Letter to the Editors-in-Chief

Automated APTT cycle for the rapid identification of plasma prekallikrein deficiency

Very prolonged activated partial thromboplastin time (APTT) is a challenging issue for coagulation laboratories and warrants further investigation, once preanalytical or analytical causes have been excluded. Abnormal APTT may be due to anticoagulation therapy, primarily heparin, which can be present in the specimen as a contaminant agent, or it may be due to a coagulation factor deficiency, either congenital or acquired (e.g., presence of specific inhibitors directed against single coagulation factors or non-specific inhibitors, such as Lupus Anticoagulants). When haemophilia (A, B or C) and lupus anticoagulants (LAs) have been ruled out, abnormalities in the so-called contact system, which includes plasma prekallikrein, factor XII (FXII) and high molecular weight kininogen (HK), should be investigated. Prekallikrein, also known as Fletcher Factor, is converted to its active form (kallikrein) by FXII that can autoactivate on negatively charged artificial surfaces or in the presence of various biological substances [1–4]. Plasma kallikrein in turn further activates FXII with consequent amplification of the process. Prekallikrein may also be activated by the serine protease prolylcarboxypeptidase [5] and by heat shock protein 90 [6] in a FXII-independent way. Deficiency of prekallikrein, which is not generally associated with bleeding tendency and is inherited as an autosomal recessive trait [7,8], is characterised by severely prolonged APTT that progressively shorten and even normalise when the preincubation time of plasma with the reagent containing a contact activator is extended to at least 10-20 minutes [9]. This behaviour is due to a bypass mechanism that allows that sufficient activated FXII is generated to further activate the coagulation factor XI [10]. Shortening of prolonged APTT with extended incubation time of plasma with a contact activator before recalcification was described in LA samples but, in this case, did not achieve normalisation [11,12]. APTT assays with extended incubation times are not commonly available on automated coagulometers; furthermore, the manual tilt-tube method is not feasible in all laboratories.

We have implemented, in the ACL Elite coagulometer (Instrumentation Laboratory – IL, Milan, Italy), an APTT cycle (Prekallikrein-APTT) that simultaneously performs two APTTs for each sample, with standard (5 min) and extended (20 min) preincubation times. This modified APTT test allows for the rapid and specific identification of prekallikrein deficiency.

Plasma sampling

Blood samples, collected in vacuum tubes containing 1/10 vol of 0.109 M sodium citrate (2.7 mL Becton Dickinson Vacutainer Rutherford, New Jersey, USA) using 21 G needles, were centrifuged at 2000 g for 30 min at room temperature. Plasma was stored at −80 °C until testing.

Samples were obtained from normal subjects (40), patients with Acquired Von Willebrand Disease (1) (AVWD), patients with lupus anticoagulants (36), on oral anticoagulant (51) and patients undergoing heparin therapy (10).

In addition, commercial lyophilised plasmas from patients congenitally deficient in prekallikrein (George King Bio-Medical, Inc., Overland Park, KS, USA), in HK (Technoclone GmbH, Wien, Austria) and in FII, FV, FX, FVIII, FIX, FXI and FXII (IL) were tested.

The APTT reagent used for the study was APTT-SP (IL), which contains synthetic phospholipids and micronised silica as an activator. Plasma prekallikrein activity was determined with the ACL Elite using prekallikrein-deficient plasma as a substrate (Technoclone GmbH, Wien, Austria), APTT-SP as a reagent and an internal reference pool, defined to contain 100% prekallikrein activity.

Prekallikrein-APTT cycle

The Prekallikrein-APTT cycle, implemented in the ACL Elite, has the same characteristics (reagents, volumes, acquisition parameters and raw data elaboration algorithm) as the basic “APTT Spe” test provided by the manufacturer; however, in the Prekallikrein-APTT cycle, each sample is processed twice during the same analysis: plasma is loaded into the rotor and incubated for twenty minutes with the cephalin-activator reagent; meanwhile, the same plasma sample is loaded again with the reagent and incubated for five minutes (Table 1). Calcium chloride is then added. At the end of the Prekallikrein-APTT cycle, two APTT results are obtained: one for the 20-min incubation time and one for the 5-min incubation time.

Statistical analysis

The Wilcoxon test for paired data was used for within-group comparisons of 5-min APTT vs. 20-min APTT. The Kruskal-Wallis and Mann-Whitney U tests were used for between-group comparisons of the 20-min APTT results. Significances of all multiple comparisons were adjusted by Bonferroni. Stata/SE 11.0 (The StataCorp, College Station, Texas, USA) was used for all statistical analyses.

