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# Altered Platelet Function in Patients with Severe Congestive Heart Failure\*

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*Platelet function was assessed in 15 patients with severe congestive heart failure (CHF) and in 26 control subjects of similar ages. The platelet count (mm<sup>3</sup>), surface reactivity, aggregometry studies, release factors, and circulating aggregates were investigated. The mean number of circulating platelets was normal, but a hyperactive platelet response was found in 53% of the CHF patients. CHF patients had a 42% mean for the spread type platelet, and the average number of aggregates was 64; control subjects had a 12% mean for the spread type platelet, and the average number of aggregates was 40 ( $p < 0.05$ ). Aggregation with all of the inducers was normal, although 27% of CHF patients showed spontaneous aggregation. The mean plasma levels of both platelet factor 4 and beta-thromboglobulin were abnormally elevated. No circulating platelet aggregates were detected. Our studies indicate that platelet function is abnormal in patients with CHF. The abnormal platelet reactivity found might contribute to the increased incidence of thromboembolic events observed in CHF patients. (Henry Ford Hosp Med J 1986;34:156-9)*

Thromboembolism is an important cause of morbidity and mortality in patients with congestive heart failure (1). The pathophysiologic mechanisms responsible for these events are not clearly delineated. An increased incidence of thromboembolic episodes was observed in patients with larger cardiothoracic ratios and in those with chronic atrial fibrillation (2). Because of the established association between congestive heart failure and thromboembolism as well as the accepted role of blood platelets in the formation of a thrombus (3), we studied the platelet response of 15 patients with severe congestive heart failure. Their results were compared to data obtained for controls of similar ages.

Scant information is available regarding platelet function in patients with heart failure (4). The objective of our study was to determine if platelet function was normal or abnormal in a group of patients with severe congestive heart failure.

## Materials and Methods

### Patient information

Fifteen patients, 11 men and four women, with chronic congestive heart failure (New York Heart Association functional class III to IV) whose mean age was 61 years (range 42 to 78 years) were included in the study. The etiology for heart failure was coronary artery disease in ten patients and cardiomyopathy in the other five patients. Two patients had a history of thromboembolic events, one of whom showed atrial fibrillation. Patients selected for this study did not have hypertension, a recent acute myocardial infarction, a recent cerebrovascular accident, unstable angina, primary valvular disease, or significant hepatic or renal dysfunction.

Six study patients received either a single antiplatelet agent or two platelet-active agents. The antithrombotic agents used were aspirin (1), heparin subcutaneously (3), and ibuprofen (2). Blood samples obtained from the heart failure patients were drawn through an indwelling arterial catheter.

### Control groups

Two groups of control subjects were used to establish our normal values. Data for evaluation of surface reactivity and aggregation using electron microscopy, platelet release factors including platelet factor 4 and beta-thromboglobulin, platelet count/mm<sup>3</sup>, as well as the platelet aggregate ratio were obtained from 26 asymptomatic subjects who came to a general medical clinic for a routine physical examination. This group's mean age was 60 years (range 42 to 77 years), which was not significantly different from the mean age of our heart failure patients. These control subjects, 19 men and seven women, did not have any disease processes that produced recognizable clinical symptoms, and none were on any drugs known to alter platelet function.

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Normal values for the platelet aggregation studies were established from data collected on 50 asymptomatic subjects of various ages. For the controls, peripheral blood was drawn by a venipuncture using a siliconized butterfly infusion set and a 21-gauge needle.

#### **Platelet count**

The number of circulating platelets (platelet count/mm<sup>3</sup>) was determined by using platelet-rich plasma obtained from whole blood anticoagulated with ethylenediamine tetraacetic acid (EDTA) processed in a Thrombo-fuge (Coulter Electronics, Inc, Hialeah, FL). The platelet-rich plasma was diluted with Isoton II and evaluated with a Coulter Counter, Model ZB1.

#### **Platelet electron microscopic survey**

Our standardized in vitro method (5) provided a morphologic assessment of three separate phases of the platelet response: adhesion, surface activation, and aggregation. The platelets' morphologic states were evaluated at the magnification and resolution capability of the transmission electron microscope. Three distinct types of platelets were observed: round, dendritic, and spread types. A platelet differential count included the percent of round, dendritic, and spread types of platelets found on examination of 100 single platelets. The number of platelet aggregates seen during evaluation of these platelets was also determined. Based on the limits of our control data ( $\pm$  two standard deviations), a hyperactive platelet response was defined as greater than 36% of the spread type platelet and/or more than 94 aggregates/100 single platelets counted.

