-factor-Xa activity levels compared with control patients after administration of subcutaneous heparin.
- The clinical relevance of the different anti-factor-Xa levels after prophylactic doses of low molecular weight heparin (LMWH) in critically ill patients is not completely understood.

WHAT THIS STUDY ADDS

- The standard dose of 40 mg enoxaparin led to a significant increase in anti-FXa levels in this selected cohort of ICU patients with normal renal function.
- This study found only subtle pharmacokinetic differences, but a comparable pharmacodynamic action, after enoxaparin administration in critically ill and normal medical ward patients.
- Thrombin generation with TGA RC-low and TGA RC-high reagents was significantly reduced in ICU and normal ward patients after receiving LMWH. Both readouts appear equally useful for estimating the pharmacodynamics of enoxaparin.
- The *ex vivo* model of thrombosis was used for the first time in patients to evaluate the anti-thrombotic activity of LMWH. This method did not show any difference in thrombus formation after administration of enoxaparin in the individual group of patients.

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Aim

In critically ill patients, reduced anti-FXa plasma activity following subcutaneous administration of enoxaparin or nadroparin has been described. In this study, we aimed to investigate the bioactivity of enoxaparin in critically ill patients and controls.

Methods

A prospective, controlled, open label study was performed on a medical intensive care unit (ICU) and a general medical ward. Fifteen ICU patients (male = 12, median age 52 years [IQR 40–65], with a median Simplified Acute Physiology Score of 30 [IQR 18–52]) and sex- and age-matched medical ward patients were included. The anti-FXa plasma activity was measured after a single subcutaneous dose of 40 mg enoxaparin. The thrombus size of a clot formed in an *ex vivo* perfusion chamber and endogenous thrombin potential (ETP) were measured.

Results

The anti-FXa plasma activity increased significantly after enoxaparin administration, with peak levels at 3 h after treatment, but was comparable between the ICU and medical ward groups (median 0.16 IU ml⁻¹ [IQR 0–0.22 IU ml⁻¹] vs. 0.2 IU ml⁻¹ [IQR 0.15–0.27 IU ml⁻¹], respectively, $P = 0.13$). The area under the anti-FXa activity curve from 0–12 h was similar between the groups (median 0.97 IU ml⁻¹ h [IQR 0.59–2.1] and 1.48 IU ml⁻¹ h¹ [IQR 0.83–1.62], $P = 0.42$ for the ICU group compared with the control group, respectively). The ETP was lower in the ICU group ($P < 0.05$) at baseline, but it was comparable at 3 h between the groups. Thrombus size decreased at 3 h compared with predose ($P = 0.029$) and was not different between the groups.

Conclusion

Similar bioactivity was achieved with a standard dose of subcutaneous enoxaparin in this selected cohort of ICU and general ward patients with normal renal function.

Introduction

Venous thromboembolism (VTE) is an important cause of morbidity and mortality in critically ill patients [1, 2]. A few clinical trials have shown the efficacy of pharmacological thromboprophylaxis in reducing the risk of VTE in the intensive care unit (ICU) setting [3–5]. Current recommendations emphasize the need for VTE prevention in the ICU using either unfractionated heparin (UFH) or low molecular weight heparin (LMWH) [6]. In the majority of European ICUs, the standard treatment is mainly LMWH [7], while UFH is preferred in Australia and North America [8].

There is increasing evidence that the doses of LMWH established for VTE prophylaxis in medical and surgical patients have different effects in the critically ill [9]. Differences in the anti-FXa profile between critically ill and medical patients have been shown for nadroparin [10] and enoxaparin [11]. However, single measurements of anti-FXa activities most likely do not accurately reflect the true influence of enoxaparin on coagulation [12]. Lower anti-FXa activities indicate pharmacokinetic differences but might not imply less protection in critically ill patients as the association between plasma anti-FXa activity and clinical outcome is weak [13, 14].

However, the assessment and comparison of the anti-thrombotic bioactivity of enoxaparin in critically ill and 'normal' medical ward patients can be used as a surrogate for the pharmacologic comparison of LMWHs. The aim of this study was to determine if a single subcutaneous dose of 40 mg enoxaparin exerts equivalent antithrombotic effects on fibrin formation in critically ill and general medical ward patients with normal renal function under low shear-rate flow conditions in an *ex vivo* perfusion chamber model of thrombosis. The pharmacodynamic (PD) effects of enoxaparin were further analyzed using the thrombin generation assay (endogenous thrombin potential, ETP). Enoxaparin pharmacokinetic data were assessed through the anti-Xa activity. This is an established method for assessing systemic exposure to LMWH because heparin concentrations are not directly measurable [15]. Moreover, this method has the advantage of providing a measure of the PD response to the drug.

