Thrombin and Plasmin Activity and Platelet Activation in the Development of Venous Thrombosis

By John Owen, David Kvam, Hymie L. Nossel, Karen L. Kaplan, and Peter B.A. Kernoff

This study was designed to determine whether venous thrombosis is associated with a characteristic pattern in the plasma concentrations of fibrinopeptides and platelet alpha granule proteins. Patients undergoing elective cranioectomy for tumor or vascular abnormality were studied prospectively for venous thrombosis with the 125I-fibrinogen uptake test and with daily measurement of the plasma concentrations of FPA (fibrinopeptide A), TIFPB (thrombin-increasable fibrinopeptide B reflecting Bβ 1-42), and βTG (β-thromboglobulin). Eighteen patients developed venous thrombosis and 14 did not. In the 14 patients who did not develop thrombosis, the mean FPA level was highest on the day after surgery and exceeded the TIFPB level so that the ratio of FPA to TIFPB, which is <0.5 in normals, was 1.2. During the next 14 days, both FPA and TIFPB levels were elevated, but the FPA/TIFPB ratio was not significantly different from the preoperative value. βTG and PF4 levels showed no clear pattern of change in the postoperative period. In 10 of the patients who developed thrombosis, the time of onset of thrombosis was documented by a change in the leg scan from negative to positive. In these 10 patients, the mean FPA level rose to equal the TIFPB level, producing an FBP/TIFPB ratio of approximately 1.0 for the 4 days preceding the change of the leg scan from negative to positive (p < 0.001). Changes in the βTG and PF4 levels were not statistically significant. We interpret the FPA and TIFPB levels as a distinct pattern that reflects sustained imbalance between thrombin and plasmin proteolysis of fibrinogen in association with the development of venous thrombosis. The finding of such sustained imbalance between FPA and TIFPB levels suggests that thrombosis is associated with impaired plasmin formation and/or action in addition to excessive fibrin formation. The finding that this pattern was evident several days before the leg scan became positive strongly supports efforts to develop a prognostic blood test. The blood tests used in this study showed adequate sensitivity, but low specificity for the detection of asymptomatic thrombosis. For this reason we suggest that use of these tests be confined to investigating the pathophysiology of thrombosis.

Understanding of the pathophysiology of naturally occurring venous thrombosis is limited, and it has not been possible to select blood tests useful in predicting, diagnosing, and monitoring thrombosis on a logical basis.1 Improved understanding of the biochemistry of hemostasis and the development of tests that reflect specific biochemical reactions now permit more detailed analysis of the pathophysiology of fibrinogen proteolysis and platelet release in patients with thrombosis. These assays include those for fibrinopeptide A (FPA), which reflects fibrin I formation by thrombin proteolysis of fibrinogen,2 Bβ 1-42,3 which reflects fragment X formation by plasmin proteolysis of fibrin I or fibrinogen,3 and beta-thromboglobulin (βTG) and platelet factor 4 (PF4), which reflect platelet alpha granule release.4,5 Based on our previous findings in patients undergoing saline-induced abortion, the ratio of FPA/TIFPB was used as an index of relative thrombin to plasmin action on fibrinogen.

The study reported here was designed to determine whether development of venous thrombosis is associated with a characteristic pattern in the plasma concentrations of fibrinopeptides and platelet alpha granule proteins. In order to correlate the temporal relationships of the blood changes and the occurrence of venous thrombosis, the time of onset of thrombosis was defined with the 125I-fibrinogen uptake test, which was used as the reference test for thrombus detection.6

MATERIALS AND METHODS

Patients

The subjects for this study were adults scheduled for craniotomy, all of whom had given written informed consent to participate. Information was analyzed only if blood sampling and fibrinogen scanning were carried out for 5 days or more.

Detection of Venous Thrombosis

The 125I-fibrinogen uptake test was used to indicate the presence or absence of venous thrombosis.9 On admission to the study, patients received 100 μCi of 125I-fibrinogen (Sensor, Abbott, Chicago, Ill.) intravenously. Thyroid uptake of 125I was blocked by the administration of either 100 mg sodium iodide intravenously 1 hr before, or 150 mg potassium iodide by mouth twice daily for 2 days.
analyses presented. Venography performed according to the tech-
nique of Rabinov and Paulin was used to confirm the presence and
locations of thrombus in patients with a positive 1271-fibrinogen test.

