ABSTRACT

Background. During haemodialysis, anticoagulants are required to prevent clotting in the extracorporeal circuit. Low-molecular weight heparins (LMWH) are frequently used because of the ease of a single injection at the start of dialysis. Disadvantages of LMWH include the lack of a reliable bedside assay for measuring their anticoagulant effect.

Methods. We investigated a bedside test for LMWH activity. The relationship between anti-Xa (chromogenic assay) and Hemonox point-of-care assay was evaluated in 21 dialysis patients (12 men and 9 women) with a median age of 71 years, receiving tinzaparin at the start of a haemodiafiltration session.

Results. At the start, before tinzaparin administration, median (interquartile ranges) of Hemonox values were 74 (67–82) s. Thirty minutes after tinzaparin administration, Hemonox values were increased to 496 (360–736) s, followed by a decrease to 149 (135–301) s after 120 min, 102 (97–144) s after 180 min and 92 (83–100) s after 240 min. Corresponding anti-Xa activities were 0 (0–0), 1.12 (0.9–1.29), 0.74 (0.57–0.96), 0.47 (0.31–0.7) and 0.31 (0.16–0.49) IU/mL. Hemonox values showed an exponential relation to anti-Xa levels. Interchangeability of tests was shown by Bland–Altman plot.

Conclusion. Point-of-care Hemonox test is a valuable bedside method for monitoring anti-Xa activity in dialysis patients anticoagulated with tinzaparin.

Keywords: anti-Xa activity, haemodialysis, Hemonox, low-molecular weight heparin, tinzaparin

INTRODUCTION

In haemodialysis, anticoagulants are required to prevent clotting of the extracorporeal circuit. In Western Europe, low-molecular weight heparin (LMWH) is most widely used for this purpose [1]. LMWH is preferable to unfractionated heparin (UFH) [2] mainly because of the ease of a single injection in the dialysis circuit at the start of the session. Disadvantages of LMWH over UFH include the lack of an easily applicable assay to measure anti-Xa activity [2]. Chromogenic anti-Xa activity level determination is the recommended laboratory assay for monitoring the anticoagulant effect of LMWH [3]. It has been demonstrated that Hemonox point-of-care assay could be used effectively to monitor enoxaparin [4–6] and dalteparin [5] anticoagulant effect in the setting of percutaneous coronary intervention. To the best of our knowledge, however, this method was never assessed in a dialysis setting.

Therefore, in the present study we investigated, in the setting of chronic haemodiafiltration patients treated with tinzaparin, the relation between results obtained with the Hemonox point-of-care assay and anti-Xa activity as measured by chromogenic assay. We also evaluated whether the test was able to identify patients at risk of underdosing or overdosing of their anticoagulation with the anti-Xa activity as reference.

PATIENTS AND METHODS

Patients

Twenty-one chronic haemodialysis patients (12 men and 9 women) with a median age of 71 (64–80) years and body
weight of 67.8 (63–78) kg were included in the study protocol. The causes of renal failure were diabetic nephropathy (n = 8) renovascular disease (n = 6) and other (n = 7). Exclusion criteria for the study were: anaemia with haematocrit below 30%, increased risk of bleeding, overt inflammation, active infection or malignancy, hepatic failure, thrombocytopenia below 150 000/µL, treatment with vitamin K antagonists, administration of LMWH for other reasons than anticoagulation during dialysis or the use of heparin as catheter lock. Ten patients received antiplatelet therapy, aspirin (n = 9) and/or clopidogrel (n = 2). Residual renal function measured as average of creatinine and urea clearance was 2.4 (0–5.3) mL/min. All patients but one had a native arterio venous fistula; one patient was dialysed with a double lumen tunnelled cuffed catheter (Hemoglide, Bard) and in one patient the inlet bloodline was connected to the arterio venous fistula, whereas the outlet line was connected to a catheter (Hemoglide, Bard).

During the sessions under study no intravenous therapies such as antibiotics, parenteral nutrition, iron, colloid plasma volume expanders or packed cells were administered.

Written informed consent of the patients and approval of the local ethic committee were obtained.

Methods

Haemodiafiltration

The sessions were performed on an AK 200 Ultra S (Gambro, Lund, Sweden) with single use FX800 Helixone membranes (Fresenius Medical Care, Bad Homburg, Germany).