Results

The observed ranges (min-max) for the 5-min and 20-min Prekallikrein-APTT obtained for all samples are reported in Table 2. The Prekallikrein-deficient samples showed a rather wide range of 5-min APTT results with prekallikrein activity from 0.66 to 1.31.

The 20-min APTT results were not significantly different from the 5-min APTT results in the contact phase factor deficiencies (FXII, HK) and in the normal subjects (Table 2, within-group comparisons). In all other cases, the 20-min APTT results were significantly different from the 5-min APTT ones; however, the 20-min incubation led to shortened coagulation times only in the prekallikrein-deficient and LA samples, whereas in all other cases an even further prolongation of APTT was observed.
Prekallikrein-APTT cycle: analysis parameters.

<table>
<thead>
<tr>
<th>Loading step</th>
<th>Timing constraint</th>
<th>Ramp</th>
<th>Centrifugation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-min incubation-sample</td>
<td>Delay at completion 1 sec</td>
<td>Enabled*</td>
<td>60 sec</td>
</tr>
<tr>
<td>Optical reference</td>
<td>Step length 900 sec</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5-min incubation-sample</td>
<td>Set timer 300 sec</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*inter-ramp interval: 3 seconds; sec, seconds.

Table 2

Prekallikrein-APTT: observed ranges (seconds) and statistical analyses.

<table>
<thead>
<tr>
<th>Groups of samples studied</th>
<th>n</th>
<th>Prekallikrein-APTT Observed ranges</th>
<th>Within-group# comparisons</th>
<th>Between-group§ comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-min sec</td>
<td>20-min sec</td>
<td>p-values</td>
</tr>
<tr>
<td>Prekallikrein deficiency*</td>
<td>6</td>
<td>82.2 – 159.0</td>
<td>26.7 – 36.0</td>
<td>0.0312</td>
</tr>
<tr>
<td>FXII, HK (contact phase) factor deficiencies*</td>
<td>7</td>
<td>40.5 – 247.0</td>
<td>38.5 – 196.0</td>
<td>0.813 ns</td>
</tr>
<tr>
<td>Other factor deficiencies*</td>
<td>33</td>
<td>39.2 – 151.0</td>
<td>37.7 – 172.0</td>
<td>0.0159</td>
</tr>
<tr>
<td>(III, IV, FX, FVII, IX, FXI, AVWD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Oral Anticoagulant therapy</td>
<td>51</td>
<td>37.5 – 92.9</td>
<td>37.2 – 112.0</td>
<td>0.0317</td>
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<tr>
<td>Lupus anticoagulants</td>
<td>36</td>
<td>48.7 – 217.0</td>
<td>36.2 – 202.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heparin therapy</td>
<td>10</td>
<td>38.5 – 147.0</td>
<td>43.5 – 247.0</td>
<td>0.020</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>40</td>
<td>23.7 – 34.5</td>
<td>23.5 – 33.0</td>
<td>0.3922 ns</td>
</tr>
</tbody>
</table>

* commercial plasmas included.
# comparison of 5-min APTT with 20-min APTT (Wilcoxon test for paired data; significance at p < 0.05).
§ comparison of 20-min APTT results of normal subjects with all the other groups (Mann-Whitney U test; significance at p < 0.00238).

Only the 20-min APTT values provided by the prekallikrein-deficient samples were not statistically different from those obtained for the normal subjects, whereas values provided by all the other groups of patients maintained a statistical difference (Table 2, between-group comparisons). These data showed that, when incubation of plasma with the activator reagent was extended to 20 minutes before recalcification, the normalisation of the very prolonged APTT was specific for prekallikrein deficiency and it was not observed in FXII and HK deficiencies.

In summary, a simple and completely automated screening cycle (lasting about 30 minutes), with the simultaneous determination of APTT after 5-min and 20-min preincubations of plasma with an activator, was implemented in the automated coagulometer ACL Elite. Normalisation of prolonged APTT with the 20-min preincubation time was observed only in prekallikrein-deficient samples and not in all the other conditions investigated (contact phase or congenital/acquired factor deficiency, LA presence, anticoagulation). This modified APTT cycle implemented in the ACL Elite could represent a rapid, specific and inexpensive screening test for prekallikrein deficiency in the workup of severely prolonged APTT.

Conflict of interest statement

The authors have no conflict of interest to report.

References


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