Plasma and Urine Beta-Thromboglobulin Concentration in Patients With Deep Vein Thrombosis

By A. C. de Boer, P. Han, A. G. G. Turpie, R. Butt, A. Zielinsky, and E. Genton

Plasma and urine beta-thromboglobulin (BTG) were measured in 52 patients with established deep vein thrombosis (DVT) and in 100 patients with clinically suspected DVT but with a negative venogram. Both plasma BTG (geometric mean 54; 95% range 12–239 ng/ml) and urine BTG (0.25; 0.03–3.1 ng/ml) were significantly elevated (p < 0.005) in patients with DVT compared to asymptomatic patients with a negative venogram (plasma BTG 32, 9–112 ng/ml; urine BTG 0.12, 0.02–0.58 ng/ml). Sensitivity (35%) and specificity (80%) of the plasma BTG assay for the diagnosis of DVT were low. The urine BTG assay had a sensitivity of 37% but a specificity of 100%. There was a significant correlation between plasma and urine BTG (r = 0.68, p < 0.005). Serial BTG measurements were made in

The role of platelets in the pathogenesis and progression of venous thrombosis remains controversial.1 Until recently, available platelet tests were inadequate to conclusively determine the participation of platelets in venous thrombosis. In clarifying the role of platelets in venous thrombosis, in addition to providing fundamental information on the process of thrombogenesis, it might be possible to identify tests that would detect thrombosis in the early phase and might be of value in monitoring subsequent treatment. When platelets are activated, they release the contents of their granules, some of which contain platelet-specific proteins, which may now be detected and quantified in plasma or urine by sensitive or specific radioimmunoassays recently developed.2 4 One of the most promising of these is beta-thromboglobulin (BTG), a protein located in the alpha granules of platelets that is released into plasma when platelets are activated and is partially excreted in urine. Thus, increased concentrations of beta-thromboglobulin in plasma or urine would indicate in vivo platelet activation and release.3 A number of studies have reported that BTG is elevated in plasma in a variety of thromboembolic disorders including venous thrombosis.5–9

This investigation was carried out to evaluate BTG concentration in plasma or urine in high-risk patients to define the contribution of platelet activation and release in the pathogenesis of venous thrombosis and in symptomatic patients to determine the value of BTG in the diagnosis of deep vein thrombosis. To do this, BTG was measured in plasma and urine in patients who had been screened during high-risk periods with fibrinogen leg scanning and impedance plethysmography and in symptomatic patients referred for venography.

Because the techniques used to detect or diagnose deep vein thrombosis might affect the BTG levels, the effect of the investigations on the measurement of BTG and in vivo platelet release was documented.

Materials and Methods

Assay of BTG

Plasma BTG was measured by radioimmunoassay with a sensitivity of 3 ng/ml as described by Han et al.10 Blood (2.7 ml) for the assay of BTG was collected with minimal stasis, using 21-gauge needles, into plastic tubes containing 0.3 ml anticoagulant mixture (0.1 disodium ethylene diamine tetra-acetate (British Drug Houses, Toronto, Canada) 0.21 M, 0.1 ml theophylline (Sigma Chemical Co., St. Louis, Mo.) 30 mM, 0.1 ml prostaglandin E1 (Sigma Chemical Co.) 10 μM). Platelet-free plasma (PFP) was prepared by centrifugation at 2000 g for 60 min at 4°C. The middle third of the PFP was collected and stored at –70°C until the assay was performed.

Normal value of plasma BTG in 80 healthy volunteers (mean age 28 ± 11 SD yr) was 27; 12–54 ng/ml (geometric mean with 95% range) and results above 54 ng/ml were subsequently considered abnormal. In 93 hospital control patients (mean age 47 ± 8 yr) admitted for nonthrombotic diseases and with a negative fibrinogen leg scan to exclude silent venous thrombosis, mean plasma BTG was 27; 9–82 ng/ml.

