Thrombin generation test in patients under anticoagulant therapy with vitamin K antagonists

Isabel Brocal; Pascual Marco; Javier Lucas; José Verdú; Fabián Tarín
Thrombosis and Haemostasis Unit, University General Hospital of Alicante, Alicante, Spain

Letters to the Editor

Dear Sir,
The anticoagulant treatment with vitamin K antagonists (VKA) is commonly indicated in patients with atrial fibrillation, heart valves prosthesis, venous thromboembolic disease, antiphospholipid syndrome and occasionally, after a myocardial infarction. In order to control bleeding risks and loss of treatment effectiveness patients are monitored by the international normalised ratio (INR). However, in clinical practice some patients with INR values out of range do not have haemorrhagic symptoms or thrombotic events. Moreover, even patients in INR in the therapeutic range could show bleeding or thrombotic signs with no local causes and/or clinical risk factors (1). Thrombin generation test (TGT) has been proposed as a new functional global test of haemostasis system able to detect hypo- and hypercoagulability states (2). Recent studies have suggested that thrombin generation assays are better than traditional coagulation tests for monitoring anticoagulation (3, 4). To our knowledge, few studies have focused on patients with VKA and TGT (5–8), and only occasionally its correlation with INR has been studied (7, 8).

In our study, 188 consecutive patients under chronic anticoagulant therapy with VKA and a control group of health volunteers with INR < 1.20 (n = 58) were included. To prepare platelet-poor plasma (PPP), citrated blood was double-centrifuged at room temperature at 2,200 g each time for 15 minutes (min). All samples were stored at −80°C until analysis. Continuous thrombin generation was measured using automated coagulation system BCS (Siemens Dade Behring, Marburg, Germany) by a chromogenic method as described by the manufacturer. Conversion of the chromogenic substrate was measured continuously over 20 min at 405 nm. Substrate cleavage by the thrombin-thrombin complex is automatically subtracted from the conversion curve with Curves software (Siemens Dade Behring). Endogenous thrombin potential (ETP) results were expressed in optical mA, as the area under the thrombin formation curve and ETP % was calculated as percentage ETP value of the control group. Standard consists of lyophilized normal human plasma calibrated by the manufacturer. Another BCS parameters are defined as Lag time (the time until thrombin burst, Tlag, s), maximum thrombin velocity of thrombin generation curve (Cmax, mA/min) and time to get it (Tmax, s). INR was measured on the STA coagulometer using a certified commercial tissue factor (Neoplastin plus, Diagnostica Stago, Paris, France). The Mann-Whitney test was used to evaluate the significance of the differences between patients and control data. Multiple comparisons among groups of patients classified according to INR value were performed using the H of Kruskal-Wallis test. Correlation coefficients between tests were analyzed using Spearman’s test. Data are given as median and first and third percentile, and a p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 14.0 software package (SPSS Inc, Chicago, IL, USA).

Gender parity and a predominance of age over 70 years old are observed in the patient group. Atrial fibrillation is the predominant diagnosis regarding to anticoagulation causes with VKA (55.9%) and over 60% of patients analyzed are within 2.00–3.50 range (median INR range).

We found statistically significant differences between control group and patients group in all parameters obtained from TGT (Table 1). Tlag and Tmax were significantly higher in patients than in the control group. Cmax, ETP and %ETP were significantly lower in patients than in the control group. Moreover, in the patient group this difference increased according to INR. The percentage of thrombin generation regarding to control group (ETP %) was 58.0 (53.8, 69.6), 45.1 (40.8, 49.4) and 34.8 (32.3, 38.8) in low (INR 1.20–1.99), medium (INR 2.00–3.50) and high (INR 3.51–6.00) INR ranges, respectively. This results are in accordance with similar studies (7, 8) that show a reduction in parameters related to the quantity of thrombin generated (Cmax and ETP) and a progressive prolongation of parameters related to coagulation times (tlag and tmax) as INR increases. Interestingly, patients with INR 1.20–1.99 already present a reduction of thrombin generation of 58.0 (53.9, 69.6) in respect to control group. These findings could explain why patients with low INR values exhibit few thromboembolic complications.

On the other hand, a significant positive correlation (p < 0.001) between INR value and Tlag or Tmax (r = 0.832 and r = 0.837, respectively), and a significant negative correlation (p < 0.001) between INR and Cmax or ETP (r = −0.807 and r = −0.756, respectively) were observed. A similar study using fluorogenic method showed that the best correlation coefficient was 0.6 (7). We consider that these differences could be due to the different protocol used. Moreover, other authors (8) that compared chromogenic and fluorogenic methods have suggested that this chromogenic thrombin generation assay (Siemens, Dade-Behring) explores only the via of tissue factor.

In conclusion, high correlation coefficients in our results suggest that TGT used in this study reflects an anticoagulation level similar to INR. Recently, studies have demonstrated that TGT by using fluorogenic method detects changes in coagu-
Table 1: Thrombin generation of patients and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>T lag (s)</th>
<th>T max (s)</th>
<th>C max (mA/min)</th>
<th>ETP (mA)</th>
<th>ETP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.1 (17.6, 22.8)</td>
<td>39.8 (36.0, 45.2)</td>
<td>208.9 (195.8, 224.6)</td>
<td>371.9 (347.5, 396.3)</td>
<td>100.0 (93.5, 106.6)</td>
</tr>
<tr>
<td>INR 1.20–1.99</td>
<td>35.0 (27.2, 39.0)*</td>
<td>33.1 (45.8, 57.0)*</td>
<td>138.3 (120.4, 151.4)*</td>
<td>215.5 (200.3, 259.0)*</td>
<td>38.0 (53.8, 69.6)*</td>
</tr>
<tr>
<td>(n=40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR 1.20–1.99</td>
<td>52.1 (47.2, 57.8)* #</td>
<td>64.4 (59.9, 84.0)* #</td>
<td>88.4 (72.5, 104.4)* #</td>
<td>167.6 (151.7, 183.7)* #</td>
<td>45.1 (40.8, 49.4)* #</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR 3.51–6.00</td>
<td>75.1 (65.2, 81.7)*†</td>
<td>111.4 (96.0, 120.0)*†</td>
<td>53.5 (48.4, 69.5)*†</td>
<td>129.3 (120.3, 144.3)*†</td>
<td>34.8 (32.3, 38.8)*</td>
</tr>
<tr>
<td>(n=32)</td>
<td>4.19 (3.85, 4.51)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Median values with first and third quartiles are shown. (*), p <0.01 versus control group. (#), p <0.01 versus INR 1.20–1.99 group. (†), p < 0.01 versus INR = 2.00 – 3.50 group.

References