Methods

The present study was performed in a tertiary medical ICU in the department of internal medicine at the medical university hospital in Vienna, Austria. The study protocol was approved by the Ethics Committee of the Medical University of Vienna and conducted in accordance with the Declaration of Helsinki. For the critically ill patients, the Ethics Committee waived the requirement to obtain *a priori* informed consent for those patients who were too ill to give consent. Written information about study participation was provided after they regained full conscious-

ness. All survivors from the ICU signed the information form ($n = 4$). Therefore, no patient refused participation. Written informed consent was obtained from all conscious patients before entry into the study.

Study population

Between May 2007 and July 2008, 16 male and female patients admitted to the ICU and 16 consenting age- and sex-matched controls from a general medical ward were recruited. One patient from the ICU was excluded from the study due to deterioration of renal function on the day of the study. The criteria for inclusion were the following: indication for thromboembolic prophylaxis with enoxaparin 40 mg subcutaneously, age ≥ 18 years, prothrombin time of $\geq 30\%$ and thrombocyte count $>60 \text{ g l}^{-1}$. Normal renal function was defined as serum creatinine concentrations of $\leq 1.2 \text{ mg dl}^{-1}$ on two consecutive occasions and creatinine clearance $>80 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$. Due to the difficulty of accurately assessing urine output in general ward patients, we applied the Cockcroft–Gault formula [16] to determine creatinine clearance. The exclusion criteria were as follows: detectable plasma anti-FXa activity on the study day, requirement for full anticoagulation or antiplatelet medication, need for haemofiltration or haemodialysis, history of heparin-induced thrombocytopenia, hereditary or acquired coagulation disorders or systemic treatment with antiplatelet therapy. The studies were performed after a single injection of enoxaparin 40 mg subcutaneously, regardless of whether it was the first indicated injection or a subsequent injection. Careful attention was given for a 24 h washout period after the last injection of enoxaparin.

Study design

This prospective, controlled study had an open-label design. On the day before the study, the participants were screened by taking a medical history and undergoing a physical examination, including laboratory screening and creatinine clearance measurement.

A single dose of 40 mg enoxaparin from a pre-filled, single dose syringe (Lovenox®, Aventis Pharmaceuticals, Bridgewater, NJ) was administered subcutaneously into the right upper thigh of each participant at 08.00 h. Care was taken to ensure complete emptying of the syringe. Plasma anti-FXa concentrations were obtained before (time point 0) and at 1, 3, 6 and 12 h after the enoxaparin injection. The perfusion chamber experiments and venous blood samplings were performed before (time point 0) and 3 h after enoxaparin administration. The time points were based on predicted peak plasma anti-FXa activity.

Blood sampling

Blood analyses and perfusion chamber studies were performed with venous blood that was drawn from a central venous catheter in ICU patients and from venipuncture of an antecubital vein in general ward patients using an 18G cannula. All central and arterial lines in the ICU are

routinely flushed with 0.9% saline. For all coagulation parameters (anti-FXa, f1 + 2, plasma D-Dimer), the blood was collected using 3.8% sodium citrate tubes. Anti-FXa activity was measured using the colorimetric Rotachrom HBPM/LMWH assay (Diagnostica Stago, Asnieres-sur-Seine, France). The measurements of prothrombin fragment f1 + 2 and plasma D-Dimer concentrations were performed in platelet-poor plasma, obtained after 15 min of centrifugation of citrated blood at 3500 *g*. Aliquots of plasma were transferred to plastic tubes and stored frozen at -30°C until analysis with commercial assays (Enzygost®, F1 + 2 micro, ELISA, Dade Behring, Marburg, and Asserachrome, D-Dimer by ELISA Method, Roche Diagnostics, Mannheim, Germany).

