Wide variability in the sensitivity of APTT reagents for monitoring of heparin dosage

S Kitchen, I Jennings, T A L Woods, F E Preston on behalf of the Steering Committee of the UK National Quality Assessment Scheme for Blood Coagulation

Abstract

Aim—To assess the sensitivity of activated partial thromboplastin time (APTT) reagents for monitoring heparin dosage using data from the UK National External Quality Assessment Scheme (NEQAS) for blood coagulation.

Methods—Data were reviewed from four surveys using samples prepared by addition of heparin to normal plasma in vitro and from two surveys in which samples were prepared using plasma from patients receiving heparin therapy (ex vivo samples).

Results—For both in vitro and ex vivo samples, notable differences between APTT reagents with respect to heparin sensitivity were noted. This indicates that a uniform therapeutic range of 1-5-2-5 calculated by the APTT ratio may not be appropriate for all reagents. Reagent sensitivity in ex vivo samples was substantially different to that in in vitro samples.

Conclusions—The results of this large series of laboratories clearly indicate that reagent specific therapeutic ranges may be necessary, and that samples prepared by the addition of heparin to normal plasma in vitro can be misleading and should not be used.

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Keywords: heparin, activated partial thromboplastin time, blood coagulation.

Unfractionated intravenous heparin is widely used for the treatment of venous thromboembolism, with the activated partial thromboplastin time (APTT) being frequently used for monitoring of heparin dosage. A relation between inadequate heparinisation and recurrent thrombosis has been demonstrated clearly. A sensitive and accurate method for heparin monitoring, therefore, is clearly necessary.

Many centres use the APTT and a therapeutic range calculated by the APTT ratio (test/normal APTT) of 1-5-2-5 irrespective of reagent. However, APTT reagents vary in their responsiveness to heparin and this is influenced further by whether the heparin is added in vitro or whether it is assessed in samples from patients receiving heparin.

The assessment of laboratory control of heparin therapy is an important component of the UK National Quality Assessment Scheme (NEQAS) for blood coagulation. In this scheme lyophilised plasma samples are distributed to over 420 laboratories carrying out tests for assessing heparin dosage. Participants are requested to assess heparin dosage using their routine method. The APTT is used by 98% of participating laboratories for this purpose, although a considerable number of reagents and end point detection methods are in use. Statistical analysis of data returned allows comparisons to be made between different reagents.

Samples distributed through the College of American Pathologists and through the UK NEQAS have traditionally been prepared by the addition of heparin to normal plasma in vitro. In recent UK NEQAS surveys, however, samples for heparin dosage assessment have been prepared using samples from patients receiving heparin. In this report we have compared the responsiveness of APTT reagents for measuring heparin dosage in the two types of samples (in vitro and ex vivo) tested by a large number of laboratories.

Methods

ADDITION OF HEPARIN TO NORMAL PLASMA IN VITRO

Plasma was collected from normal subjects as described elsewhere. About 600 ml blood was collected over 45 minutes into citrate phosphate dextrose anticoagulant using a Haemoflowics Ultralight instrument. Blood was then centrifuged twice at 2500 x g at 4°C for 30 minutes and stored in bulk at -40°C for up to 12 weeks. The plasma was then thawed and buffered with 0-034 M hydroxyethylpiperazine ethane sulphonic acid. Unfractionated sodium heparin was then added prior to lyophilisation in 0-5 ml aliquots for six days. These lyophilised samples were then stored at -20°C for no longer than 12 months prior to despatch by post to participants, who reconstitute the
plasma with distilled water at room temperature.

Four different samples were prepared in this way, with heparin concentrations in the range 0.07–0.30 IU/ml by anti-Xa assay. These samples were distributed as part of UK NEQAS survey numbers 73, 75, 77, and 79 between September 1991 and February 1993.

