No influence of heparin plasma and other (pre)analytic variables on D-dimer determination

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SUMMARY

BACKGROUND Sample material for D-dimer, as for most other coagulation tests, is citrate plasma, which is inconvenient when the test is performed on a clinical chemistry analyzer. Other (pre)analytic variables might influence the D-dimer determination, but are not studied extensively. We investigated the effect of heparin plasma and different (pre)analytic variables on the D-dimer concentration.

METHODS Heparin plasma for D-dimer concentration, using the Tina-quant® latex assay, was compared with citrate plasma in 190 patients. The possible influence of pneumatic mail was investigated in heparin plasma from 25 patients: one tube was transported by pneumatic mail and the other was carried by hand. For sample stability analysis, D-dimer concentration in random citrate plasma samples was measured immediately, after 16 and 24 hours and after having been frozen once or stored for two years at –70°C. For analyzer analysis, we compared D-dimer results from a Hitachi 917 and an Integra 700 analyzer in citrate and heparin samples from 190 patients; in 145 other heparin samples, we compared the data obtained from a Hitachi 717 and an Integra 400 analyzer.

RESULTS D-dimer concentration in citrate and heparin plasma showed a perfect correlation (r=1.00). The D-dimer concentrations in heparin were 19% higher than in citrate plasma, which can be attributed to the dilution by the citrate solution. No influences of pneumatic dispatch, time of measurement, freezing or type of analyzer on D-dimer concentrations were found.

CONCLUSIONS Our data show the validity of the determination of D-dimer concentrations using heparin plasma. The D-dimer assay remains valid under different (pre)analytic conditions.
INTRODUCTION

D-dimer, a classical coagulation parameter, is increasingly used in the exclusion of venous thromboembolism and the evaluation of coagulopathies. As with all other coagulation tests, the sample material is citrate plasma. In particular when the D-dimer test is performed on a clinical chemistry analyzer, the use of citrate plasma is a burden. Depending on whether other coagulation tests are requested, either an extra tube for the D-dimer determination or sample splitting is needed. Furthermore, as citrate plasma used for coagulation tests is centrifuged differently from heparin plasma and serum, it disturbs the clinical chemistry workflow. Because D-dimer will be used in an emergence setting, where a short turn-around time (TAT) is mandatory, it would be advantageous if D-dimers could also be determined in heparin plasma.

Although many investigators have studied different D-dimer assays mainly in search of clinical outcomes as sensitivity and negative predictive value (1-3), it is possible that differences in the (pre)analytic processes can lead to different results of the D-dimer assay. The influence of (pre)analytic variables on the D-dimer concentration has not been studied extensively. There are reports on the effect of freezing on D-dimer concentration (4-7), but the effects of transport and type of analyzer have been less studied (8;9).

The first objective of this study is the validation of D-dimer determinations using heparin plasma. The second objective of this study is to investigate whether the results of a D-dimer assay are influenced by different (pre)analytic processes, such as transportation, time of measurement, freezing and the type of analyzer used.
METHODS

D-dimer measurement
We used the Tina-quant® (Roche, Germany) quantitative latex assay for determination of D-dimer concentrations. Samples were collected into S-Monovette® 9NC tubes (0.106 mol/L sodium citrate; Sarstedt, Rommelsdorf, Germany) and S-Monovette® lithium heparin tubes (15 IU/mL lithium-heparin). Citrate samples were centrifuged according to the standard protocol for coagulation samples (3000g for 15 minutes without brake) and lithium-heparin samples were centrifuged according to the protocol for clinical chemistry samples (2200g for 10 minutes with brake). Measurements were performed on a Roche Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and a Cobas Integra 700 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Pre-analytic conditions
Comparison of citrate versus heparin plasma:
Citrate and heparin plasma samples were randomly obtained from 190 patients (139 in Munich and 51 in Nieuwegein). The samples in Munich were analyzed on a Hitachi 917 and the samples in Nieuwegein were analyzed on an Integra 700 analyzer.