Perfusion chamber experiments

Ex vivo thrombus formation was assessed using a perfusion chamber model [17–19]. The perfusion chambers were made of a plexiglas block, through which a cylindrical hole 0.2 cm in diameter was drilled. Each chamber contained a thrombogenic surface (pig aorta tunica media). The shear rate of the chamber was 212 s^{-1} , mimicking venous flow conditions. Before blood perfusion, the system was perfused with 0.9% sodium chloride to remove air bubbles. Venous blood (obtained from the central venous catheter for ICU patients or from the antecubital vein for controls) was passed through the chamber for 5 min at a constant rate of 10 ml min^{-1} (Masterflex® L/S™, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA), followed by 30 s of perfusion with 0.9% sodium chloride. Before each perfusion, 5 ml of blood was discarded. Fibrin formation on the thrombogenic surface was evaluated by measuring the level of D-Dimers ($\mu\text{g ml}^{-1}$) in the supernatant of the plasmin-degraded thrombus (Asserachrome, Roche, Mannheim, Germany) [20]. This variable was named thrombus size and represents the total amount of fibrin in the *ex vivo* thrombus. This approach is more accurate than the traditional morphometric analysis of *ex vivo* thrombus formation [21, 22].

Thrombin generation potential

The thrombin generation potential was measured with the TECHNOTHROMBIN® fluorogenic test (Technoclone, Vienna, Austria) to determine thrombin generation over time in platelet-poor plasma [23–25]. Venous blood was collected into 0.1 volume of 3.8% trisodium citrate, centrifuged for 20 min at 2000 *g* and stored at -30°C until analysis. The test principle is based on monitoring the fluorescence generated after cleavage of a fluorogenic substrate by thrombin over time after activation of the coagulation cascade by different concentrations of tissue factor and negatively charged phospholipids in plasma. From the changes in fluorescence over time, the concentration of thrombin (nM) formed in the sample was calculated using the respective thrombin calibration curve. To demonstrate the effect of LMWH, we evaluated thrombin

generation using three different reagents provided in this kit: 1) TGA RC-low: a low concentration of phospholipid micelles containing recombinant human TF (71.6 pM) in a Tris-Hepes-NaCl buffer, 2) TGA RC-high: a high concentration of phospholipid micelles containing recombinant human TF (71.6 pM) in a Tris-Hepes-NaCl buffer and 3) TGA RD: does not contain human TF but has a different phospholipid composition. For the analysis of differences between the reagents, the maximum concentration of thrombin generated (peak thrombin) was used.

Statistical analysis

The sample size calculation was based on the observed mean (2.63) of anti-factor-Xa activity in ICU patients, the observed mean (4.26) of anti-factor-Xa activity in normal ward patients and the SD (1.5) of anti-factor-Xa activity [11]. We calculated that at least 15 patients needed to be included to detect a 40% relative difference in anti-factor-Xa activity with a power of 85% and a two-sided alpha value of 0.05. Most of the data were not normally distributed and, therefore, non-parametric tests were used for statistical testing. For all descriptive analyses, continuous data are shown in the format of median (interquartile range, IQR). The anti-FXa activity within groups over time was assessed using Friedman ANOVA. Comparisons within groups were performed with the paired Wilcoxon test, while comparisons between groups were assessed using the Mann–Whitney U-test. The Spearman rank correlation test was used for computations of associations. We calculated the correlation of the difference in (Δ) PD parameters (difference between time point 0 [baseline] and time point 3 h), consisting of Δ thrombus size, Δ TGA RC-low and Δ TGA RC-high with PK (anti-FXa activity) at 3 h to correct for baseline differences. A two-tailed *P* value of <0.05 was considered significant. All statistical calculations were performed using commercially available statistical software (SPSS Version 16.0; Chicago, USA). A Bland–Altman analysis was performed to determine the 95% limits of agreement between the test methods of thrombin generation [26, 27]. The mean difference between the measures was plotted, with lines representing $\pm 1.96 \times \text{SD}$ of the difference, giving the 95% confidence range in which the values would be expected to fall.

Results

Table 1 presents demographic and laboratory data for all study participants. The age and sex distributions were similar between the groups. The differences in clinical and laboratory data represent the differences in the clinical condition of patients in the ICU vs. those in the regular ward. The main admission diagnoses of patients are listed in Table 2. All of the ICU patients required mechanical ventilation, with five patients on additional vasopressor therapy. C-reactive protein, fibrinogen and liver