Urine spot specimens were spun at 2000 g for 10 min within 1 hr after collection, and the supernatant was subsequently stored at –70°C for future batch analysis. Assay of BTG in urine was performed as previously described.1 The sensitivity of the assay was 0.04 ng/ml, the intraassay variation 9.7%, and the interassay variation 11.4%.

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patients with renal insufficiency and for this reason, patients with an elevated serum creatinine were excluded from the study.

**BTG Values in Patients at High Risk of Developing DVT**

Forty neurosurgical patients who were screened with IPG and leg scanning for the detection of DVT had daily early morning urine samples collected for assay of BTG, beginning at the first postoperative day. In 20 of these patients, serial plasma samples were also studied. Again, only patients with a normal serum creatinine were studied. In addition, all patients were in stable condition throughout the study period in regard of their cardiovascular and renal systems.

Ten patients developed DVT as detected by 125I-fibrinogen leg scanning and subsequently proven by venography. In 30 patients, the screening tests remained normal.

**Statistical Analysis**

The means of the various diagnostic groups were compared using Student's t test for unpaired data. Because of the non-normal distribution of the data, all results were transferred to the natural log before statistical analysis was performed. All results are expressed as geometric mean with 95% probability range.

**RESULTS**

**BTG and Established Venous Thrombosis**

Of the 52 patients with DVT, 20 had thrombosis limited to the calf veins (CVT), and 32 showed

<p>| Table 1. Effect of 125I-Fibrinogen Added to Plasma In Vitro on Plasma BTG Measurement |
|---------------------------------|-----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Radioactivity Added (cpm)</th>
<th>NSB†</th>
<th>ΔNSB †</th>
<th>BTG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>392</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>766</td>
<td>592</td>
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<td>22</td>
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<td>782</td>
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<td>19</td>
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<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>5,126</td>
<td>1,202</td>
<td>810</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>6,874</td>
<td>1,396</td>
<td>1,004</td>
<td>14</td>
</tr>
</tbody>
</table>

*Increasing concentration of 125I-fibrinogen added to the same plasma sample.
†Total nonspecific radioactive binding, including that due to 125I-fibrinogen, present in plasma.
‡Nonspecific radioactive binding due to 125I-fibrinogen (total NSB – NSB of blank tube: 392 cpm).

Normal urine BTG concentration in healthy volunteers (n = 97) was 0.09; 0.01–0.56 ng/ml or 0.09; 0.01–0.60 ng BTG/mg creatinine. Urine BTG levels above 0.56 ng/ml were considered abnormal. In hospital controls (n = 75) mean urine BTG concentration was 0.24; 0.02–0.95 ng/ml. Macroscopic, hematuria was observed to significantly elevate urine BTG concentration, but the presence of a urinary catheter or urinary tract infection did not affect the results.

**Effect of Diagnostic Procedures on BTG**

The effect of bilateral ascending venography on BTG release was studied by measuring plasma BTG in 10 patients before and 1 and 24 hr after venography. No changes in BTG were observed, either 1 or 24 hr after venography. 125I-fibrinogen, used in leg scanning and circulating in plasma, might coprecipitate with the bound radioactive ligand in the plasma BTG assay. The effect of radiolabeled fibrinogen on BTG measurement was studied by adding 125I-fibrinogen in vitro to assay tubes (Table 1). Because of nonspecific coprecipitation of radiolabeled fibrinogen with the bound moiety of the assay, the measured concentration of BTG was reduced. For this reason, a correction factor was applied to plasma samples from patients scanned with 125I-fibrinogen by incubating labeled BTG and the plasma sample in the absence of antibody. The value obtained was corrected for the nonspecific binding inherent in the assay, and this final count was subtracted from the counts of the samples.