Method for transport conditions:
From 25 patients, 2 tubes of heparin plasma were collected. One of the heparin-plasma samples was sent to the laboratory by pneumatic mail (a distance of approximately 100m); the other sample was carried by hand. The samples were centrifuged as clinical chemistry samples. D-dimer concentration was measured on an Integra 700 analyzer. In another experiment, transport was simulated by placing sample tubes on an oscillating roller. One tube citrate plasma and two tubes of lithium-heparin plasma from three healthy donors were obtained. The citrate sample and one of the lithium-heparin samples were centrifuged immediately. The citrate sample was left standing at room temperature. The centrifuged lithium-heparin plasma was split into two tubes; one was left standing at room temperature, the other tube was placed on a roller. The second lithium-heparin tube was not centrifuged but placed directly on the roller (anticoagulated whole
blood) and centrifuged afterwards. D-dimer concentration in all samples was determined using a Hitachi 917 analyzer.

Method for sample stability:
Random clinical citrate (n=15) and heparin (n=17) plasma samples were collected from patients. The D-dimer concentration was measured directly and measured again after the samples had stood at room temperature for 16 and 24 hours and after having been frozen once (snap-frozen in liquid nitrogen). In addition, five citrate plasma samples from a two year old clinical study, which had been stored at \(-70^\circ\text{C}\), were measured. All measurements except for the long term stability study were performed on a Hitachi 917. For the 2 year stability study, the D-dimer measurement was done on an Integra 700.

Analytic conditions

Comparison of analyzers:
The first experiment was to compare a Hitachi 917 and an Integra 700 analyzer by which D-dimer concentration was measured using citrate and heparin plasma of 190 patients.

In a second experiment, from 145 patients fresh heparin samples were taken. D-dimer concentrations were measured on an Integra 400 and a Hitachi 717 analyzer. The standard centrifugation protocol for clinical chemistry samples was used, as described previously.

Statistics
For comparison of D-dimer results, the Pearson and Kendall correlation coefficient and the Passing-Bablok regression were used. The Student t-test was used to determine the influence of simulated transport on the D-dimer concentration and to determine the sample stability of D-dimer concentration.
RESULTS

Pre-analytic conditions

Comparison of citrate versus heparin plasma:
In Fig. 1, D-dimer concentrations in citrate plasma are compared with heparin plasma. We found that the results in heparin were higher than in citrate plasma: mean 2.51 vs 2.06 mg/L. There was a high correlation between the citrate- and heparin plasma samples and between the two analyzers (r=1.0, slope=1.196, Kendall tau=0.959, md(95)=0.18).

Influence of transport:
The effect of transporting heparin samples by pneumatic mail is shown in Fig. 2. We found no difference between the two sample groups (mean 1.57 vs 1.59 mg/L; r=0.999, slope=0.993, Kendall tau=0.967, md(95)=0.09, p=0.4). The results of simulated transport by placing sample tubes on an oscillating roller are given in Table 1; there was no difference in D-dimer concentrations among the samples.

Sample stability:
Results of sample stability are given in Fig. 3 for citrate plasma and Fig. 4 for heparin plasma. No differences were found between the different times of measurement (p=0.4 for 16 h and 0.557 for 24 h in citrate plasma; p=0.3 for 16 h and 0.3 for 24 h in heparin plasma). There was no effect of freezing (p=0.3 for citrate-plasma samples frozen once; p=0.1 for citrate-plasma samples stored frozen for 2 years; p=0.3 for heparin-plasma samples frozen once).
Figure 1. D-dimer concentration in citrate versus heparin plasma on Integra 700 (□) and Hitachi 917(△) analyzers (n=190)

Figure 2. D-dimer concentration in lithium-heparin plasma with and without transport by pneumatic mail (n=25)
Figure 3. Sample stability in citrate plasma (○ after 16 hours (n=11), □ after 24 hours (n=13), △ frozen 1 time (n=9), ★ frozen 2 years (n=5))

Figure 4. Sample stability in heparin plasma (○ after 16 hours (n=13), □ after 24 hours (n=16), △ frozen 1 time (n=10))
Table 1. Sample stability in simulated transport by an oscillating roller (average of 3 donors). Values in lithium-heparin plasma have been corrected for citrate references. Values are reported as mean ± SD. P-values indicate differences compared to citrate plasma.