Table 1

Baseline group characteristics

Characteristics	Intensive care (<i>n</i> = 15) median (IQR)	Medical ward (<i>n</i> = 16) median (IQR)	<i>P</i> value
Age (years)	52 (40–65)	56 (42–65)	
Gender M (F)	13 (3)	13 (3)	
Body mass index (kg m ⁻²)	26 (22–26)	28 (24–31)	0.037
SAPS score	30 (18–52)	NA	
Mechanical ventilation (<i>n</i>)	15	NA	
Vasopressors (<i>n</i>)	5	0	
Norepinephrine dosage (μg kg ⁻¹ min ⁻¹ , <i>n</i> = 6)	0.00 (0.00–0.09)	NA	
Systolic blood pressure (mmHg)	124 (104–131)	132 (122–155)	0.019
Diastolic blood pressure (mmHg)	56 (49–67)	74 (69–84)	<0.001
Heart rate (beats min ⁻¹)	80 (68–89)	75 (63–80)	
Serum creatinine (mg dl ⁻¹)	0.76 (0.59–0.89)	0.96 (0.83–1.0)	0.011
Creatinine clearance (ml min ⁻¹ /BSA)	128 (101–145)	113 (92–138)	
Leucocyte count (g l ⁻¹)	9.3 (6.8–11.6)	6.5 (4.6–7.6)	0.033
Haemoglobin (g dl ⁻¹)	9.7 (8.7–11.3)	14.2 (13.3–14.6)	<0.001
Platelet count (10 ⁹ l ⁻¹)	257 (105–323)	221 (194–250)	
C-reactive protein (mg dl ⁻¹)	13.4 (4.7–17.7)	0.5 (0.3–0.9)	<0.001
Fibrinogen (mg dl ⁻¹)	539 (356–667)	300 (280–400)	0.002
Prothrombin time (%)	68 (53–80)	96 (84–122)	<0.001
aPTT (s)	34.9 (32.3–41.5)	35.9 (33.1–38.8)	
Antithrombin III activity (%)	83 (70–116)	97 (88–109)	
ASAT (U l ⁻¹)	44 (22–135)	19 (16–26)	<0.001
ALAT (U l ⁻¹)	47 (23–110)	22 (15–30)	0.009
γGT (U l ⁻¹)	147 (72–188)	24 (19–32)	<0.001

Groups were compared using the Mann–Whitney U-test; SAPS, Simplified Acute Physiology Score; aPTT, activated thromboplastin time; ASAT, aspartate-aminotransferase; ALAT, alanine-aminotransferase; γGT, gamma-glutamyl-transferase; BSA, body surface area; NA, not applicable.

Table 2

Admission diagnoses

Admission diagnoses	ICU (<i>n</i> = 15)	Medical ward (<i>n</i> = 16)
Pulmonary disease	4	0
Septicaemia	1	0
Infectious disease	0	1
Neoplasm	1	6
Cardiopulmonary resuscitation (CPR)	8	0
Conditions that led to CPR		
Cardiac	5	
Pulmonary	2	
Septicaemia	1	
Endocrine disease	0	9
Intoxication	1	0

transaminases were higher in the ICU patients. Serum creatinine concentrations were within the normal range in both groups.

Anti-FXa activity

The anti-FXa plasma activity increased significantly after administration of enoxaparin (Figure 1, *P* < 0.001 in both groups). The peak anti-FXa activity (*C*_{max}) was not significantly different between the ICU and control patients

(0.16 IU ml⁻¹ [IQR 0–0.22 IU ml⁻¹] vs. 0.2 IU ml⁻¹ [IQR 0.15–0.27 IU ml⁻¹], respectively, *P* = 0.13). The time to peak anti-FXa activity (*t*_{max}) was significantly delayed in the ICU group compared with the control group (6 h [3–12 h] vs. 3 h [3–6 h], respectively, *P* = 0.038; Figure 1). There was a significant change over time in the anti-FXa activity in both groups, but no difference between the groups (*P* < 0.05 within groups, Figure 1). Likewise, the area under the anti-FXa activity vs. time curve from 0–12 h was similar in both groups (median 0.97 IU ml⁻¹ h⁻¹ [IQR 0.59–2.1] and 1.48 IU ml⁻¹ h⁻¹ [IQR 0.83–1.62], *P* = 0.42; ICU group vs. normal ward, respectively). Within the ICU group, subgroup analyses of patients with and without vasopressors were performed, but these subgroups did not show any difference in the course of anti-factor-Xa activity after enoxaparin administration.

Plasma f1 + 2 and D-Dimer concentrations

As expected, baseline f1 + 2 and D-Dimer concentrations were significantly higher in the plasma of the ICU patients (Table 3). Enoxaparin did not affect plasma concentrations of f1 + 2 or D-Dimer.

