Anti Xa Monitoring during Treatment with Low Molecular Weight Heparin or Danaparoid: Inter-assay Variability

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Summary

If laboratory monitoring of low molecular weight heparin (LMWH) therapy is required the test of choice is the anti Xa activity assay. The relationship between anti Xa results obtained using different techniques is unknown. The aim of the present study was to compare anti Xa results obtained with eight different commercially available anti Xa activity assays (five chromogenic and three clotting based assays) in samples from patients receiving either therapeutic or prophylactic treatment with two types of LMWH (enoxaparin or dalteparin) or danaparoid.

We have demonstrated that highly significant differences exist between results obtained using different techniques. The mean anti Xa activity in patients receiving treatment or prophylaxis with enoxaparin ranged from 0.28 to 0.64 iu/ml. A similar relationship was present in samples from patients treated with dalteparin, mean anti Xa results ranging from 0.43 to 0.69 iu/ml. The Heptest clotting assay as used here in combination with the Automated Coagulation Laboratory instrument, was associated with lower results than other clotting or chromogenic techniques. In patients receiving danaparoid for heparin induced thrombocytopenia (HIT) mean results with three clotting based assays were 0.30 to 0.36 u/ml, compared to mean results of 0.47 to 0.65 u/ml for chromogenic assays.

Our data clearly indicate that the selection of anti Xa assay method could influence patient management since the dose required to achieve the therapeutic range would differ according to the assay employed. This is particularly important since the frequency of haemorrhagic side effects has been shown by others to be dose dependent, irrespective of the concomitant anti Xa activity results. In danaparoid therapy the clotting assays studied here should not be employed for monitoring without a modified target range, unless it can be demonstrated that the higher doses required to achieve the therapeutic range are safe.

Introduction

There is an increasing body of evidence that low molecular heparins (LMWH) are at least as effective as unfractionated heparin (UFH) in preventing and treating deep vein thrombosis (1, 2), pulmonary embolism (3) and arterial thrombotic disorders (4, 5).

Since the inter patient variability in dosage requirements is much lower for LMWH than for UFH (6) and since the half life of LMWH (unlike UFH) is essentially independent of dose (7) it is unlikely that laboratory monitoring of LMWH will need to be as frequent as for UFH. Monitoring may only be required in selected cases, for example in the treatment of patients in whom pharmacokinetic parameters differ from the majority of patients. This could include paediatric, pregnant or obese patients, or where renal insufficiency occurs. In any patients with complications or with high risk of complications such as haemorrhage it may also be helpful to document anti Xa levels.

LMWH prolong the activated partial thromboplastin time (APTT) or thrombin time weakly compared to UFH and the test of choice if laboratory monitoring is deemed necessary is therefore the anti Xa activity assay even though the relationship between anti Xa and efficacy or risk of haemorrhage is not established (2).

A number of studies evaluating anti Xa results during LMWH therapy have been published (8-11) and there is evidence that some clotting based anti Xa assays may underestimate anti Xa activity relative to chromogenic assays (12). The number of different assays in each of these studies is small. Furthermore, there are no comparative studies in relation to therapy with the heparinoid, danaparoid sodium in patients with HIT, where the target therapeutic range recommended in the literature of 0.5-0.8 u/ml (13) is based on a chromogenic anti Xa assay. It is not known whether this range can be used if clotting assays are employed. The aim of the present study was to compare results of a range of different clotting and chromogenic anti Xa assays (a total of eight) during therapy with two types of LMWH or with danaparoid.

Patients, Materials and Methods

Samples

Venous blood samples were obtained using an evacuated collecting system (Vacutainer, Becton-Dickinson Ltd). In each case 4.5 ml blood was mixed with 0.5 ml 0.105 M buffered trisodium citrate and centrifuged at 2500 g for 10 min at room temperature. Plasma was removed and stored at −70° C prior to testing. All tests were performed on plasma which had been frozen and thawed only once.

Patients

Patients receiving enoxaparin (Clexane, Rhone-Poulenc Rorer Ltd). Thirteen samples were analysed. Six samples were obtained from 4 patients receiving enoxaparin as treatment for deep venous thrombosis (DVT) with an initial dose of 100 iu/kg every 12 h as recommended by the manufacturer, which was adjusted if anti Xa activity was outside 0.5-1.0 iu/ml at 4-5 h post injection (range of doses 75-100 iu/kg). Seven samples were obtained from 6 patients receiving enoxaparin as DVT prophylaxis (4 receiving 4000 iu and 2 receiving 2000 iu every 24 h). Samples were collected a median of 4 h after injection (range 3-8 h).