<table>
<thead>
<tr>
<th>Material and condition</th>
<th>D-dimer (µg FEU/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate plasma(^a)</td>
<td>0.207 ± 0.081</td>
<td></td>
</tr>
<tr>
<td>Lithium-heparin plasma(^b)</td>
<td>0.253 ± 0.045</td>
<td>0.5</td>
</tr>
<tr>
<td>Lithium-heparin plasma roller(^c)</td>
<td>0.217 ± 0.081</td>
<td>0.2</td>
</tr>
<tr>
<td>Lithium-heparin plasma whole blood roller(^d)</td>
<td>0.223 ± 0.076</td>
<td>0.1</td>
</tr>
</tbody>
</table>

\(^a\) centrifuged immediately and left standing at room temperature  
\(^b\) centrifuged immediately and left standing at room temperature  
\(^c\) centrifuged immediately and placed on an oscillating roller  
\(^d\) immediately placed on a roller (anti-coagulated whole blood) and centrifuged afterwards.
Analytic conditions

Comparison of analyzers:

Fig. 1 shows the comparison of D-dimer measurements on a Hitachi 917 and an Integra 700 analyzer. A good correlation was found (r=1.000, slope=1.196, Kendall tau=0.959). Fig. 5 shows the comparison of D-dimer measurements in heparin plasma on a Hitachi 717 and Integra 400 analyzer. No differences were found (r=0.999, slope=1.0165, Kendall tau=0.950).

Figure 5. D-dimer determination in lithium-heparin plasma on Integra 400 and Hitachi 717 analyzers (n=145)
DISCUSSION

Our data confirm and extend the previous report of Vukovich et al (10) on the validity of performing the Tina-quant D-dimer test using heparin plasma rather than the standard measurements in citrate plasma. This finding substantially decreases the TAT of the D-dimer test by reducing pre-analytic processing. We estimate that, in our hospital, this TAT has been reduced from 60 to 30 minutes. Our observation has an important consequence for the use of D-dimer measurements in emergency settings, where it is a prerequisite to perform a reliable test with a short TAT. It also implicates that a D-dimer test performed on a chemical analyzer can compete with the newer point-of-care D-dimer tests: the latter D-dimer tests have less extensively been validated and their advantage from a time-consuming point of view is now being diminished.

We found the D-dimer concentration in heparin plasma to be higher than in citrate plasma, with an average of 19%. This can be explained by the fact that there is no dilution in the heparin plasma, whereas this is the case in the citrate solution. This percentage was identical in both centers that participated in this study. As all reports in literature are based on the standard citrate solution, we therefore multiply the results in the heparin samples by a factor of 0.84 (=1/1.19) to avoid the need for a change in reference and cut-off values. This correction factor is in accordance with the correction factor that has been found by Vukovich et al (10) and corresponds with what one would theoretically expect when 1 part citrate solution and 9 parts blood with a hematocrit of 42% are taken together (11).

The D-dimer concentration in lithium-heparin plasma in our study is unaffected by transport by pneumatic mail or by rough handling, simulated by placing the sample on an oscillating roller. This opens the possibility of pneumatic dispatch of the sample tubes and thus a gain in time at the emergency department. It also indicates that samples obtained by general practitioners or local hospitals with limited capacity can be transported to a central laboratory for D-dimer measurements without decreasing their quality. That means that patients do not necessarily have to go to the emergency room for D-dimer determinations.

We found that the D-dimer concentrations in citrate and heparin plasma were stable regardless of the time of measurement, freezing or the type of analyzer used. This indicates that it is valid to perform studies on frozen
material and that test results of the Tina-quant® assay, obtained under different (pre)analytic conditions, can be compared without loss of reliability. In conclusion, measurement of D-dimer concentrations with the Tina-quant® D-dimer test using heparin plasma is valid and provides a reduction in TAT. The D-dimer assay remains valid under different (pre)analytic conditions.