Perfusion lung scans using 99Tc-albumin were performed before and
7-10 days after surgery.

Normal perfusion lung scans were accepted as evidence that
embolization had not occurred. Abnormal perfusion scans, showing
one or more well defined lobar or segmental defects in regions
without corresponding abnormalities on chest radiograph were fur-
ther investigated either by ventilation scanning or by pulmonary
angiography. Multiple lobar or segmental defects on perfusion
scanning in areas with no defect on the ventilation scan were
considered to indicate a high probability of pulmonary embolism.

Blood Collection and Processing

Blood samples were obtained from an antecubital vein using a
21-gauge butterfly needle. For measuring FPA, PF4, and tTG
levels, 9 ml of blood was collected into a 10-ml siliconized vacutain-
er, which had been previously injected with 1 ml of anticoagulant
solution containing heparin (1400 U/ml), Trasylol (1000 U/ml),
theophylline (20 mM), and adenosine (10 mM). For measuring
TIFPB levels, 4.5 ml of blood taken into a syringe was immediately
added to 0.5 ml of the same anticoagulant in a polystyrene tube.
Both tubes were immediately placed in melting ice and were
centrifuged for 10 min at 1700 g within 30 min of collection. For
measuring PF4 and tTG, plasma from the 1700 g centrifugation was
centrifuged to dryness in a Brinkmann concentrator to remove the alcohol.
The residue was dissolved in one-half volume of distilled water and
stored at -80°C until assayed. All centrifugation was at 4°C.

Assays

The FPA assay, TIFPB assay, PF4 and tTG assays were
performed as previously described with the following modifications.

FPA and TIFPB. Bound and free radioactive tracer were sepa-
rated with 12.5 g/liter charcoal (Fisher Scientific, Springfield, N.J.)
suspension with incubation on melting ice for 15 min. Separation
was more reproducible with this modification.

PF4. Sodium sulfate at 18.5% (w/v) rather than 20% was used
to separate bound and free radioactivity to obviate poor packing on
centrifugation in plasma with high levels of lipoprotein. Plasma
proteins at the dilution used (1/6) produce coprecipitation of
unbound tracer by sodium sulfate. This artifact was controlled by
diluting standards and controls in buffer containing 15% v/v horse
serum (Grand Island Biological Co., Grand Island, N.Y.).

Analysis of Data

The distribution of values of FPA, TIFPB, PF4, and tTG, and the
molar ratio of FPA/TIFPB were positively skewed and were ade-
quately described by log-gaussian distributions. For this reason,
geométric means were used to describe the findings in Figs. 1 and 2.
Statistical analyses were performed using appropriate tests as
described by Sokal and Rohlf. Analysis of variance was used for
the plasma levels of fibrinopeptides and platelet proteins. In these
analyses, values of p greater than 0.01 were considered not
significant.

RESULTS

General clinical characteristics of patients studied

Forty-seven patients were entered into the study and
sufficient data for analysis were obtained on 32. Eight-
teen patients had positive 1271-fibrinogen leg scans, and
14 patients had consistently negative leg scans. Clin-
ical data on the 32 patients who were included in the
analysis are shown in Table 1. The sex, age, and
diagnosis of these patients are shown in Table 2. There
were no significant differences between the two groups
with regard to sex distribution or age, though patients
with thrombosis tended to be older. There was a
significant difference (p = 0.014) in the frequencies of
the diagnosis of tumor versus vascular anomalies, with
tumor more common in the patients with thrombosis.

Of the 14 patients with vascular anomalies, 5 received
eACA during the period before surgery and 4 of these
had positive 1271-fibrinogen leg scans, 3 before and 1
after surgery.

Results of Tests for Thromboembolism

The results of tests for thrombosis in the 18 patients with
positive 1271-fibrinogen tests are summarized in
Table 1. Six patients had a positive leg scan before
surgery, 8 patients developed a positive scan from 1 to
30 days after surgery, and in 4 patients the first leg
scan was performed after surgery and was positive. In
Table 1. Clinical Data and Evidence for the Presence or Absence of Thrombosis