Patients receiving dalteparin sodium (Fragmin, Pharmacia & Upjohn). Twenty-four samples from 9 different patients were analysed. All patients were receiving the product as treatment for DVT. The initial dose of 100 iu/kg every 12 h was adjusted if anti-Xa activity was outside 0.5-1.0 iu/ml at 4-5 h...
Each sample was analysed in duplicate using 3 different clotting and 5 different chromogenic assays. Details of assays used are given in Table 1. Assays were coded A to H and are referred to by letter hereafter. All were calibrated using pooled normal plasma (prepared from 20 healthy normal subjects and stored deep frozen at –70°C) following addition of the WHO 1st International Standard (IS) for LMWH (NIBS & C, Potters Bar, UK) to a final concentration of 1.0 iu/ml anti Xa activity for analysis of samples from patients receiving enoxaparin or dalteparin. This plasma was then diluted in pooled normal plasma to concentrations of 1.0, 0.5, 0.25, 0.125, 0.06 and zero iu/ml. Assays from patients receiving danaparoid were calibrated using 6 dilutions of normal plasma as above, but containing added danaparoid at 1.0 u/ml.

In all assays other than E and F activated factor X (Xa) is added to plasma or dilutions of plasma, some of which is neutralised by heparin/anti thrombin (AT). Residual factor Xa is quantified either by colour development after cleavage of chromogenic substrate (chromogenic assays) or by the time taken for fibrin formation in the presence of phospholipids, Ca++ and plasma components (clotting assays). Assay E (chromogenic) had all components of the assay co-lyophilised in a reaction cuvette. In assay F substrate was added before factor Xa, so that the inhibition of factor Xa and cleavage of substrate by residual factor Xa were competing reactions in both E and F. Assay B was performed using the Automated Coagulation Laboratory (ACL) instrument as described by Ozawa and co-workers (14).

It has been reported that some anti Xa assays are linear up to 1.0 iu/ml but not above that level (10). For this reason, and because the calibration curves in each assay did not extend beyond 1.0 iu/ml, any sample with an initial assay result of greater than 1.0 iu/ml was repeated after dilution in normal plasma and the original result disregarded.

**Results**

**Patients Receiving Enoxaparin**

The anti Xa assay results of individual samples are shown in Fig. 1. The mean, median and range of results obtained by each technique are shown in Table 2. Mean anti Xa activity ranged from 0.28 to 0.64 iu/ml for the three clotting assays and from 0.42 to 0.60 iu/ml for the five chromogenic assays. Results obtained with methods A, C, and G, were significantly greater than results with method B (ANOVA, \( p < 0.01 \)). Results with method C were more than twice those with method B. Results of individual patients by methods B and C (the techniques with highest and lowest mean anti Xa results) are shown in Fig. 2.

**Patients Receiving Dalteparin**

The anti Xa assay results of each patient are shown in Fig. 3. The mean, median and range of results obtained by each technique are shown in Table 2. Mean anti Xa activity ranged from 0.43 to 0.69 iu/ml for the three clotting assays and from 0.42 to 0.60 iu/ml for the five chromogenic assays. Results obtained with methods A, C, and G, were significantly greater than results with method B (ANOVA, \( p < 0.05 \)). Results with method C were also significantly greater than results with methods D, E, F and H (\( p < 0.05 \)). The maximum difference (60%) was between methods B and C, as for patients receiving enoxaparoid. Individual results for these two techniques are shown in Fig. 2.

**Patients Receiving Danaparoid**

The anti Xa assay results of each patient are shown in Fig. 4. The mean, median and range of results obtained by each technique are

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**Table 1**  
Source and principles of analysis of anti Xa assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Principle of analysis</th>
<th>Endpoint detection</th>
<th>Commercial Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Clotting</td>
<td>Manual</td>
<td>Diagnostic reagents, Thame, UK</td>
</tr>
<tr>
<td>B</td>
<td>Chromogenic</td>
<td>ACL</td>
<td>Organon Teknika, Cambridge, UK</td>
</tr>
<tr>
<td>C</td>
<td>Chromogenic</td>
<td>Specrophotometer</td>
<td>Coaguate/Chromogenic Quadrtech, Epsom, UK</td>
</tr>
<tr>
<td>D</td>
<td>Chromogenic</td>
<td>ACL</td>
<td>Organon Teknika, Cambridge, UK</td>
</tr>
<tr>
<td>E</td>
<td>Chromogenic</td>
<td>ACL</td>
<td>Instrumentation Laboratory, Warrington, UK</td>
</tr>
<tr>
<td>F</td>
<td>Chromogenic</td>
<td>ACL</td>
<td>Stachrom/Stago, Shild, Dunide, UK</td>
</tr>
<tr>
<td>G</td>
<td>Chromogenic</td>
<td>ACL</td>
<td>Instrumentation Laboratory, Warrington, UK</td>
</tr>
<tr>
<td>H</td>
<td>Chromogenic</td>
<td>Stachrom/Stago, Shild, Dunide, UK</td>
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</tr>
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</table>

