

Short report

## Beta-thromboglobulin in cerebral infarction

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**SUMMARY** Plasma beta-thromboglobulin (BTG) was significantly elevated in the acute phase of 116 atherosclerotic thrombotic ( $p < 0.0001$ ) and 36 cardioembolic ( $p < 0.005$ ) infarcts but normal for 96 lacunes compared with controls. This elevation persisted into the 6th week after the acute event. Among atherosclerotic thrombotic infarcts, the acute beta-thromboglobulin level showed a tendency to correlate with infarct size on CT and predicted mortality at 6 weeks. These results suggest that platelet aggregation plays a primary role in the pathogenesis of atherothrombosis.

Platelet aggregation plays a significant role in normal haemostasis by sealing defects in the vessel wall. Pathological extension of platelet aggregation may, on the other hand, contribute to the development of occlusive vascular diseases. Recent studies have shown that antiplatelet agents are effective in improving the outcome of patients with cerebral<sup>1,2</sup> or coronary arterial diseases.<sup>3,4</sup> It seems likely that these antiplatelet agents would be most effective in those patients with increased platelet reactivity.

Platelet aggregation is associated with the release of a number of proteins including beta-thromboglobulin (BTG), which is a small protein stored in the  $\alpha$ -granules of platelets. Its function is unclear but it is possibly a "granule packing protein" stabilising the active constituents in the  $\alpha$ -granules. Measurement of this platelet released material provides an index of platelet activation in vivo in atherosclerotic vascular disease and arterial thrombosis.<sup>5</sup>

We hypothesise that the role played by platelet aggregation in cerebral infarct varies with the pathological nature of the ischaemic insult. Thus, atherosclerotic thrombotic infarction, a large-vessel disease that arises as a result of artery-to-artery embolism of platelet thrombi from the main carotid arteries or following thrombotic occlusion of a major cerebral artery, should be associated with significant platelet activation and hence an elevated BTG level. Lacunar infarction, on the other hand, is a small-vessel disease with lipohyalinosis of the penetrating

end-arteries with in situ thrombosis.<sup>6</sup> Platelet aggregation does not play any role in its pathogenesis and thus the BTG level should remain normal. Cardioembolic infarction arises from fibrin-platelet clots in the heart so that platelet activation may be expected to be present. We tested the hypothesis by measuring the BTG level in a consecutive series of ischaemic stroke patients.

### Patients and methods

#### Patients

All Chinese patients with acute cerebral infarction or transient ischaemic attack admitted to the University Department of Medicine, Queen Mary Hospital over a 6-month period were studied. Patients were examined within 7 days of onset of ictus and classified as transient ischaemic attacks (TIAs), atherosclerotic thrombotic infarcts (AIs), cardioembolic infarcts (EIs) or lacunar infarcts (LIs) by clinical criteria and computed tomographic (CT) or necropsy confirmation:

*Transient ischaemic attacks* (TIAs) required complete resolution of the neurological deficit within 24 hours;

*Atherosclerotic thrombotic infarcts* (AIs) required clinical evidence of cortical deficits (dysphasia, dyspraxia, visual field defects, gaze paresis) and CT/necropsy evidence of recent cortical infarction, sinus rhythm and no source for cardiac emboli;

*Cardioembolic infarcts* (EIs) were clinically the same as AIs except that all these patients had either a cardiac arrhythmia or a definite emboligenic abnormality;<sup>7</sup>

*Lacunar infarcts* (LIs) required the clinical lacunar syndromes (pure motor hemiparesis, pure sensory stroke, ataxic-hemiparesis, dysarthria-clumsy hand syndrome, sensorimotor stroke) without cortical deficits in whom CT was normal or showed a lucency characteristic of a lacune.