Perfusion chamber

The thrombus size was comparable between the groups at baseline and 3 h after enoxaparin administration (Figure 2, *P* = 0.26 and 0.42, respectively), and it did not change over

Figure 1

Anti-FXa activity before and 1, 3, 6 and 12 h after subcutaneous administration of 40 mg enoxaparin in ICU (A) and normal ward patients (B). Median anti-FXa activity (red line) did not differ between the groups at any time point

time when comparing before and 3 h after enoxaparin administration within the groups ($P = 0.18$ for ICU and $P = 0.158$ for normal ward, Figure 2). The thrombus size in the pooled data decreased 3 h after administration of enoxaparin compared with baseline, with a median difference of $-10 \mu\text{g ml}^{-1}$ ($2.9, -15.9 \mu\text{g ml}^{-1}$, $P = 0.029$). The baseline values of thrombus size correlated with the plasma fibrinogen concentrations in ICU patients ($r = 0.685$; $P = 0.005$) and in normal ward patients ($r = 0.589$; $P = 0.021$).

Thrombin generation

The values of thrombin generation using the TGA RC-low reagent were higher in normal ward patients at baseline, but there was no difference between the groups 3 h after administration of enoxaparin (Figure 3, Table 3). Comparisons within the groups reflected a clear response to enox-

aparin, with a significant reduction in the ETP using both TGA RC-low and TGA RC-high reagents (Figure 3, Table 3). The ETP using the TGA RD reagent, with a special composition of phospholipids, did not show any difference at any time point between the groups. However, we could observe a decrease in the thrombin peak within the ICU group (Figure 3, Table 3). There was a strong correlation between the analyses using TGA RC-low and TGA RC-high ($r = 0.822$, $P < 0.001$; Figure 4A). The coefficient of variation between these two test methods was 14%. The mean difference was -87 nM (SD 97, Figure 4B). The difference between measurements using these two reagents was within 277 and -103 nM (95% agreement). There was no correlation between anti-FXa activity and Δ thrombus size ($r = -0.004$), but there was a positive correlation between anti-FXa activity and Δ TGA RC-low ($r = 0.369$, $P = 0.041$) and Δ TGA RC-high ($r = 0.372$, $P = 0.039$).

Discussion

In this selected cohort of critically ill patients with normal renal function, a single subcutaneous dose of 40 mg enoxaparin demonstrated comparable antithrombotic bioactivity, as assessed by three different methods, compared with normal ward patients. The anti-FXa activity significantly increased in both groups after treatment. While there was no significant difference in the C_{max} of anti-FXa activity, the t_{max} was delayed in the ICU group. We observed systemic coagulation activation in the ICU patients, evidenced by increased f1 + 2 and D-Dimer venous plasma concentrations. Accordingly, *ex vivo* thrombus size was slightly larger in the ICU patients than in the general medical ward controls. Although we noted a decrease in thrombus size when data from all study participants (ICU and control) were pooled, comparisons of time points within each group and between groups did not show any statistically significant differences. The values of thrombin generation using the TGA RC-low reagent were higher in normal ward patients at baseline, but not at 3 h. Comparisons within the groups reflected a clear response to enoxaparin, with a significant reduction using the TGA RC-low and TGA RC-high reagents.

The anti-FXa activity of the ICU patients was comparable with previously published data [11, 28–30]. Although the peak anti-FXa concentrations in the ICU patients were within the expected range, they were approximately 50% lower than previously reported [11]. This finding might be explained, primarily, by the small number of patients on vasopressors and the substantially lower dose of norepinephrine used in the current study population ($n = 5$) compared with previous studies [10, 11] and, secondly, by the much lower SAPS II scores [11].

The significantly prolonged t_{max} for anti-FXa in critically ill patients suggests that some pharmacokinetic differences exist in this set of patients with low doses of

Table 3

Outcome parameters at baseline and 3 h after subcutaneous administration of 40 mg enoxaparin in ICU and normal ward patients. Data are presented as the median (IQR)

	Baseline (t = 0 h)		Enoxaparin (t = 3 h)	
	ICU	Normal ward	ICU	Normal ward
Thrombus D-Dimer ($\mu\text{g ml}^{-1}$)	54 (27–117)	43 (32–89)	37 (22–110)	44 (24–72)
Plasma f1 + 2 (nM)	368 (87–991)	177 (64–1709) [†]	327 (97–841)*	198 (81–1647)
Plasma D-Dimer ($\mu\text{g ml}^{-1}$)	945 (945–1302)	225 (94–1098) [†]	945 (945–1305)	258 (91–945)
Peak thrombin (nM)				
RC Low	270 (121–566)	379 (101–552) [†]	137 (4–337)*	208 (12–624)*
RC high	344 (150–657)	487 (161–644) [†]	269 (34–482)*	339 (102–725)*
RD	730 (462–1168)	665 (533–963)	626 (438–1137)*	648 (498–971)*

* $P < 0.05$ within groups (baseline vs. 3 h post enoxaparin). [†] $P < 0.05$ between groups (ICU vs. normal ward).

