Plasma $\beta$-Thromboglobulin and Platelet Factor 4 Concentrations in Patients with Atrial Fibrillation

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SUMMARY

The clinical significance of beta-thromboglobulin ($\beta$-TG) and platelet factor 4 (PF-4) levels were evaluated in 26 patients with atrial fibrillation (af) complicated by valvular heart disease (VHD), 73 patients with af but without valvular heart disease and 57 normal subjects. The $\beta$-TG level was significantly higher in af patients without VHD than in normal subjects (49.4 ± 35.8 ng/ml vs 31.2 ± 14.0 ng/ml, p < 0.01) and in af patients with VHD than in normals (64.1 ± 52.8 ng/ml vs 31.2 ± 14.0 ng/ml, p < 0.01). Af patients with or without VHD tended to show high levels of PF4 compared with normals (af patients without VHD: 34.1 ± 45.5 ng/ml, af patients with VHD: 18.6 ± 27.2 ng/ml, normals: 11.6 ± 8.2 ng/ml). There was no correlation between $\beta$-TG levels and age in af patients without VHD or in normals. There was also no correlation between $\beta$-TG levels and heart rate in af patients without VHD.

The activation of platelets was suggested in patients with atrial fibrillation on the basis of increased levels of platelet releasing substances, especially in those with VHD. The high levels of $\beta$-TG and PF4 in patients with atrial fibrillation may be one explanation for the high incidence of thromboembolism in these patients, indicating the necessity of antiplatelet therapy.

Additional Indexing Words:
$\beta$-thromboglobulin Platelet factor 4 Atrial fibrillation Thromboembolism Valvular heart disease

PATIENTS with atrial fibrillation (af) run a high risk of thromboembolism that has the clinical features of sudden onset without premonitory symptoms. It is clinically very important to detect those with a high risk of thromboembolism. It is thought that thromboembolism is caused by platelet-fibrin emboli originating from the atrium in patients with af, and platelets

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Received for publication August 21, 1985.
Manuscript revised January 29, 1986.
play an important role in the formation of thrombi. Beta-thromboglobulin (β-TG) is a platelet-specific protein that is liberated from α-granules during the platelet release reaction. Platelet factor 4 (PF4) is another platelet-specific protein released during platelet degranulation. It has been reported that the plasma concentration of these substances was elevated by the platelet release reaction\(^1\) and it is thought useful to assess platelet activation in vivo.

The ultimate purpose is to detect those AF patients with a high risk of thromboembolism. In the present preliminary study, the plasma concentrations of both substances were measured to evaluate the significance of platelet-specific proteins in AF patients with and without valvular heart disease.

**Materials and Methods**

The normal control group was made up of 51 men and 6 women with a mean age of 36 years (age range: 18–70 years) who were screened on the basis of history, and routine physical and laboratory examinations, for the absence of detectable diseases which might affect platelet function.

Ninety-nine patients with atrial fibrillation were separated into 2 groups (i.e., 73 AF patients without valvular heart disease (VHD), and 26 AF patients with VHD). The mean ages of patients with and without VHD were 55 years (age range: 31–77 years) and 47 years (age range: 28–71 years), respectively. The 26 patients with VHD included 7 with mitral stenosis (MS), 9 with both MS and mitral regurgitation (MR), 2 with MS and aortic regurgitation (AR), 5 with MS and AR, 1 with MR and 2 with AR. None of these patients were on antiplatelet therapy. Digitalis and diuretics had been discontinued at least 1 week before blood sampling. No one with acute thromboembolism was included in the present study.

Measurement of plasma β-TG levels was undertaken in all subjects studied. PF4 levels were measured in 23 normals and in 17 patients without VHD and 11 with it. Both substances were measured in platelet-poor plasma obtained and prepared as follows: 5 ml of blood obtained by venipuncture without occlusion were aspirated into a polystyrene syringe using a 20-gauge needle. Blood samples were immediately transferred to cooled assay tubes containing an anticoagulant/antiplatelet mixture of Na EDTA and theophyllin. The tubes were mixed by gentle inversion and cooled (0–4°C). Within the following 2 hours, the blood samples were centrifuged at 2,000 g and cooled at 2–4°C for 30 min to obtain platelet-poor plasma.

Plasma concentrations of β-TG were assayed using a β-thromboglobulin RIA kit from Amersham International Limited and those of PF4 by RIA kits from Abbott Laboratories. Measurements were made twice, and the
mean of the two values was considered as the final result. Results were expressed as mean ± SD. The difference in the mean values among the 3 groups was analyzed by Student’s t-test. A p value of less than 0.05 was considered statistically significant.