The cardiac status of each patient was assessed by history,

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Table Distribution of risk factors and the BTG level among stroke subtypes and controls

	TIA	AI	EI	LI	Normal controls	Patient controls
N	16	116	36	96*	73	35
Age (mean, SD)	63.9, 9.4 <sup>a</sup>	65.9, 9.3 <sup>a</sup>	67.1, 7.1 <sup>a</sup>	63.9, 9.6 <sup>a</sup>	64.8, 9.6 <sup>a</sup>	52.4, 14.5
Sex (% male)	58.3	55.2	36.1 <sup>b, c</sup>	63.5	49.3	60.0
Hypertension (%)	38.9	43.9	33.3	48.9	6.8	6.8
Ischaemic heart disease (%)	5.6	9.6 <sup>d</sup>	25.7	33.1	—	2.3
Diabetes (%)	16.7	21.6	11.1	12.5	1.4	—
Smoking (%)	27.8	33.3	23.5 <sup>e</sup>	43.8	31.5	45.5
Cardiac arrhythmia (%)	—	—	100 <sup>f</sup>	—	—	—
Valvular heart disease (%)	—	—	22.2	—	—	—
BTG, ng/ml (Mean, SD)	28.9, 14.1	32.8, 16.4 <sup>g, h</sup>	35.6, 17.6 <sup>b, i</sup>	23.5, 9.5	25.7, 6.9	24.2, 7.2

TIA = Transient ischaemic attack. AI = Atherosclerotic thrombotic infarct. EI = Cardioembolic infarct. LI = Lacunar infarct.

\*: 74 pure motor hemiparesis, 10 sensorimotor stroke, 5 pure sensory stroke, 4 dysarthria-clumsy hand syndrome, 3 ataxic-hemiparesis.

†: 34 atrial fibrillation, 2 sick sinus syndrome.

Compared with EI: <sup>d</sup>:  $p < 0.05$ .

Compared with LI: <sup>b</sup>:  $p < 0.01$ , <sup>c</sup>:  $p < 0.05$ , <sup>f</sup>:  $p < 0.0001$ , <sup>h</sup>:  $p < 0.001$ .

Compared with normal controls: <sup>g</sup>:  $p < 0.0001$ , <sup>i</sup>:  $p < 0.005$ .

Compared with patient controls: <sup>a</sup>:  $p < 0.0001$ , <sup>e</sup>:  $p < 0.01$ .

examination and a 12-lead electrocardiographic (ECG) examination. Two-dimensional echocardiography and 24-hour Holter monitoring were performed whenever there was any clinical suspicion of an underlying cardiac abnormality.

Cerebral angiography was not used as a criterion to distinguish between the various stroke subtypes; it was performed only when clinically indicated to exclude a surgical lesion.

Excluded were patients: (1) with disability from previous stroke or other neurological diseases; (2) taking aspirin or other antiplatelet agents; and (3) who could not be classified with certainty into any one of the four ischaemic subtypes.

#### Patient controls

Thirty five patients with various non-vascular neurological diseases served as patient controls; these included Parkinson's disease (13), meningioma (7), glioma (6), alcoholic dementia (4), head injury (3) and Huntington's chorea (2).

#### Normal controls

The spouses of the stroke patients served as normal controls; none had a history of cardiovascular, cerebrovascular, or peripheral vascular diseases, although five subjects were known to have hypertension and one diabetes mellitus.

#### Plasma collection, preparation and assay of BTG

Plasma collection and preparation for BTG assay were performed according to standard procedures.<sup>8</sup> Venous blood (4.5 ml) was withdrawn from the antecubital vein using a gauge-21 needle without venous occlusion. The blood was immediately transferred into a pre-cooled plastic tube containing ETP (edetic acid EDTA 33 g/l, theophylline 1.8 g/l, prostaglandin E<sub>1</sub> PGE<sub>1</sub> 1000 µg/l). The tube was inverted gently two to three times and then transferred to an ice-water bath for 15 minutes. It was then centrifuged at 1200 g for 20 minutes at 4°C. The top 0.5 ml of platelet-poor plasma was collected and stored at -70°C until assay.