**Table 2**  
Anti Xa activity results by different techniques

<table>
<thead>
<tr>
<th></th>
<th>Mean (iu/ml)</th>
<th>Median (iu/ml)</th>
<th>Range (iu/ml)</th>
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</thead>
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<tr>
<td>Enoxaparin</td>
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<tr>
<td>Mean (iu/ml)</td>
<td>0.58</td>
<td>0.52</td>
<td>0.64 - 1.65</td>
</tr>
<tr>
<td>Median (iu/ml)</td>
<td>0.28</td>
<td>0.24</td>
<td>0.45 - 0.64</td>
</tr>
<tr>
<td>Range (iu/ml)</td>
<td>0.08 - 1.00</td>
<td>0.44 - 0.64</td>
<td>0.13 - 2.24</td>
</tr>
<tr>
<td>Dalteparin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (iu/ml)</td>
<td>0.32</td>
<td>0.32</td>
<td>0.28 - 0.64</td>
</tr>
<tr>
<td>Median (iu/ml)</td>
<td>0.46</td>
<td>0.46</td>
<td>0.22 - 0.52</td>
</tr>
<tr>
<td>Range (iu/ml)</td>
<td>0.15 - 1.65</td>
<td>0.38 - 1.00</td>
<td>0.38 - 1.38</td>
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<tr>
<td>Danaparoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (iu/ml)</td>
<td>0.34</td>
<td>0.34</td>
<td>0.15 - 0.62</td>
</tr>
<tr>
<td>Median (iu/ml)</td>
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<td>0.44</td>
<td>0.17 - 0.48</td>
</tr>
<tr>
<td>Range (iu/ml)</td>
<td>0.08 - 1.38</td>
<td>0.13 - 0.22</td>
<td>0.13 - 0.22</td>
</tr>
</tbody>
</table>

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Fig. 1  
Patients receiving enoxaparin therapy: Anti Xa assay results with eight different techniques. Results are presented as box-plots indicating 10, 25, 50, 75 and 90 percentiles. The box therefore represents 50% of results post injection according to manufacturers recommendations (range of doses 70-130 iu/kg). Samples were obtained at 4 h post injection in 17/24 cases (range 3-7 h).

Patients receiving danaparoid sodium (Orgaran, Organon Teknika Ltd). Twenty samples from 9 patients were analysed. All patients had developed HIT during therapy with unfractionated heparin (UFH). Patients were initiated on 150 to 190 u per h by intravenous infusion which was adjusted to maintain an anti Xa activity (by chromogenic assay) of 0.5-0.8 u/ml. Samples were collected at least 4 h after initiation of infusion with one exception which was collected 21 h after the infusion was discontinued.
shown in Table 2. In contrast to results in patients receiving LMWH there was no significant difference between results with the three clotting assays (mean anti Xa activity 0.30, 0.36 and 0.36 u/ml). The range of results with the five chromogenic assays was 0.47 to 0.65 u/ml. Results with all chromogenic assays were significantly greater (ANOVA, \( p < 0.001 \)) than results with all clotting assays with the exception of chromogenic methods D and F compared against clotting assays A and C. Individual results for the techniques giving greatest and lowest results (methods E and B) are shown in Fig. 5. For these two assays there was a high degree of discordance between therapeutic information. Taking 0.5-0.8 u/ml as the target for therapy (13) there were 7 samples (from 4 different patients) in which results obtained with method E indicated over-dosage (>0.8 u/ml) and results with method B indicated under-dosage (<0.5u/ml).

Discussion

Monitoring of LMWH may not always be necessary (1) and is not usually required in thromboprophylaxis. It may, however, be required in the treatment of patients in whom pharmacokinetic parameters differ from the majority of patients, for example in paediatric or obese patients, during pregnancy, or where renal insufficiency occurs. Haemorrhage is an important complication of heparin therapy and it has been shown that the risk of bleeding during treatment of DVT with dalteparin increases (by 60%) if the anti Xa activity exceeds 1.0 iu/ml, at least when administered by continuos infusion (15). The same study also demonstrated an increased risk of haemorrhage with high doses of the drug and independent of the concomitant anti Xa level. Thus there are a number of clinical situations where laboratory monitoring may be helpful.
LMWHs prolong APTT (or thrombin time) weakly compared to UFH and the anti Xa activity assay is a more appropriate and sensitive test. The majority of studies in which anti Xa activity has been determined have employed chromogenic anti Xa assays and these have been reported in both enoxaparin (16-20) and dalteparin therapy (15, 21, 22).