Figure 2

Thrombus size (thrombus D-Dimer concentration [$\mu\text{g ml}^{-1}$]) in ICU and normal ward patients before and 3 h after 40 mg subcutaneous enoxaparin administration. The horizontal box lines indicate the median and 25th and 75th quartiles. The whiskers are the minimum and maximum values

vasopressors. Whether this delay is of clinical relevance needs to be determined, and a larger sample size than that recruited for pharmacokinetic studies will be necessary to answer this question. The t_{max} was estimated from our pharmacokinetic model, but precision was limited by the number of sampling times. In this study, anti-FXa activity was measured after prophylactic dosing of enoxaparin, which is not generally monitored. Even for the therapeutic application of LMWH, a therapeutic window of anti-FXa activity has not been established in a controlled outcome study evaluating thromboembolic events. So far, therapeutic dosing of LMWH is applied according to body weight. For future studies, it would be plausible to monitor the PK of LMWH in the ICU at different time points after dosing. According to this study, monitoring of anti-FXa activity

should not be limited to a single time point after subcutaneous administration when differences in absorption cannot be excluded.

The perfusion chamber method has been used and validated to investigate the effect of antithrombotic drugs administered to healthy subjects [17–19]. This is the first study to evaluate *ex vivo* thrombus size by measurement of D-Dimer concentrations of the plasmin-degraded thrombus in patients. D-Dimer content is directly related to the amount of fibrin formed in the thrombus and hence the size of the thrombus [20]. Although this method could have been affected by many variables in the ICU cohort, such as higher fibrinogen and vWF concentrations due to the presence of systemic inflammation, activated coagulation and endothelial function, we did not detect any difference in thrombus formation between the groups. The small sample size and the low dose of enoxaparin might explain the lack of sensitivity of this method within this setting.

The adjustment of LMWH therapy by determination of the anti-FXa activity does not correlate with clinical efficacy. The indirectly acting LMWHs interfere with several steps of the blood coagulation cascade, but measurement of the anti-FXa activity does not reflect the activity of other clotting factors, such as thrombin. Assessment of endogenous thrombin generation has been established as a tool to estimate drug effects on the formation of thrombin [12, 31–35]. This method can monitor the initiation, propagation and decay phases of thrombin generation [36]. This study confirms that the ETP can be used to characterize the anticoagulant activity of enoxaparin. However, it also demonstrates that the reagents used determine the sensitivity of assays. The TGA RC-low and TGA RC-high revealed a high correlation across the range of measurements, whereas the TGA RD did not provide consistent results. According to these data, TGA RC-low and TGA RC-high appear equally useful to estimate the PD of enoxaparin. Furthermore, there was good agreement between the TGA readouts, as demonstrated by a high coefficient of correlation. Importantly, these results may differ when other drugs are being

Figure 3

Thrombin generation in platelet-poor plasma with different reagents (TGA RC-low: white boxes, TGA TC-high: striped boxes, TGA RD: dotted boxes; * $P < 0.005$ before and 3 h after 40 mg subcutaneous enoxaparin administration). The horizontal box lines indicate the median and 25th and 75th quartiles. The whiskers are the minimum and maximum values

Figure 4

(A) Correlation of measurements of thrombin generation between TGA RC-low and TGA RC-high reagents. (B) Bland–Altman plot for measurements of thrombin generation using TGA RC-low and TGA RC-high reagents. ● ICU patients, ○ normal ward patients

used, and the method therefore needs to be critically evaluated in each setting.

In conclusion, we observed similar bioactivity in a selected cohort of ICU patients and normal ward patients

from a standard dose of 40 mg subcutaneous enoxaparin. We found only subtle pharmacokinetic differences, but a comparable pharmacodynamic action, between critically ill and general ward patients with normal renal function

after this low dose of an LMWH preparation. Thrombin generation was shown to be a suitable method for demonstrating the anticoagulant effect of a prophylactic dose of enoxaparin.

Competing Interests

There are no competing interests to declare.