Plasma was collected as soon after admission as possible (acute BTG) and again at 6 weeks among the survivors (chronic BTG). The plasma concentration of BTG was measured by radioimmunoassay (RIA). The BTG RIA kit was purchased from the Radiochemical Centre, Amersham, England.

#### STATISTICAL METHODS

Statistical analysis was performed with the aid of the Statistical Package for the Social Sciences (SPSS-X). Data were analysed by Student's t-test or paired t-test where appropriate.

#### Results

##### Patients

The demographic characteristics of each stroke subtype and their distribution of risk factors are shown in the table. The stroke subtype was confirmed on CT in 255 (96.6%) patients and on necropsy in nine (3.4%) patients. Of the 255 patients who had CT confirmation, scanning was performed within the first 48 hours in 60.5%, between 3-7 days in 13.6%, in the second week in 15.2% and between 3-4 weeks in the remaining 10.7%.

##### BTG levels

Acute BTG was determined within the first 48 hours of onset of ictus in 67.4% of patients, between days 3-4 in 23.8% and between days 5-7 in the remaining 8.8%. The acute BTG levels are indicated in the table and fig 1. The mean (SD) value for the normal controls was 25.7 (6.9) ng/ml and that for the patient controls was 24.2 (7.2) ng/ml; there was no difference between these two groups of controls in spite of a significantly younger patient control population (52.5 (14.5) vs 64.8 (9.6) years,  $p < 0.0001$ ).

The acute BTG (mean, SD) value was 28.9 (14.1) ng/ml for TIAs, 32.8 (16.4) ng/ml for AIs, 35.6 (17.6) ng/ml for EIs and 23.5 (9.5) for LIs. Both AI and EI patients had significantly higher BTG levels than lacunes or controls. The mean BTG level for TIA patients lay between those for AI and LI patients, although not reaching any statistically significant difference. Acute BTG levels in lacunes were not significantly different from controls.

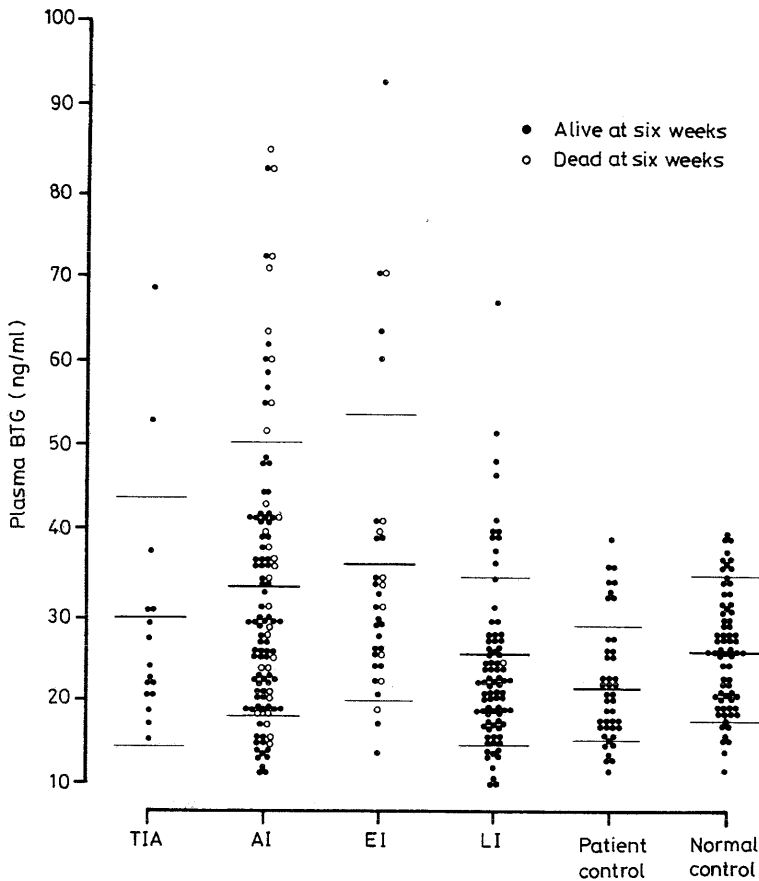


Fig 1 Distribution of acute BTG levels in the various stroke subtypes and controls. The horizontal bars represent the mean and one standard deviation.