**EX VIVO SAMPLES FROM PATIENTS RECEIVING INTRAVENOUS UNFRACTIONATED HEPARIN FOR ACUTE VENOUS THROMBOEMBOLISM**

Material was prepared by pooling plasma from samples collected for routine patient management. All patients were receiving intravenous unfractionated sodium heparin (PumpHep, Leo Laboratories Limited, Princes Risborough, Bucks, UK) for thromboembolic disease. Each patient had an international normalised ratio of less than 1.4. Venous blood samples were obtained using an evacuated collecting system (Vacutainer, Becton Dickinson, Oxford, UK). In each case 4.5 ml blood was mixed with 0.5 ml 0.105 M buffered trisodium citrate and centrifuged at 2500g for 10 minutes at room temperature. Plasma was removed and centrifuged a second time to ensure adequate platelet removal, pooled and stored at −70°C prior to lyophilisation using the same procedure as for in vitro samples. Each pool consisted of plasma samples from about 200 subjects. Two different pools were prepared in this way and distributed as part of survey numbers 82 and 84 in October 1993 and February 1994, respectively.

### Table 1 UK NEQAS heparin dosage assessment: APTT ratios analysed according to reagent used

<table>
<thead>
<tr>
<th>APTT reagent</th>
<th>Average number of laboratories</th>
<th>Median APTT ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survey number</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>1.69 2.02 4.01 2.81 1.63 1.67</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>1.49 1.89 2.89 2.31 1.30 1.37</td>
</tr>
<tr>
<td>DB</td>
<td>42</td>
<td>1.58 1.99 3.45 2.77 1.55 1.59</td>
</tr>
<tr>
<td>DK</td>
<td>65</td>
<td>1.31 1.60 2.60 2.47 1.45 1.53</td>
</tr>
<tr>
<td>IL</td>
<td>109</td>
<td>1.58 1.75 3.08 2.69 1.93 2.08</td>
</tr>
<tr>
<td>M</td>
<td>54</td>
<td>1.70 2.05 3.20 2.50 1.67 1.73</td>
</tr>
<tr>
<td>ML</td>
<td>9</td>
<td>2.0 2.65 4.50 4.60 1.92 1.96</td>
</tr>
<tr>
<td>OA</td>
<td>21</td>
<td>1.54 1.76 2.87 2.44 1.87 1.93</td>
</tr>
<tr>
<td>OP</td>
<td>20</td>
<td>1.55 1.67 2.83 2.49 1.75 2.36</td>
</tr>
<tr>
<td>All reagents</td>
<td>413</td>
<td>1.55 1.80 3.00 2.55 1.73 1.88</td>
</tr>
</tbody>
</table>

Surveys 73 to 79 used normal plasma heparinised in vitro. Surveys 82 and 84 used samples from heparinised patients.

### Table 2 Ranking of APTT reagents by response to heparin for samples to which heparin had been added in vitro and samples from patients receiving heparin

<table>
<thead>
<tr>
<th>NEQAS samples prepared by the in vitro addition of heparin to normal plasma (mean result of four NEQAS surveys)</th>
<th>NEQAS samples prepared by pooling samples from patients receiving heparin (mean result of two NEQAS surveys)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>Mean APTT ratio</td>
</tr>
<tr>
<td>ML</td>
<td>3.43</td>
</tr>
<tr>
<td>A</td>
<td>2.63</td>
</tr>
<tr>
<td>DB</td>
<td>2.45</td>
</tr>
<tr>
<td>M</td>
<td>2.36</td>
</tr>
<tr>
<td>OP</td>
<td>2.33</td>
</tr>
<tr>
<td>IL</td>
<td>2.27</td>
</tr>
<tr>
<td>OA</td>
<td>2.15</td>
</tr>
<tr>
<td>B</td>
<td>2.14</td>
</tr>
<tr>
<td>DK</td>
<td>2.02</td>
</tr>
</tbody>
</table>

**SURVEY SAMPLES**

The levels of clotting factors relevant to the APTT (factors II, V, VIII:C, IX, X, XI, XII, and fibrinogen) were determined in the lyophilised samples distributed in each of the six surveys.

Factors II, V and X assays were performed using rabbit brain thromboplastin (PT Fib HS-Plus Instrumentation Laboratory, Warrington, UK). One stage assays of factors VIII:C, IX, XI, and XII were performed using Kaolin/platelet substitute (Diagnostic Reagents, Tuam, UK) with five minute activation and 0.025 M calcium chloride. All assays were carried out on the Automated Coagulation Laboratory (ACL) instrument, using three dilutions of test or standard plasma and human deficient plasma. Assays were calibrated using the following standard plasmas: 19th British Standard for factor VIII:C; 6th British Standard for Blood Coagulation Factors (IX); snap frozen pooled plasma derived from 25 normal donors and stored at −70°C for factors II, V, X, XI, and XII. Fibrinogen concentration was determined by Claus’s method. The heparin concentration was determined using a clotting anti-Xa assay validated with the 4th International Standard for unfractionated heparin as described previously.