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REFERENCES

- 1 Cook D, Crowther M, Meade M, Rabbat C, Griffith L, Schiff D, Geerts W, Guyatt G. Deep venous thrombosis in medical-surgical critically ill patients: prevalence, incidence, and risk factors. *Crit Care Med* 2005; 33: 1565–71.
- 2 Ibrahim EH, Iregui M, Prentice D, Sherman G, Kollef MH, Shannon W. Deep vein thrombosis during prolonged mechanical ventilation despite prophylaxis. *Crit Care Med* 2002; 30: 771–4.
- 3 Fraisse F, Holzapfel L, Couland JM, Simonneau G, Bedock B, Feissel M, Herbecq P, Pordes R, Poussel JF, Roux L. Nadroparin in the prevention of deep vein thrombosis in acute decompensated COPD. The Association of Non-University Affiliated Intensive Care Specialist Physicians of France. *Am J Respir Crit Care Med* 2000; 161: 1109–14.
- 4 Leizorovicz A, Cohen AT, Turpie AG, Olsson CG, Vaitkus PT, Goldhaber SZ. Randomized, placebo-controlled trial of dalteparin for the prevention of venous thromboembolism in acutely ill medical patients. *Circulation* 2004; 110: 874–9.
- 5 Cade JF. High risk of the critically ill for venous thromboembolism. *Crit Care Med* 1982; 10: 448–50.
- 6 Geerts WH, Bergqvist D, Pineo GF, Heit JA, Samama CM, Lassen MR, Colwell CW. Prevention of venous thromboembolism: american College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008; 133: 381S–453S.
- 7 Hilbert P, Teumer P, Stuttmann R. [Prevention of thromboembolism in German intensive care units : results of a nationwide survey.]. *Anaesthesist* 2008; 57: 242–50.
- 8 Shorr AF, Williams MD. Venous thromboembolism in critically ill patients. Observations from a randomized trial in sepsis. *Thromb Haemost* 2009; 101: 139–44.
- 9 Robinson S, Zincuk A, Strom T, Larsen TB, Rasmussen B, Toft P. Enoxaparin, effective dosage for intensive care patients: double-blinded, randomised clinical trial. *Crit Care* 2010; 14: R41.
- 10 Dorffler-Melly J, de Jonge E, Pont AC, Meijers J, Vroom MB, Buller HR, Levi M. Bioavailability of subcutaneous low-molecular-weight heparin to patients on vasopressors. *Lancet* 2002; 359: 849–50.
- 11 Priglinger U, Delle Karth G, Geppert A, Joukhadar C, Graf S, Berger R, Hulsman M, Spitzauer S, Pabinger I, Heinz G. Prophylactic anticoagulation with enoxaparin: is the subcutaneous route appropriate in the critically ill? *Crit Care Med* 2003; 31: 1405–9.
- 12 Gerotziakas GT, Petropoulou AD, Verdy E, Samama MM, Elalamy I. 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: 955–62.
- 13 Levine MN, Planes A, Hirsh J, Goodyear M, Vochelle N, Gent M. The relationship between anti-factor Xa level and clinical outcome in patients receiving enoxaparin low molecular weight heparin to prevent deep vein thrombosis after hip replacement. *Thromb Haemost* 1989; 62: 940–4.
- 14 Nieuwenhuis HK, Albada J, Banga JD, Sixma JJ. Identification of risk factors for bleeding during treatment of acute venous thromboembolism with heparin or low molecular weight heparin. *Blood* 1991; 78: 2337–43.
- 15 Guillet B, Simon N, Sampol JJ, Lorec-Penet AM, Portugal H, Berland Y, Dussol B, Brunet P. Pharmacokinetics of the low molecular weight heparin enoxaparin during 48 h after bolus administration as an anticoagulant in haemodialysis. *Nephrol Dial Transplant* 2003; 18: 2348–53.
- 16 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41.
- 17 Wahlander K, Eriksson-Lepkowska M, Nystrom P, Eriksson UG, Sarich TC, Badimon JJ, Kalies I, Elg M, Bylock A. Antithrombotic effects of ximelagatran plus acetylsalicylic acid (ASA) and clopidogrel plus ASA in a human *ex vivo* arterial thrombosis model. *Thromb Haemost* 2006; 95: 447–53.
- 18 Sarich TC, Osende JJ, Eriksson UG, Fager GB, Eriksson-Lepkowska M, Ohlsson L, Carlsson S, Wahlander K, Gustafsson D, Badimon JJ. Acute antithrombotic effects of ximelagatran, an oral direct thrombin inhibitor, and r-hirudin in a human *ex vivo* model of arterial thrombosis. *J Thromb Haemost* 2003; 1: 999–1004.
- 19 Zafar MU, Vorchheimer DA, Gaztanaga J, Velez M, Yadegar D, Moreno PR, Kunitada S, Pagan J, Fuster V, Badimon JJ. Antithrombotic effects of factor Xa inhibition with DU-176b: phase-I study of an oral, direct factor Xa inhibitor using an *ex vivo* flow chamber. *Thromb Haemost* 2007; 98: 883–8.
- 20 Bossavy JP, Sakariassen KS, Barret A, Boneu B, Cadroy Y. A new method for quantifying platelet deposition in flowing native blood in an *ex vivo* model of human thrombogenesis. *Thromb Haemost* 1998; 79: 162–8.
- 21 Orvim U, Barstad RM, Stormorken H, Brosstad F, Sakariassen KS. Immunologic quantification of fibrin deposition in thrombi formed in flowing native human blood. *Br J Haematol* 1996; 95: 389–98.
- 22 Wolzt MEU, Gouya G, Eriksson UG, Gouya G, Leuchten N, Kapiotis S, Elg M, Schützer KM, Zetterstrand S, Holmberg M,