Sex had no effect on BTG level. The mean (SD) value for the 36 male normal controls was 25.3 (6.8) ng/ml, and that for the 37 female normal controls was 25.3 (6.8) ng/ml ( $p > 0.1$ ). Similarly, no difference was found between the male and female patients of each stroke subtype, although there were significantly more male LI and more female EI patients.

There was a considerable degree of overlap (fig 1) in the acute BTG levels among the various stroke subtypes. A single BTG level in the acute phase did not predict the nature of the ischaemic disorder although there were no values greater than 70 ng/ml in lacunar infarcts.

By 6 weeks after ictus, 28 patients out of the initial 116 AI patients had died. Of the 88 survivors, 82 had BTG level repeated (fig 2). The acute BTG level of patients still alive at 6 weeks was 30.5, 14.2 ng/ml, while that of patients who died was 39.9, 20.4 ng/ml ( $p < 0.05$ ).

The 6-week chronic BTG level in the 82 AI patients who had the test repeated was 30.9, 13.8 ng/ml. There

was no significant difference from their acute BTG levels.

By contrast with AI, there was no significant difference in the acute BTG level between those EI patients who survived 6 weeks (26/36) and those who succumbed at 6 weeks (10/36), and there was no difference between the acute and 6-week BTG levels in the 22 EI patients who had a repeat BTG level taken (fig 3).

#### Correlation between BTG and infarct size on CT

**TIA:** All 16 TIA patients had normal CT scans.

**AI:** The infarct size on CT of the 116 AI patients ranged from 0.5 to 199.8 ml, with a median of 4.35 ml. By dividing into two subgroups according to the median infarct size (4.35 ml), the subgroup with larger infarcts (infarct size above median,  $n = 58$ , BTG 35.1, 16.7 ng/ml) had a higher acute BTG level than the subgroup with smaller infarcts (infarct size below median,  $n = 58$ , BTG 29.3, 14.1 ng/ml), with a significance level of  $p = 0.06$  (fig 4). Linear regression

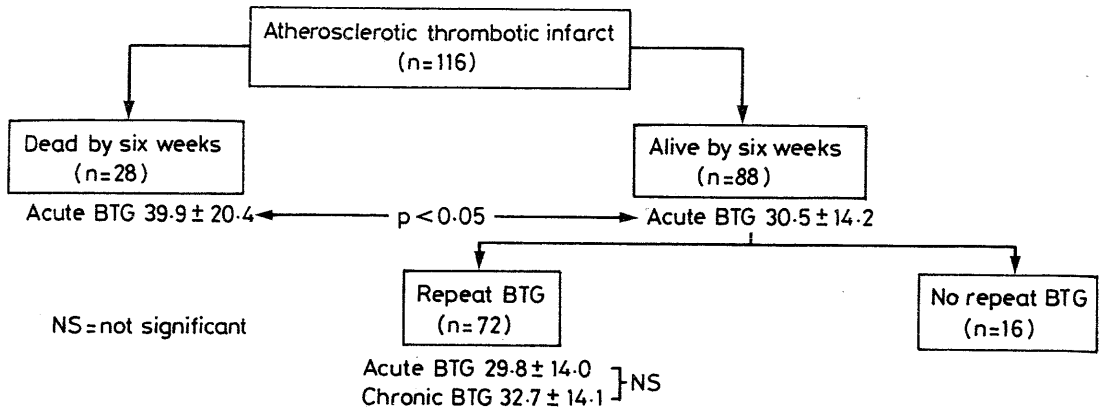


Fig 2 Acute and chronic BTG levels in the 116 patients with atherosclerotic thrombotic infarcts.

analysis between BTG and infarct size in these 116 patients also revealed a borderline significance ( $r = 0.25$ ,  $p = 0.08$ ).