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Perfusion</th>
<th>Lung Scan</th>
<th>Venography</th>
</tr>
</thead>
<tbody>
<tr>
<td>No thrombosis (125I-fibrinogen leg scan negative throughout)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>18</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>27</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>64</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>22</td>
<td>Vascular malformation</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>57</td>
<td>Meningioma</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>46</td>
<td>Glioblastoma</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>74</td>
<td>Meningioma</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>47</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>58</td>
<td>Cranial nerve compression</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>53</td>
<td>Aneurysm</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>37</td>
<td>F</td>
<td>27</td>
<td>Vascular malformation</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>38</td>
<td>M</td>
<td>58</td>
<td>Vascular malformation</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>29</td>
<td>Meningioma</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thrombosis (125I-fibrinogen leg scan positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) Preoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>51</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>60</td>
<td>Meningioma</td>
<td>Pos (VQ)</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>34</td>
<td>F</td>
<td>62</td>
<td>Aneurysm</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>36</td>
<td>F</td>
<td>49</td>
<td>Aneurysm</td>
<td>ND</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>43</td>
<td>F</td>
<td>52</td>
<td>Meningioma</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>70</td>
<td>Meningioma</td>
<td>ND</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>(B) Postoperative</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>71</td>
<td>Glioblastoma</td>
<td>Pos</td>
<td>Pos</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>59</td>
<td>Metastatic prostatic carcinoma</td>
<td>Pos</td>
<td>Pos</td>
<td>13</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>55</td>
<td>Glioblastoma</td>
<td>Pos</td>
<td>Pos</td>
<td>30</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>44</td>
<td>Glioblastoma</td>
<td>Pos</td>
<td>Inadequate</td>
<td>4</td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>69</td>
<td>Meningioma</td>
<td>Pos</td>
<td>ND</td>
<td>15</td>
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<tr>
<td>32</td>
<td>F</td>
<td>76</td>
<td>Meningioma</td>
<td>Pos</td>
<td>Pos</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>55</td>
<td>Vascular malformation</td>
<td>Pos (VQ)</td>
<td>Pos</td>
<td>6</td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>37</td>
<td>Aneurysm</td>
<td>ND</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>(C) Indeterminate onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>54</td>
<td>Colloid cyst</td>
<td>Pos</td>
<td>Pos</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>54</td>
<td>Glioblastoma</td>
<td>Pos</td>
<td>Pos</td>
<td>6</td>
</tr>
<tr>
<td>39</td>
<td>M</td>
<td>45</td>
<td>Meningioma</td>
<td>ND</td>
<td>Inadequate</td>
<td>3</td>
</tr>
<tr>
<td>42</td>
<td>M</td>
<td>63</td>
<td>Glioblastoma</td>
<td>ND</td>
<td>Pos</td>
<td>10</td>
</tr>
</tbody>
</table>

13 of these 18 patients the presence of venous thrombosis was confirmed by venography, in 3 patients venograms were not done, and in 2 others the venograms were inadequate for interpretation. Lung scans performed on 6 patients with negative leg scans were normal in all cases. Lung scans were abnormal in 10 of 12 patients with a positive leg scan. In 4 of the 10 patients the diagnosis of pulmonary embolism was confirmed—in 2 by simultaneous ventilation perfusion scans, in 1 by angiography, and in 1 at autopsy.

Results of Blood Tests

Changes in relation to surgery. Data from the 14 patients who did not develop thrombosis are plotted in relation to the day of operation (Fig. 1). Analysis of variance demonstrated highly significant ($p < 0.001$)

Table 2. Summary of Patients With Positive and Negative 125I-Fibrinogen Leg Scans

<table>
<thead>
<tr>
<th>125I-Fibrinogen Uptake Test</th>
<th>n</th>
<th>Sex M/F</th>
<th>Age (yr) Median</th>
<th>Range</th>
<th>Tumor Benign</th>
<th>Malignant</th>
<th>Vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>18</td>
<td>12/6</td>
<td>55</td>
<td>37–76</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>8/6</td>
<td>47</td>
<td>18–74</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>
BLOOD CHANGES IN VENOUS THROMBOSIS

Changes associated with surgery for FPA, TIFPB, and the FPA/TIFPB ratio. On the day after surgery, the mean FPA level was highest and exceeded the mean TIFPB level, which had little changed from the mean level found in preoperative samples. On that day, the ratio of FPA/TIFPB was 1.2. On the second day after surgery the FPA level was slightly lower and the TIFPB higher, decreasing the mean FPA/TIFPB ratio to less than 0.5. Over the next 13 days, the mean FPA/TIFPB ratio remained close to the preoperative value of 0.5, though both FPA and TIFPB concentrations were increased over their preoperative levels. PF4 and βTG levels showed no clear pattern of change in the postoperative period.