**BTG in Patients With Established DVT**

Plasma BTG was measured in 52 patients with venographically confirmed deep vein thrombosis. Of these patients, 30 had urine samples collected for BTG determination. All blood and urine samples were collected on the day of presentation, before anticoagulant therapy was started. These patients were part of a group of 152 patients with clinically suspected DVT and were not immediately treated with anticoagulants, but were screened daily with IPG and 125I-fibrinogen leg scanning. A venogram was performed after 3 days or before that if the IPG became positive or the leg scanning showed uptake of radiolabeled fibrinogen in the thigh, and anticoagulant therapy was started if indicated.

Blood samples for serial determination of plasma BTG were taken for 3 days in 40 of these patients, 9 of whom had DVT and 31 no DVT on subsequent venography. Serial urine samples were collected from 2 patients with venous thrombosis and from 9 patients without DVT.

It has been observed that plasma and urine BTG are elevated in
involvement of the popliteal, femoral, or iliac veins (PVT).

Mean plasma BTG concentration of the initial sample in patients with CVT was 48, 9–250 ng/ml, and in patients with PVT 57, 13–249 ng/ml. This difference between the two groups is not significant, however, it was significantly elevated ($p < 0.005$) compared to the patients with a negative venogram (32; 9–112 ng/ml) as well as compared to the hospital control patients and normal controls (Fig. 1).

Mean urine BTC concentration was 0.25, 0.03–3.1 ng/ml, in 30 patients with DVT and there was no difference between the patients with proximal or calf vein thrombosis. Urine BTG concentration in 42 patients with a negative venogram was 0.12, 0.02–0.58 ng/ml, which is significantly different from values in patients with thrombosis ($p < 0.005$) (Fig. 2). When the results were expressed as ng BTG/mg creatinine, the same difference between patients with DVT (0.31, 0.03–3.7 ng/ml) and without DVT (0.14, 0.03–0.76 ng/ml) was observed, and for this reason, the data are presented as ng/ml.

Plasma and urine BTG concentration of samples collected at the same time from the group of symptomatic patients were compared and a significant correlation between plasma and urine BTG levels ($r = 0.68, p < 0.005$) was found.

There was no relation between BTG values in plasma or urine and duration of symptoms, but in the patients with established DVT, those with a positive leg scan ($n = 22$) had significantly higher BTG levels at first presentation (63, 11–365 ng/ml) compared with 9 patients with a positive venogram but a negative fibrinogen leg scan (32, 14–72 ng/ml, $p < 0.001$).

Plasma BTG was measured serially in 9 patients with venous thrombosis and in 31 patients with symptoms of venous thrombosis but a normal venogram. Mean plasma BTG of the 3-day period in the thrombosis group (48, 14–159 ng/ml) was significantly elevated ($p < 0.025$) compared to the mean BTG of the group of 31 patients with a negative venogram (34, 13–91 ng/ml). About one-third of the patients both with and without DVT showed wide fluctuations in plasma BTG during the study period. Compared to the initial sample, both in the DVT and control group, initially elevated BTG levels became normal, or normal BTG levels became abnormal, during follow-up. No consistent pattern could be observed in the two patient groups.

Urine aliquots were collected serially from 2 patients with DVT. In both there was an elevated urine level on at least 1 day. Serial urine aliquots were also collected from 9 symptomatic patients with a negative venogram. No urine concentrations above 0.54 ng/ml were seen in this group of patients.

Changes in BTG in Patients at High-Risk of Developing DVT

In 30 patients with negative screening tests, urine BTG was elevated postoperatively but returned to normal within 2 or 3 days (Fig. 3). A similar pattern was noted in plasma BTG levels.

One patient who underwent a craniotomy for a large intracerebral hemorrhage and with an intracere-$
bral drain for 6 postoperative days had elevated urine BTG levels for a longer period of time.