- Wählander K. Effect on perfusion chamber thrombus size in patients with atrial fibrillation during anticoagulant treatment with oral direct thrombin inhibitors, AZD0837 or ximelagatran, or with vitamin K antagonists. *Thromb Res* 2012; 129: e83–91.
- 23** Siller-Matula JM, Plasenzotti R, Spiel A, Quehenberger P, Jilma B. Interspecies differences in coagulation profile. *Thromb Haemost* 2008; 100: 397–404.
- 24** Eslam RB, Reiter N, Kaider A, Eichinger S, Lang IM, Panzer S. Regulation of PAR-1 in patients undergoing percutaneous coronary intervention: effects of unfractionated heparin and bivalirudin. *Eur Heart J* 2009; 30: 1831–6.
- 25** Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. *JAMA* 2006; 296: 397–402.
- 26** Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307–10.
- 27** Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995; 346: 1085–7.
- 28** Mayr AJ, Dunser M, Jochberger S, Fries D, Klingler A, Joannidis M, Hasibeder W, Schobersberger W. Antifactor Xa activity in intensive care patients receiving thromboembolic prophylaxis with standard doses of enoxaparin. *Thromb Res* 2002; 105: 201–4.
- 29** Jochberger S, Mayr V, Luckner G, Fries DR, Mayr AJ, Friesenecker BE, Lorenz I, Hasibeder WR, Ulmer H, Schobersberger W, Dunser MW. Antifactor Xa activity in critically ill patients receiving antithrombotic prophylaxis with standard dosages of certoparin: a prospective, clinical study. *Crit Care* 2005; 9: R541–8.
- 30** Rommers MK, Van der Lely N, Egberts TC, van den Bemt PM. Anti-Xa activity after subcutaneous administration of dalteparin in ICU patients with and without subcutaneous oedema: a pilot study. *Crit Care* 2006; 10: R93.
- 31** Drouet L, Bal dit Sollier C, Martin J. Adding intravenous unfractionated heparin to standard enoxaparin causes excessive anticoagulation not detected by activated clotting time: results of the STACK-on to ENOXaparin (STACKENOX) study. *Am Heart J* 2009; 158: 177–84.
- 32** Kakkar VV, Hoppenstead DA, Fareed J, Kadziola Z, Scully M, Nakov R, Breddin HK. Randomized trial of different regimens of heparins and *in vivo* thrombin generation in acute deep vein thrombosis. *Blood* 2002; 99: 1965–70.
- 33** Bostrom SL, Hansson GF, Sarich TC, Wolzt M. The inhibitory effect of melagatran, the active form of the oral direct thrombin inhibitor ximelagatran, compared with enoxaparin and r-hirudin on *ex vivo* thrombin generation in human plasma. *Thromb Res* 2004; 113: 85–91.
- 34** Al Dieri R, Alban S, Beguin S, Hemker HC. Fixed dosage of low-molecular-weight heparins causes large individual variation in coagulability, only partly correlated to body weight. *J Thromb Haemost* 2006; 4: 83–9.
- 35** al Dieri R, Alban S, Beguin S, Hemker HC. Thrombin generation for the control of heparin treatment, comparison with the activated partial thromboplastin time. *J Thromb Haemost* 2004; 2: 1395–401.
- 36** Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoort R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003; 33: 4–15.