Results

The mean plasma concentration of β-TG was higher in af patients without VHD than in normal subjects (49.4 ± 35.8 ng/ml vs 31.2 ± 14.0 ng/ml, p<0.01, Fig. 1). The af patients with VHD showed a tendency to a higher level of β-TG as compared with the af patients without VHD (64.1 ± 52.8 ng/ml vs 49.4 ± 35.8 ng/ml, Fig. 1). The β-TG values in 14 of 73 af patients without VHD (19%) and 10 of 26 with VHD (38%) were abnormally high.

There was no significant correlation observed between age and β-TG levels in normal subjects (r=0.19). In af patients without VHD there was

![Graph showing plasma β-thromboglobulin levels in normals and atrial fibrillation patients with and without valvular heart disease. Asterisks indicate significance of the mean difference between normals and af patients.](image)

Fig. 1. Plasma β-thromboglobulin levels in normals and atrial fibrillation patients with and without valvular heart disease. Asterisks indicate significance of the mean difference between normals and af patients.
also no age-dependent tendency in β-TG levels, although the β-TG level was higher in patients in their fifties than in those in their forties (Fig. 2).

There was no significant correlation between β-TG levels and heart rates in AF patients without VHD.

The mean plasma concentrations of PF4 tended to be higher in AF patients with and without VHD than in normal subjects (34.1 ± 45.5 ng/ml vs 11.6 ± 8.2 ng/ml, 18.6 ± 27.2 ng/ml vs 11.6 ± 8.2 ng/ml, respectively).

A significant correlation was observed between the levels of β-TG and PF4 in 23 normals and 28 AF patients (r = 0.64, p < 0.001, Fig. 3).

**DISCUSSION**

Platelets play a significant role in hemostasis through the formation of a thrombus on the injured vascular wall. Thrombus formation, especially in arteries, is initiated by platelet reactions. Injury to the vessel wall and loss of endothelium allow platelets to come into contact with exposed subendothelial structures. In this environment, platelets show a series of morphological changes: disc-sphere transformation, pseudopod extension and degranulation. Subsequently, platelets release ADP from their dense granules and
generate biologically active derivatives of arachidonic acid, which diffuse into the plasma causing platelets to aggregate.\(^3\) This release reaction also liberates other biologically active materials such as serotonin, PF4, \(\beta\)-TG and platelet mitogenic factor.

From these facts, it is suggested that an increase of platelet releasing substances in circulating blood, especially platelet-specific PF4 and \(\beta\)-TG, will be indexes of the presence of activated platelets and of the tendency to thromboembolism.\(^4\) In fact, there are some studies reporting elevated plasma levels of \(\beta\)-TG and PF4 in patients with various forms of vascular disease and thromboembolism.\(^4\)-\(^13\)

Atrial fibrillation is generally accepted as one of the commonest of the arrhythmias that predispose to systemic embolism. According to Wolf et al.,\(^14\) patients with chronically established atrial fibrillation, with or without rheumatic heart disease, run a highly increased risk of stroke, which is probably due to embolism. Chronic atrial fibrillation in the absence of rheumatic heart disease is associated with more than a 5-fold increase (5.6\%) in the incidence of stroke, while atrial fibrillation with rheumatic heart disease has a 17-fold increase (17.6\%). The high incidence of embolic stroke in patients with atrial fibrillation prompted us to study its influence on platelet function.

In the present study, high levels of \(\beta\)-TG and PF4 were demonstrated in af patients, whether or not VHD was present, indicating that platelets could
be activated in AF patients. Because of the insufficient number of cases, the present study could not specify the basic disease or pathophysiological state causing high levels of both substances. However, it is suggested that platelets in patients with mitral valve disease, especially with mitral stenosis, tended to be activated compared with those in patients with aortic valve disease alone. Patients with high plasma levels of β-TG and PF4 are thought to be at a higher risk of systemic embolism, and therefore antiplatelet therapy or anticoagulant therapy is necessary for these patients.

Although the mechanism of platelet activation in AF is not known, a possible explanation for the higher plasma levels of β-TG and PF4 may be platelet activation due to blood stasis in the atria. In valvular heart disease, platelet activation will be strengthened by endothelial damage in the atria and valve. High blood flow resistance between the atrium and the left ventricular cavity may also be a predisposing factor for platelet activation.

There was no significant correlation between plasma β-TG levels and age in normal subjects. In AF patients without VHD, the β-TG level was higher in patients in their fifties than in those in their forties. Because this difference may have been due to the unequal number of cases in the 2 groups, the difference may have little practical importance. These results suggest that the changes in cardiovascular system due to aging have minimal effect on platelet activation. Heart rate was not considered to be an important determinant for activation of platelets since no significant correlation was observed between heart rate and β-TG level in AF patients without VHD.

References