**Results**

The most widely used test for monitoring of heparin dosage by participants in the UK NEQAS was the APTT. This was used by 98% of participating laboratories. A wide range of different reagents and methods of end point detection were in use. To facilitate comparison between reagents, the ratio of sample result to the mid point of the local normal range was calculated. The median APTTs calculated from all results are shown in table 1.

**USE OF DIFFERENT APTT REAGENTS**

The number of different reagents in use increased during the study period from 15 in September 1991 (survey 73) to 22 in February 1994 (survey 84). In survey 84 nine reagents were used by 10 or more laboratories. These were as follows: Actin FS(A); Boehringer Mannheim (B); Diagen Bell and Alton (DB); Diagen KPS (DK); Instrumentation Laboratory (IL); Manchester (M); Manchester Low Opacity (ML); Organon Teknika Auto APTT (OA); and Organon Teknika Platelet LS (OP).

The median APTT ratios (survey sample result divided by the mid point of the local normal range for APTT) for all users of each of these reagents are shown in table 1. This includes manual and automated methods of end point detection.

Marked reagent differences were observed with respect to APTTs determined on samples to which heparin had been added in vitro (surveys 73–79) and on samples from patients receiving heparin (surveys 82 and 84). When the least responsive reagent (lowest APTT ratio) was compared with the most responsive (highest APTT ratio) for each of the six surveys,
highly statistically significant differences were noted (unpaired t test, p<0.0001).

Reagents were ranked by heparin sensitivity based on the mean results in four surveys using plasma to which heparin has been added in vitro and the mean of two surveys using pooled samples from patients receiving heparin (table 2). The relation between reagents with respect to heparin sensitivity was different for the two types of sample—for example, reagent ML was associated with ratios 51% higher than IL for samples heparinised in vitro, and 3% lower than IL for pooled ex vivo samples. Ratios obtained by users of DB were 13% higher than ratios with OA in samples heparinised in vitro, and 21% lower than OA for samples from patients receiving heparin. Reagent DB ranked third and seventh (out of nine) for the two types of sample. Reagent IL ranked sixth and second. A number of other such discrepancies were also present (table 2).

In heparin dosage assessment exercises UK NEQAS participants are provided with brief clinical details and are asked to interpret their result as "no heparin detected", "inadequate", "adequate", or "overdose" of heparin. For survey 82, in which the sample distributed was a plasma pool from patients receiving heparin, 33 (94%) of the 35 users of reagent B (median ratio 1:30) regarded the sample as inadequate with respect to the local therapeutic range, whereas 126 (90%) of 140 users of IL reagent (median ratio 1:93) considered the heparin dosage to be adequate. The median APTT ratio for this sample based on results obtained with all reagents was 1:73. In survey 75 the median APTT based on results with all reagents was 1:80. The sample in this survey was prepared by addition of heparin to plasma. Despite the similar median APTT ratios, the discrepancy in clinical interpretation between users of reagents B and IL for the sample from patients receiving heparin (survey 82) was not present in survey 75, which used a sample prepared by in vitro heparinisation. In survey 75 more users of reagent IL (35%) considered heparin therapy to be inadequate (insufficient) than users of reagent B (20%).

Influence of coagulometer used to determine APTT
UK NEQAS participants used a range of different methods of end point detection and 22 types of coagulometer. Three reagents (DK, DB, M) were used manually and in combination with the KC series (Amelung Limited) of coagulometers in sufficient numbers to permit meaningful comparison (table 3). The results obtained by users of KC instruments were not significantly different from those obtained by users of the manual technique in all six surveys for reagents DK and M, and in five of six surveys for reagent DB (table 3). Results obtained by users of KC instruments were significantly (unpaired t test, p=0.018) lower than those obtained by users of the manual technique for reagent DB in one of six surveys (table 3).