ET: The infarct size on CT of the 36 EI patients ranged from 0.8 to 273.5 ml, with a median of 26.35 ml. By dividing into two subgroups according to the median infarct size (26.35 ml), there was still a tendency for the subgroup with larger infarcts to have a higher mean BTG level (38.4, 20.9 ng/ml vs 32.4, 14.3 ng/ml, fig 4), although it did not reach statistical significance ( $p > 0.1$ ).

LI: There was no difference in the acute BTG level between those LI patients with an infarct demonstrated on CT ( $n = 46$ , BTG 23.1, 8.5 ng/ml) and those with a normal CT ( $n = 50$ , BT 23.8, 10.4 ng/ml) (fig 4).

**Discussion**

Our study showed that in the acute phase after a

stroke, the BTG level is significantly elevated among those with a cortical infarct, whether of atherosclerotic or cardioembolic origin, but the level was within the normal range for an age-matched population among those with a lacune. While other workers<sup>10-13</sup> have reported similar findings, our population is the largest cohort of patients with methodical timing of blood sampling to ensure meaningful results. The finding of elevated BTG levels in atherosclerotic thrombotic infarcts compared with that in lacunes is in keeping with the concept that the former represent large-vessel diseases in which platelet aggregation is important and that the latter represent small-vessel diseases with lipohyalinosis and in situ thrombosis without significant platelet activation. The fact that cardioembolic infarcts also have elevated BTG levels, signifying platelet activation, is not surprising. Pathologically, the hearts of these patients have mural clots consisting of fibrin-platelet mixtures. In addition, it had been

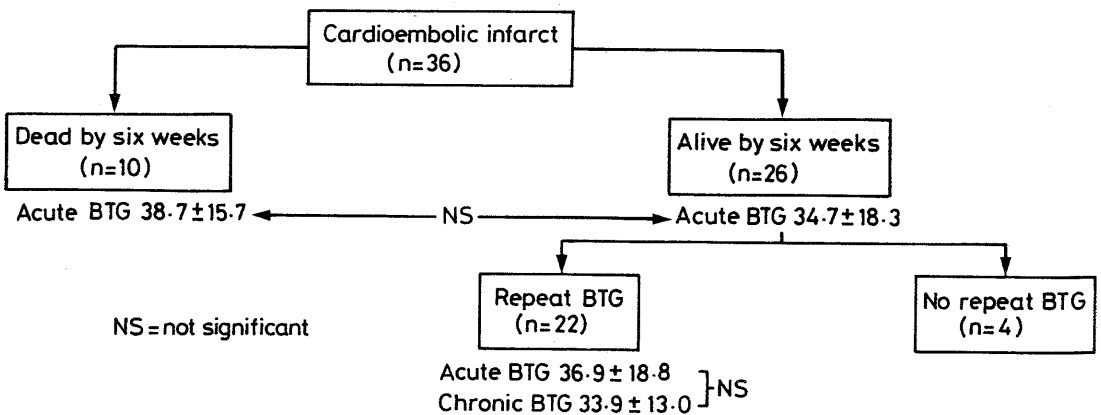


Fig 3 Acute and chronic BTG levels in the 36 patients with cardioembolic infarcts.

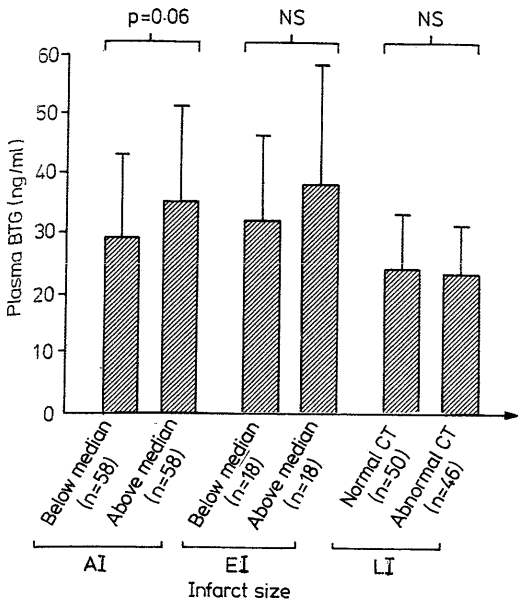


Fig 4 Mean acute BTG levels in the various stroke subtypes according to infarct size on CT. The bars represent one standard deviation.