Changes in relation to thrombosis. The data for 10 patients, 8 from group B and 2 (34 and 36) from group A, in whom the time of onset of venous thrombosis could be documented are shown in Fig. 2. The data were arranged so that the day of the first positive 125I-fibrinogen leg scan for each patient coincided. Analysis of variance demonstrated highly significant \((p < 0.001)\) changes in the FPA/TIFPB ratio in association with thrombosis. Changes in FPA and TIFPB levels were apparent, but these did not reach statistical significance. No significant changes were found in the levels of PF4 and βTG. On days 7–5 before thrombosis, mean values of FPA, TIFPB, and FPA/TIFPB were not significantly different from
values seen on days 2–14 after surgery, in the absence of thrombosis. During the 4 days immediately preceding thrombosis, the FPA level was further increased beyond the postsurgical value, without equivalent increases in the TIFPB. During this period, the FPA/TIFPB ratio was approximately 1.0. On the day that thrombosis was detected, the FPA levels were lower and TIFPB levels higher, so that the ratio of FPA/TIFPB fell to the baseline value of 0.4 and remained near that value for the next 7 days.

Changes with extension of thrombosis. Patient 30 (Fig. 3) had evidence of thrombosis in the first scan performed 1 day after surgery. On days 10–15 he showed major extension of thrombosis with ultimate involvement on the whole limb. On days 6 and 7, he had markedly elevated levels of βTG but not PF4. The FPA/TIFPB ratio was elevated on days 2 and 3 and on days 11–15. Except for the fifth postoperative day, the patient had elevated levels of FPA for 2 wk after surgery.

Discriminant Analysis

The data were examined for ability to discriminate between patients who developed thrombosis and those who did not. Using single variables for classifying, we found greatest discrimination by the FPA/TIFPB ratio. Using a critical level of 1.6 maximized the number of patients correctly classified at 74% (23/31). These data are shown in Table 3. Multiple discriminant analysis was not performed.

DISCUSSION

The specificity and sensitivity of the 123I-fibrinogen uptake test for leg vein thrombosis has been amply documented. Although its validity has not been proven in the clinical setting, the timing of the onset of venous thrombosis by the time of conversion of the leg scan is the most sensitive and specific method currently available. The frequency of thrombosis in this study was high, but consistent with other reports of post-neurosurgical thrombosis. An unexpectedly high frequency of asymptomatic thrombosis preceding surgery has been reported previously, and in this study was found in 6 patients. Three of these patients had cerebral artery aneurysms, and all 3 had received the fibrinolytic inhibitor EACA in an attempt to minimize rebleeding. Overall, 4 of 5 patients with aneurysms who received EACA had venous thrombosis, compared to 1 of 7 patients with aneurysms who did not receive EACA. Since administration of EACA might have produced changes in thrombin/plasmin balance independent of the thrombotic event, the data were reanalyzed after eliminating patients who received EACA. Analysis of variance still showed significant changes in the blood levels of fibrinopeptides, both with surgery and thrombosis. However, EACA administration was not identified as an independent variable in the design of this study, so the data from the patients who received EACA are included in our analyses of the blood changes.
BLOOD CHANGES IN VENOUS THROMBOSIS

Table 3. Classification of Patients by Plasma FPA/TIFPB Ratio

<table>
<thead>
<tr>
<th>FPA/TIFPB Ratio</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1.6</td>
<td>14</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>&lt;=1.6</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>14</td>
<td>31</td>
</tr>
</tbody>
</table>

Classification of patients described in Table 1 according to the occurrence of at least one FPA/TIFPB ratio greater than or equal to 1.6 (one patient with thrombosis has been omitted because no blood tests were obtained before the positive leg scan was observed). Data for patients with thrombosis are for the time preceding the finding of a positive leg scan.

The clinical model used for these studies is complex, since the hematologic findings may be influenced by both the disease process and by the operation. Another problem is inability of the leg scan to detect thrombosis occurring elsewhere. This, however, would lead to false negative diagnosis in the no thrombosis group, potentially obscuring significant differences between this group and the group with thrombosis.