As anticoagulant, tinzaparin (Innohep, Leo Pharmaceutical Corp, Ballerup, Denmark) was administered as a single dose in the outlet (venous) bloodstream prior initiation of the session. No heparin was added in the priming solution of the circuit.

The haemodiafiltration sessions lasted 240 min with an effective blood flow of 300 mL/min, a dialysate flow of 600 mL/min. Volume controlled postdilution haemodiafiltration was applied with a substitution rate of 25% of the blood flow. The sessions under study were performed on the first dialysis of the week or during a midweek session in 10 and 11 patients, respectively.

Sample collection

Blood was sampled at the start of the session before heparin injection from the vascular access, and at 30, 120, 180 and 240 min from the inlet (arterial) bloodstream. All samples, except at the start, were taken with the blood pump rotating at 300 mL/min. In 13 out of 21 patients, baseline samples were taken at the occasion of an additional session. Blood samples were collected in citrated tubes (Venosafe™ 3.6 mL, 0.109 M buffered sodium citrate, Terumo Europe NV, Leuven, Belgium) for measurement of anti-Xa and antithrombin and in tubes with gel and clotting activator (VenoSafeAutoSep, Terumo, Belgium) for C-reactive protein (CRP) and in K-EDTA tubes (BD Plymouth, UK) for haematocrit and platelet count.

For measuring Hemonox clotting time, blood was sampled from the inlet bloodstream in a 3 mL syringe (BD, Franklin Lakes, NJ, USA) with an 18G needle (BD Microlance™, BD, Drogheda, Ireland) and injected immediately on the Hemonox cuvette (Hemochron™ JR LMWH, Edison, NJ, USA). Samples for other coagulation tests were centrifuged and kept at −80°C until analysis.

Analytical techniques

Hemochron Jr. Hemonox™ (ITC, Edison, NJ, USA) assay is a microcoagulation system based on the whole blood clotting time. The test cuvette is preloaded with dried lipitated recombinant rabbit brain tissue factor, stabilizers and buffers. Following introduction of whole blood, the instrument measures clotting time expressed in seconds. The clot detection mechanism consists of light-emitting diode optical detectors aligned with the test channel of the cuvette. The speed at which the blood sample moves between the detectors is measured. As clot formation begins, blood flow is impeded and the movement slows down. A clot endpoint is achieved when the movement decreases below a predetermined rate. Electronic quality control was performed before use.

Anti-Xa activity was measured by a chromogenic method measured at 405 nm (Biophen Heparin, Hyphen BioMed, Neuville-sur-Oise, France) on an STA-C (Diagnostica Stago, Asnières, France). This is a kinetic method based on the inhibition of a constant amount of factor Xa (FXa) by the tested heparin in the presence of endogenous antithrombin, and hydrolysis of an FXa-specific chromogenic substrate (SXa-11), by the FXa in excess. Para-nitroanilide is then released from the substrate in relation to the residual FXa activity. The anti-Xa assay was calibrated with the Biophen Heparin Calibrator (Hyphen). This is a set of calibration plasmas supplemented with LMWH (five different levels from 0 to 2.0 IU/mL) for the assay of heparin (UFH or LMWH) using chromogenic anti-Xa assays. A five point calibration curve was constructed, and was controlled before analysis by three levels of control material (zero, low and high level). Results are expressed as international units (IU)/mL with a detection limit of 0.05 IU/mL.

Analysis of antithrombin was carried out using a chromogenic method (Coamatic Antithrombin, Chromogenix, Instrumentation Laboratory, Milan, Italy).

CRP was measured through a particle-enhanced immunoturbidimetric assay (CRPLX, Roche Diagnostica, Mannheim, Germany).

Platelet count and haematocrit were measured by a fully automated haematology analyser Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan) using hydrodynamic focusing with impedance for red blood cell (haematocrit) and platelet counting.

Calculations and statistics

Data are expressed as median and interquartile ranges. Paired data were analysed with repeated measures analysis of variance (Friedman) followed by Wilcoxon in the case of significance. Unpaired data were analysed with Mann–Whitney U-test. Significance was accepted if P was < 0.05. The relation between anti-Xa and Hemonox values was fitted by applying a one-phase exponential analysis. For that purpose, out of range values were set at 900. Anti-Xa and natural
logarithm-transformed Hemonox values were compared as standardized values with Bland–Altman analysis.