Several studies of LMWH have included more than one type of anti Xa assay (8, 9, 11, 12, 23, 24) but in some LMWH was added to plasma in vitro. Testing of plasma to which unfractionated heparin has been added in vitro can lead to unreliable or misleading results compared to testing of plasmas from patients receiving the same drug (25). This may also be the case for LMWH.

The present study compared directly anti Xa assay results by eight different commercially available techniques, in samples from patients receiving enoxaparin or dalteparin (LMWH). Assays of LMWH were calibrated using the first International Standard for LMWH.

During LMWH therapy with either enoxaparin or dalteparin there were highly significant differences between anti Xa activity results obtained with different assays. In samples from patients receiving enoxaparin for DVT treatment or prophylaxis, the mean of results by one technique were more than twice those by another. In patients receiving treatment doses of dalteparin results differed by up to 60%. The poor level of agreement between results obtained with some anti Xa assays suggests that the management of patients may be influenced by the laboratory technique that is performed to monitor them. The largest difference between results with different chromogenic techniques was 43% in the case of enoxaparin therapy and 27% in dalteparin treatment. It is not clear to what extent, if any, these smaller differences would influence patient management, since the relationship between anti Xa activity and efficacy or risk of haemorrhage is complex.

The level of agreement between results with different sources of anti Xa assays could be influenced by the use of different instruments or by the use of different heparin preparations to prepare calibration curves (9). In the present study all assays were calibrated with the 1st International Standard for LMWH and all chromogenic assays were performed on the same coagulometer. Clotting assays were performed with different methods of endpoint detection. The lowest results were obtained with the Heptest assay used in combination with an ACL instrument (14). It is not known whether results with this assay would have been different if combined with a different coagulometer.

The reason for differences between results with one clotting assay and other clotting or chromogenic assays is unknown but may relate to the influence of thrombin inhibition during the assay (2, 26). The clearance of heparin chains of different lengths after administration varies with its molecular weight. The longer heparin chains supporting both anti Xa and anti IIa activities are cleared twice as rapidly as the shorter chains, supporting only anti Xa activity (27, 28). Thus the composition of LMWH changes after administration with the rapid loss of anti IIa activity. Some clotting based assays are probably influenced by the anti IIa activity which remains in the heparin added to plasma to construct the calibration curve. This material is largely missing from the test sample, collected from patients 4-6 h after injection. Thus the clotting times used to establish the calibration curve are prolonged in relation to the test sample, leading to a systematic underestimation of the anti Xa activity. Only assays uninfluenced by anti IIa activity would not show this effect. This mechanism may have contributed to the underestimation of the anti Xa activity by the Heptest technique employed relative to the chromogenic assays. Our observation that results with the Staclot and Diagnostic reagents clotting assays are not associated with this underestimation would suggest that they are not sensitive to this anti IIa effect. This is supported in the case of the Staclot assay by the observations of Dignac and co-workers (26) who also noted similar anti Xa results with this clotting assay and the Stachrom chromogenic technique.

One important potential complication of heparin therapy is the development of HIT, which requires rapid replacement of heparin with alternatives such ashirudin or danaparoid (29). Danaparoid sodium is a mixture of heparan sulphate and dermatan sulphate and has been used successfully in the treatment of HIT (13). Although Magnani (13) recommended a therapeutic range of 0.5-0.8 u/ml anti Xa (based on chromogenic assays) to our knowledge there are no studies comparing anti Xa assay results with different techniques.

Our study clearly demonstrates the presence of highly significant and marked differences between results of clotting and chromogenic assays in patients treated with danaparoid. For the assays associated with the highest and lowest mean anti Xa activity there was high degree of discordance between therapeutic information. If we adopt the recommendations of Magnani (13), there were seven samples in which the one assay would have indicated over-dosage (anti Xa >0.8 u/ml) whereas results of another assay would have indicated under-dosage (ant Xa <0.5 u/ml). Monitoring with one method would therefore have led to a reduction in dosage whereas use of the other method would have led to an increase.

Assays of danaparoid were calibrated using the material received by patients as recommended by Warkentin et al. (29) since the composition of this material is fundamentally different from the international standard for LMWH. The reason for the differences between results with different techniques is unknown but as for LMWH the anti IIa activity of danaparoid is cleared much more rapidly than the anti Xa activity, with half lives of 4.3 h and 24.5 h respectively in one study (30). This may have contributed to underestimation of anti Xa activity by clotting assays relative to chromogenic techniques for the reason discussed in relation to LMWH results above. The anti Xa/IIa ratio of danaparoid is at least 28 (31). This compound therefore contains very little anti IIa activity and this may be a reason why the agreement between results of different clotting assays was better than that observed after LMWH.

In conclusion, we have demonstrated that in LMWH or danaparoid therapy the results of some anti Xa assays differed markedly from those obtained with the chromogenic techniques used in dose finding studies and the therapeutic range by anti Xa activity may be influenced by assay technique.

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