Patients who did not develop thrombosis showed major reproducible changes in FPA and TIFPB levels and in the FPA/TIFPB ratio on the day after surgery. For this reason, data collected on the day after surgery from patients who developed thrombosis were not used in constructing Fig. 2. Two patients received heparin after the finding of a positive leg scan. Since heparin causes a prompt fall in FPA levels, the results of tests taken during anticoagulant therapy were not used in constructing Fig. 2.

The platelet protein levels did not show statistically significant changes either in relationship to surgery or to thrombosis. However, this does not rule out a role for platelets in the development of thrombosis. Urinary βTG levels have been reported to be elevated for several days preceding leg scan conversions.

In normal individuals, the plasma level of TIFPB is higher than FPA and the ratio of FPA/TIFPB is <0.5. The increased FPA without significant change in TIFPB (FPA/TIFPB >1.0) seen 1 day postsurgery indicates perturbed balance between thrombin and plasmin action on fibrinogen. By the second postoperative day, plasmin activity had increased, as manifest by the increased TIFPB level, and normal balance had been restored. These data indicate that plasmin acts after fibrin formation, consistent with data showing that fibrin accelerates the conversion of plasminogen to plasmin by tissue plasminogen activator. Over the next 12 days the FPB/TIFPB ratio did not differ significantly from the presurgical value.

In patients with thrombosis, the increased FPA levels found before the leg scan became positive were not associated with increased TIFPB levels. Because TIFPB levels did not rise promptly after FPA levels became elevated, there was a period of 4 days preceding the occurrence of a positive leg scan during which the FPA/TIFPB ratio was increased to approximately 1.0. This change in the FPA/TIFPB ratio implies a sustained imbalance between thrombin and plasmin action on fibrinogen. Such imbalance would favor fibrin II formation, which has been postulated to be necessary for thrombus formation. The elevated FPA levels, due to excess thrombin action, result from failure of regulation of thrombin formation. Preceding thrombosis it is not clear whether thrombin activity is localized or generalized, though results of previous studies suggest that in established venous thrombosis, thrombin activity is not generalized.

We have previously reported that FPA levels increased and exceeded TIFPB levels after intraamniotic injection of hypertonic saline. FPA levels peaked 1–2 hr after saline infusion. This was followed by a rapid increase in TIFPB levels, with peak TIFPB levels occurring 2 hr after the FPA levels had reached their highest point. Thus, we think that the pattern of plasmin activity following thrombin activity represents a common response to injury. The time course of the changes that occur after surgery and with thrombosis is, however, quite different from that occurring after intraamniotic injection of hypertonic saline.

The failure of the TIFPB level to rise after the rise in FPA level reflects local failure of plasmin action. This could be due to the presence of an inhibitor of plasmin action or due to failure to generate plasmin. One possible explanation is failure of plasminogen activator production by endothelial cells. If this is the case, then the regulation of plasminogen activator synthesis and release by endothelial cells becomes important in the pathogenesis of thrombosis after surgery.

The fall in the FPA/TIFPB ratio that occurred at the time of thrombus detection is striking and is due mainly to a fall in the FPA level. This contrasts sharply with previously reported data that patients with symptomatic venous thrombosis have elevated FPA levels. This difference, we believe, is due to an important difference in patient populations. In this study, almost all episodes of thrombosis were asymptomatic and the majority were localized and self-limited.

We interpret the fall in FPA as evidence that control has been reestablished, thrombin action turned off, and the thrombotic process contained. Extension of thrombosis occurs and often produces symptoms when there is failure to reestablish control and thrombin action continues. Support for this interpretation can be found in the data shown in Fig. 3. This patient had major extension of thrombosis while showing persis-
tently elevated FPA levels. The mechanism by which control is exerted is not known, but we postulate that one rapid acting component is binding of thrombin by polymerizing fibrin, resulting in a decreased concentration of free thrombin with consequent decline in the FPA level.

The finding of changes in the blood that precede the occurrence of a positive leg scan gives support to efforts directed toward finding a predictive or diagnostic blood test. Although this study was not designed to answer the question of clinical usefulness of these fibrinopeptide assays, the data were examined for discriminant ability. Sensitivity was found to be adequate but specificity for thrombosis was lacking. Thus, we suggest that new tests involving either response to a stimulus or reflecting alteration in another part of the hemostatic system will be required to obtain specificity. We further suggest that the current use of these fibrinopeptide assays be limited to research studies concerning the pathophysiology of thrombotic disease.

ACKNOWLEDGMENT

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REFERENCES

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