In 10 patients the fibrinogen leg scan became positive and DVT was confirmed by venography in all 10. The same changes in urine BTG as described above were observed in the immediate postoperative period. Urine BTG did not remain normal, however, but started to rise again and significantly elevated levels (above 1.0 ng/ml) were observed in association with development of DVT in all 10 patients (Fig. 4). In 9 of the 10 patients, urine BTG rose before the fibrinogen leg scan became positive. In 4 patients, the rise began 24 hr before the leg scan became positive, but in others, there was an interval of several days. In one patient, urine BTG never returned to normal after surgery, and remained abnormal until the leg scan became positive. Six patients were studied for a longer period of time after venography was performed; in 4 of them, urine BTG returned to normal again in 4–6 days. This return to normal was not related to heparin therapy, as in one patient urine BTG was already normal again before heparin therapy was started and another patient was treated with a "Greenfield" vena cava filter only and his urine BTG concentration dropped to normal also.

Plasma BTG was measured serially in six patients and was elevated when the leg scans became positive. The results from one patient are shown in Fig. 5.

**Plasma and Urine BTG as a Diagnostic Test**

The potential value of plasma and urine BTG as a test for the diagnosis of deep vein thrombosis in patients with symptoms was assessed. Plasma BTG measured at initial presentation does not seem to be of significant value, as both the sensitivity (35%) and the specificity (80%) were low (Table 2). Using the mean BTG of 3 consecutive days instead of only the sample collected at first presentation did not improve the accuracy of the assay, as the sensitivity was only 44% and the specificity 74%.

Urine BTG measured at first presentation had a sensitivity of 37% for the diagnosis of venous thrombosis, but a specificity of 100%, as no elevated urine levels were seen in patients without DVT (Table 3).

**DISCUSSION**

These data confirm reports that increased levels of beta-thromboglobulin in plasma or urine occur in patients with acute venous thrombosis, suggesting that platelets are activated and release the contents of their granules in the process of thrombus formation. The
pattern of BTG release suggested that platelet activation occurred predominantly, if not exclusively, in the early phases of thrombus formation. Similar observations of the time course of release to thrombus formation were reported by Smith et al.18 and are compatible with the observation by Paterson14 that platelets were found mainly at the origin of venous thrombi. Further evidence that platelet activation occurs early in the formation of venous thrombi comes from the reports of Riba15 and Grossman16 that In-labeled platelets accumulate in recently formed thrombi but not in older thrombi.

The reason that platelet activation in venous thrombosis appeared to stop after a few days is not clear. Our data show that it is not related to treatment with heparin. One possible explanation is that platelet activation is stimulated by fibrin monomers but not by polymerized fibrin,17 and is in keeping with our observation that BTG levels were higher in patients with active fibrin deposition as demonstrated by fibrinogen leg scanning.

Because platelet activation in venous thrombosis was related to the time of thrombus formation, the BTG assay was of little diagnostic value, the reason being that patients may have had thrombi for some days before symptoms developed and because BTG returned to normal within a few days of active thrombus formation in high-risk patients undergoing surveillance. Furthermore, BTG was not specific because elevated levels of BTG have been reported in a number of disorders associated with thromboembolism such as coronary artery disease,18 cerebrovascular disease,19 diabetes mellitus,20,21 hypertension,13 or renal insufficiency.21

The data suggest that BTG might be of value for screening patients at risk of DVT as was suggested by Bolton et al.9 In this regard, the assay of BTG in urine rather than plasma would appear to be preferable because in vitro release with falsely high levels due to collection and preparation of specimens does not occur. However, there are disadvantages to the urine BTG assay. The patients must be in a stable condition from a cardiovascular and renal point of view, as was the case in our selected group of patients. In addition, it takes 48 hr to complete a urine assay, which would limit its use as a diagnostic test in acute deep vein thrombosis.

While assay of BTG in plasma or urine is of limited value in the management of patients with suspected venous thrombosis, these data indicate that platelets are activated in the early phase of thrombus development, but whether or not platelet activation is causally related or secondary to thrombus formation remains to be established.

REFERENCES


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