Receiver operator characteristic (ROC) curve analysis was applied to determine cut-off values of Hemonox for monitoring anti-Xa activity below and above 0.3 and 1.0 IU/mL. Although, the desired anti-Xa levels in dialysis are not firmly established, levels between 0.2 and 0.4 IU/mL are recommended [1]. In renal failure patients treated with subcutaneous LMWH, anti-Xa peak and trough levels below 1.0 IU/mL [7] and 0.3 IU/mL [8], respectively, have been suggested.

Between-run imprecision of Hemonox was assessed by calculating the per cent difference of duplicates [4], measured at baseline in 13 patients at two occasions.

Statistics and figures were generated with SPSS (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism® 4.0 (Graphpad Software, San Diego, CA, USA).

RESULTS

The administered dose of tinzaparin per kilogram bodyweight was 65.7 (55.2–69.8) IU/kg. No premature interruptions of the haemodiafiltration sessions occurred; no bleeding complications were noted. The ultrafiltered volume was 1.84 (0.79–2.39) L.

At the start of the session, haematocrit was 37 (34.4–38.2)%, platelet count 240 000 (222 000–266 000)/µL, CRP 0.2 (0.2–0.6) mg/dL and antithrombin 94 (86–113)%.

Evolution of clotting parameters during the dialysis sessions

Hemonox clotting time during the session is displayed in Figure 1A. At the start, before heparin administration, Hemonox values were 74 (67–82) s. Thirty minutes after tinzaparin administration, values were highly increased to 496 (360–736) s, followed by a gradual decrease to 149 (135–301) s after 120 min, 102 (97–144) s after 180 min and 92 (83–100) s at the end of the session (P < 0.001 between all time points). Four out of 105 measurements were out of range, meaning that no clotting occurred until 900 s. This corresponded with anti-Xa levels between 0.97 and 1.5 IU/mL. All three patients in whom this occurred received antiplatelet therapy.

Between-run imprecision of baseline Hemonox test measured as difference of duplicates [4], measured at baseline in 13 patients at two occasions.

Statistics and figures were generated with SPSS (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism® 4.0 (Graphpad Software, San Diego, CA, USA).

**Comparison between clotting parameters**

Figure 2 depicts the exponential relation between the Hemonox values (Y) and the anti-Xa activity (X), which was defined by:

\[ Y = 54.98 \cdot \exp(1.862 \cdot X) \quad \text{with} \quad R^2 = 0.713. \]

Bland–Altman analysis of standardized values of anti-Xa and the natural logarithm of Hemonox is illustrated in Figure 3. A correspondence between both tests is seen with a bias of 2.8 \times 10^{-7} and a 95% limit of agreement of −0.982 to 0.982.

**Comparison between patients with and without antiplatelet therapy**

Baseline Hemonox clotting times in patients with and without antiplatelet agents were 69 (67–76) and 80 (70–83) s, respectively (not significant). The relation between Hemonox (Y) and anti-Xa activity (X) in patients with antiplatelet therapy was \[ Y = 54.2 \cdot \exp(1.863 \cdot X) \quad \text{with} \quad R^2 = 0.705; \] for patients not on antiplatelet therapy it was \[ Y = 54.83 \cdot \exp(1.880 \cdot X) \quad \text{with} \quad R^2 = 0.722. \] The exponential increase and the start values of the individual exponential curves were not different in either group.

Using ROC analysis, Hemonox was able to discriminate for anti-Xa values below 0.30 IU/mL (area under the ROC curve of 0.938 with 95% CI: 0.896–0.981) (Figure 4). We propose a Hemonox value of 111 s as the lower cut-off, associated with a sensitivity and specificity of 0.783 and 0.972, respectively, to assess a minimal level of anticoagulation. For discriminating
anti-Xa above 1.00 IU/mL, the area under the curve was 0.959 (95% CI: 0.922–0.995). A cut-off of 260 s for the Hemonox test results in a sensitivity of 1 and a specificity of 0.918 to detect overanticoagulation (Table 1).

**DISCUSSION**

In the present study we found that the point-of-care Hemonox test can be used reliably to monitor anticoagulation with the LMWH tinzaparin during haemodiafiltration.