Clotting factor and heparin concentrations in survey samples
A summary of clotting factor and heparin concentrations determined by an anti-Xa method is shown in table 4. Concentrations of factor VIII:C and fibrinogen were more than twofold higher in pooled samples from patients receiving heparin (surveys 82 and 84) compared with normal plasma samples heparinised in vitro (surveys 73–79). The concentrations of other clotting factors were similar in the two groups of samples. Mean heparin concentrations (anti-Xa activity) were similar in the two sample types (table 4).

Discussion
Unfractionated intravenous heparin is widely used for the treatment of venous thromboembolism. Heparin dosage monitoring is usually achieved through an APTT test and with this monitoring system, a number of studies have demonstrated a correlation between inadequate heparinisation and recurrence of thrombosis. As a direct consequence of these observations most laboratories have adopted a therapeutic range of 1:5–2:5. However, APTT reagents vary in their sensitivity to heparin and the underlying rationale for this uniform approach to monitoring of heparin dosage, irrespective of reagent, seems to be misplaced.
NEQAS is an established method for assessing not only individual laboratory performance with respect to a single analyte, but also differences in reagent performance. We therefore analysed UK NEQAS results obtained with different reagents using material prepared by heparinisation of normal plasma in vitro and also by pooled ex vivo samples from patients receiving heparin as the response of APTT methods to the two sample types may differ.  

For both in vitro and ex vivo samples notable differences between APTT reagents with respect to their response to heparin were observed. For example, in one survey (84), in which ex vivo samples were used, the median ratio obtained by users of one reagent was 1.37 compared with 2.36 for users of another. This indicates that a uniform therapeutic range of 1.5–2.5 may not be appropriate. A statistical comparison between the least and most responsive of the APTT reagents in each of the six surveys revealed highly significant differences (p<0.0001). This concurs with results of other studies of APTT reagents based on in vitro and ex vivo samples.  

Reagent sensitivity in ex vivo samples was substantially different from that observed with in vitro samples. Reagent ML—for example, was clearly the most responsive reagent for in vitro samples; DB was generally the least responsive and IL had an intermediate response, with APTT ratios about 50% lower than ML for four survey samples. This is in contrast to results obtained with ex vivo samples, for which IL produced higher APTT ratios than ML. A number of other discrepancies were also present.

When the concentrations of clotting factors were determined in the two sample types, notable differences were observed for factor VII:C and fibrinogen. Concentrations of both were about twofold higher in pooled ex vivo samples compared with normal plasma samples heparinised in vitro. This is not surprising as the patients receiving heparin had acute thromboembolic disease and both of these proteins are acute phase reactants. Both may influence APTTs determined using different reagents. In particular, sensitivity to factor VIII has been reported to vary for different APTT reagents. These differences may have contributed to the observed reagent based difference in APTT ratios.

The effect of different coagulometers on APTT ratios has been described previously. Three reagents (DB, DK, M) were used manually and in combination with the KC series of coagulometers in sufficient numbers to permit meaningful comparison. Results obtained by users of this instrument were similar to those obtained by users of the manual technique, with the exception of reagent DB in one of six surveys. This suggests that this coagulometer does not generally influence results obtained with these three reagents. This, however, partly contrasts with the findings of a previous study in this department using a different coagulometer (ACL) and a study of its effect on APTTs determined with reagents ML and IL in the study by Kitchen and Preston the use of the ACL with reagent IL was not associated with differences between APTT ratios when compared with the manual technique, whereas use of this instrument in conjunction with reagent ML led to a significant increase in APTT ratios (from a mean of 2.3 to a mean of 3.1). Individual fresh samples from patients receiving heparin were used in that study.

Some of the reagents described in the present study (ML, IL, DB, DK, B, and M) were the subject of a study comparing APTT ratios (patient:mean normal APTT) with heparin concentrations in ex vivo samples from patients receiving heparin. In this study the ranking of these six reagents by sensitivity to heparin, based on the mean of 21 individual plasma samples and using the manual technique, was as follows: IL, ML, M, DB, DK, and B. This ranking related to fresh samples (analysed within two hours of collection and with varied order of testing with different reagents) from patients receiving full dose intravenous sodium heparin and with an international normalised ratio less than 1.3. This order is identical with that obtained by NEQAS participants in surveys 78 and 84 using lyophilised pooled ex vivo samples, but is different to the ranking obtained by NEQAS participants for in vitro samples (as follows: ML, DB, M, IL, B, and DK). This suggests that pooled ex vivo samples are more appropriate than the addition of heparin to normal plasma in vitro as the relation between reagents is maintained.