shown in a prospective clinical study<sup>14</sup> that the addition of dipyridamole, an antiplatelet agent, to warfarin in patients who had prosthetic heart valve replacement reduced the incidence of embolic complications. This indicates that platelet activation does have a role to play in cardioembolic disease, although it may not be the most important pathogenic element.

Our results also indicated a considerable degree of overlap in the actual BTG value (fig 1). The great majority of AI and LI patients have their BTG levels within mean 2 SD of the controls. Hence, a single BTG level in the acute phase of a stroke has little predictive value, and it is evident that BTG is a relatively insensitive indicator of the type of ischaemic injury.

It has been shown in experimental animals<sup>15</sup> that cerebral ischaemia can stimulate platelet aggregation. One may argue that the elevated BTG level in acute stroke is only an epiphenomenon and not causally related to the ischaemic event. However, the persistently elevated BTG levels at 6 weeks after ictus would suggest that platelet activation is a continuing process and that it is not related solely to an acute aggregating stimulus. Further, Stewart *et al's* finding of an elevated BTG level a mean of 45.5 days from the last TIA<sup>11</sup> supports our contention, as there is little or no tissue destruction in TIAs so rendering it difficult to invoke platelet activation as a result of tissue damage.

Failure to demonstrate a significant BTG elevation in our TIA cohort may be explained by the small number of patients, but it is interesting to note that their mean BTG value was approximately midway between that of atherosclerotic thrombotic infarcts and that of lacunar infarcts. This raised the possibility that our TIA patients are heterogeneous, consisting of patients with both large-vessel and small-vessel diseases. In any event, these factors would favour the fact that platelet aggregation is primarily involved in the pathogenesis of large-vessel diseases and not secondary to the ischaemic event.

While we have shown that the acute BTG levels predicted mortality at 6 weeks among atherosclerotic thrombotic infarcts, we did not find any similar observation among the cardioembolic strokes. While this may be a type II error due to the small number of cardioembolic stroke patients, it must be pointed out that platelet activation is not the only factor involved in its pathogenesis, as the clotting system also has a role to play. Moreover, cerebral embolism is a systemic disease. Although the patient presents with a stroke, it is likely that embolic materials are dispersed asymptotically in other parts of the body as well. The BTG level therefore reflects the overall extent of systemic embolism and not just the cerebrovascular insult.

In correlating acute BTG level and infarct size on CT, we showed that among atherosclerotic thrombotic infarcts, there was a tendency towards a higher BTG level to be associated with a larger infarct, although not actually reaching statistical significance. While these findings were consistent with the above observation of acute BTG predicting mortality at 6 weeks, the results had to be interpreted with caution because it is known that CT performed very early in the course of an extensive infarct may be deceptively unrevealing and we are unable, for logistic reasons, to perform CT at a standardised time after onset of ictus in every patient. Among lacunes, there was no difference in the acute BTG level in those whose CT scans were normal and those whose CT scans demonstrated a small lacunar lesion.

In conclusion, our observation of an elevated BTG level in cortical non-lacunar strokes suggests that antiplatelet therapy<sup>1,2</sup> may be beneficial only in this subgroup of patients. Although the measurement of BTG provides a relatively simple way to identify the occurrence of platelet activation, we have shown that a single BTG level has little or no predictive value on the type of ischaemic injury. Further studies are therefore needed to identify better markers of platelet aggregation to improve our understanding and care of the acute stroke patients.

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