Previously, it has been demonstrated that the point-of-care assay Hemonox could be used to monitor anticoagulant activity of enoxaparin [4–6] and dalteparin [5] in patients receiving percutaneous coronary intervention. To the best of our knowledge, this is however the first study evaluating Hemonox in dialysis patients. Also, the effect of tinzaparin was never assessed before. Tinzaparin sodium is an LMWH produced by enzymatic depolymerization of the unfractionated heparin LEO, extracted from porcine intestinal mucosa. With an average molecular weight of 4500 Da, the Xa/IIa activity ratio of tinzaparin is 1.9 [9]. As different LMWHs can have a different impact on anticoagulation, data from enoxaparin and dalteparin cannot be extrapolated as such to tinzaparin as well. In

<table>
<thead>
<tr>
<th>Anti-Xa activity (IU/mL)</th>
<th>Area under the curve</th>
<th>Suggested Hemonox value (s)</th>
<th>Sensitivity</th>
<th>1-Specificity</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.30</td>
<td>0.938</td>
<td>111</td>
<td>0.783</td>
<td>0.028</td>
<td>0.896–0.981</td>
</tr>
<tr>
<td>&gt;1.00</td>
<td>0.959</td>
<td>260</td>
<td>1.000</td>
<td>0.082</td>
<td>0.922–0.995</td>
</tr>
</tbody>
</table>

**FIGURE 2:** Exponential relationship between $X$ = anti-Xa activity (IU/mL) and $Y$ = Hemonox (s). $Y = 54.98 \cdot \exp(1.862 \cdot X)$.

**FIGURE 3:** Bland–Altman plot of normalized values of anti-Xa activity and natural logarithm of Hemonox values.

**FIGURE 4:** ROC curve: Hemonox for detection of patients with anti-Xa below 0.3 IU/mL (A) and above 1.0 IU/mL (B).
the present study, Hemonox was found to reliably estimate anti-Xa activity to be in the therapeutic range in patients treated with tinzaparin.

LMWHs have been introduced in haemodialysis mainly because of their user friendliness, as they can easily be administered intermittently. A major disadvantage of LMWHs over UFH is the lack of possibilities to monitor the degree of anticoagulation [2]. It has been repeatedly demonstrated that both activated clotting time and activated partial thromboplastin time are not appropriate to monitor LMWH [3, 10, 11]. In addition, the results of the anti-Xa chromogenic test are in most dialysis centres not readily available. The major aim of a point-of-care test would be to instantly appreciate the level of anticoagulation and identify patients who have an anticoagulation outside the desired range. Indeed, on the one hand, besides problems with vascular access, clotting is the most important limiting factor to achieve adequate dialysis [12]. On the other hand, monitoring can also identify patients who receive an LMWH overdose and therefore have an elevated residual level of anticoagulation at the end of the session. This is important as dialysis patients have an increased risk of bleeding [13] and LMWH have a prolonged effect in renal failure [14]. In general, in stable chronic haemodialysis patients, LMWH can be administered three times per week without need for monitoring. In some conditions however, monitoring is important to prevent complications with potential impact on outcomes. A typical example where point-of-care monitoring can be of use are dialysis patients who already receive LMWH subcutaneously for underlying comorbidities. In these patients with renal failure, a bedside measurement of residual baseline anti-Xa activity before the dialysis session is necessary to safely adapt the additional dose to be administered at the start of dialysis. Also, when the dialysis session needs to be prolonged, or when the system has clotted and needs to be restarted, the required dose of additional anticoagulation can only be appreciated quickly enough by a point-of-care measurement. Other typical examples where a point-of-care estimate of residual anticoagulation would be very useful are dialysis patients undergoing invasive procedures shortly after dialysis, such as removal of large bore dialysis catheters, dental procedures or biopsies.

As some of our patients received antiplatelet agents, we compared baseline Hemonox in both patients groups and found it not different. Likewise, the exponential relationship between Hemonox and anti-Xa was not different. Based on these findings, both groups were pooled for analysis in the present study.

Besides Hemonox, other point-of-care tests for monitoring the anticoagulant effect of LMWH have been described [11, 15]. In contrast to Hemonox, these tests were homemade, however [11, 15].

In conclusion, the point-of-care Hemonox test is valuable in the monitoring of anticoagulation in dialysis patients treated with tinzaparin.

CONFLICTS OF INTEREST STATEMENT

None declared.

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