Differences between in vitro and ex vivo samples with respect to the sensitivity of APTT reagents to heparin have been described in both single centre and multicentre studies of in vitro samples. The UK NEQAS data from six surveys, each including around 400 laboratories, indicate notable differences between reagents with respect to their response to heparin and that the use of samples collected from patients receiving heparin is more appropriate than the addition of heparin to normal plasma. The importance of reagent differences is emphasised by the clinical conclusions based on results of one survey (using ex vivo samples), in which 94% of users of one reagent considered the heparin dosage to be inadequate, whereas 90% of users of another reagent regarded it as adequate.

Results obtained by this large series of laboratories clearly indicate that APTT reagents vary noticeably with respect to their response to heparin, suggesting use of a reagent that is fully therapeutic ranges are required, and that samples prepared by the addition of heparin to normal plasma in vitro for this purpose can be misleading and should not be used.

3 Hull RD, Raskob GE, Hirsh J, JY RM, Leclerc JR, Geerts WH, et al. Continuous intravenous heparin compared...
with intermittent subcutaneous heparin in the initial treat-
4 Hull RD, Raskob GE, Rosenbloom D, Panju AA, Brill-
Edwards P, Ginsberg JS, et al. Heparin for 5 days as
compared with 10 days in the initial treatment of proximal
5 Turpie AGG, Robinson M, Doyle DJ, Mulji AS, Misskell
GJ, Sealey BJ, et al. Comparison of high-dose with low-
dose subcutaneous heparin to prevent left ventricular
mural thrombosis in patients with acute transmural an-
terior myocardial infarction. N Engl J Med 1989;320:
352-7.
6 Levine M, Hirsh J, Gent M, Turpie AGG, Cruikshank M,
Weitz J, et al. A randomised trial comparing activated
partial thromboplastin time with heparin assay in patients
with acute venous thrombosis requiring large daily doses
7 Brandt JT, Tripllett DA. Laboratory monitoring of heparin:
Effect of reagents and instruments on the activated partial
8 Shojania AM, Tercreault J, Turnbull G. The variations be-
 tween heparin sensitivity of different lots of activated
partial thromboplastin reagents produced by the same
9 Ray M, Carroll P, Smith I, Hawson G. An attempt to
standardise activated partial thromboplastin time reagents
used to monitor heparin therapy. Blood Coagul Fibrinolysis
10 Kitchen S, Preston FE. A comparison of activated partial
thromboplastin time methods for control of heparin ther-
11 Bain B, Forster T, Steigh B. Heparin and the activated
partial thromboplastin time—A difference between in vitro
and in vivo effects and implications for the therapeutic
12 Scialla SJ. Heparin monitoring by the activated partial
thromboplastin time: Comparison of ex vivo measurement
and in vitro standardisation. Am J Clin Pathol 1985;84:
351-4.
13 Gawoski JM, Arkin CF, Bovill T, Brandt J, Rock WA Jr,
Tripllett DA. The effects of heparin on the APTT of
the College of American Pathologists Survey specimens.
Responsiveness, precision and sample effects. Arch Pathol
14 Kitchen S, Walker ID, Woods TAL, Preston FE. Throm-
boplastin related differences in the determination of in-
ternational normalised ratio: A cause for concern? Thromb
15 Von Clauss A. Gerinnungphysiologische Schnittmethode
zur Bestimmung des Fibrinogens. Acta Haematol 1957;
17:237-46.
16 Donnachie D, Kitchen S, Preston FE. Evaluation of a
clotting anti-Xa assay for heparin [abstract]. Br J Haematol
1994;86:33.
17 Basu D, Gallus A, Hirsh J, Cade J. A prospective study of
the value of monitoring heparin treatment with the activated
partial thromboplastin time. N Engl J Med 1972;287:
324-7.
18 Gallus A, Hirsh J, Gent M. Relevance of pre-operative
and post-operative blood tests in post-operative leg vein
19 Howarth S. Activated partial thromboplastin time reagents:
20 Ciavarella N, Coccheri S, Mannucci PM, Canciani MT,
Mariani G, Mori PG, et al. Activated partial throm-
boplastin time: A multicentre evaluation of 11 reagents in
the screening of mild haemophilia A. Scand J Haematol
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