#### **Platelet aggregometry**

Aggregometry studies were performed with a Bio-Data Aggregation Profiler (PAP-4, Biodata Corp, Philadelphia, PA) using platelet-rich plasma ( $250 \times 10^9$  platelets/L) in combination with the following stimulants: epinephrine, adenosine diphosphate (ADP), collagen, and arachidonic acid. Specimens that showed an aggregation rate of over 70% were tested for spontaneous aggregation and were also studied using different concentrations of the inducers.

#### **Platelet aggregate ratio**

The presence or absence of circulating platelet aggregates was established according to the method of Wu and Hoak (6).

#### **Platelet release factors**

Plasma levels of the platelet release factors (platelet factor 4 and beta-thromboglobulin) were measured by radioimmunoassay methods (7).

#### **Statistical analysis**

The unpaired Student's t-test and chi-square test were used to determine the level of significance for various tests of platelet function. A p value of 0.05 or less was considered a significant finding.

## **Results**

#### **Platelet count/mm<sup>3</sup>**

A comparison of the control subjects and the patients with congestive heart failure showed that the number of circulating platelets was within the normal range (126,000 to 450,000/mm<sup>3</sup>) at 257,000/mm<sup>3</sup>.

#### **Electron microscope differential count**

Transmission electron microscopy demonstrated a statistically significant ( $p < 0.05$ ) decrease in the number of the dendritic type platelet and a concomitant increase in the percent of the spread type platelet in differential counts of patients with congestive heart failure. The mean number of dendritic platelets was 74% for the control group and 49% for the congestive heart failure patients. In contrast, a mean value of only 12% of the spread type platelet was found in the differential counts of the control subjects, whereas the congestive heart failure patients showed 42% of the spread type platelet (Figure) ( $p < 0.05$ ).

A significantly increased mean number ( $p < 0.05$ ) of platelet aggregates (64 aggregates) was also noted for patients with congestive heart failure compared to the control subjects (40 aggregates). A hyperactive platelet response was found in three of 26 control subjects (12%) and in eight of 15 (53%) of the congestive heart failure patients ( $p < 0.05$ ). Therefore, the surface reactivity and/or aggregation of over half of the patients with congestive heart failure were abnormally increased.

#### **Platelet aggregate ratio**

The mean platelet aggregate ratio of 0.74 found in our control subjects was not significantly different from the average ratio found for the patients with congestive heart failure (Table 1).

#### **Platelet aggregometry**

Evaluation of platelet aggregation induced by the various stimulants for the entire group of patients with congestive heart failure did not show any significant changes (Table 2). However, platelets from four of the 15 patients (27%) aggregated spontaneously. In contrast, platelets removed from five other patients showed a release-type defect with two or more inducers. All of these patients had received drugs known to inhibit platelet activity, primarily aspirin and ibuprofen.

#### **Platelet release factors**

The mean levels of both platelet factor 4 and beta-thromboglobulin were uniformly increased ( $p < 0.05$ ) (Table 2). Nine patients (60%) had an increased level of platelet factor 4, and 13 patients (87%) had an abnormal plasma concentration of beta-thromboglobulin.

#### **Subgroup analysis**

Subgroups of patients with conditions known to alter platelet function, such as gout and diabetes mellitus, and patients who received antiplatelet agents were analyzed separately. Data collected from patients with coronary artery disease were compared to results obtained from patients with cardiomyopathy. Results of all the platelet function tests were not significantly different in the subgroups compared to patients with congestive



**Table 1**  
**Tests of Platelet Function**

	Controls	CHF
Platelet count ( $\times 1000$ )	288 $\pm$ 162	257 $\pm$ 184
Electron microscope differential count		
Round type (%)	14 $\pm$ 28	9 $\pm$ 18
Dendritic type (%)	74 $\pm$ 36	49* $\pm$ 58
Spread type (%)	12 $\pm$ 24	42* $\pm$ 64
Platelet aggregates (n)	40 $\pm$ 54	64* $\pm$ 126
Platelet aggregate ratio	0.74 $\pm$ 0.38	0.87 $\pm$ 0.54

\*p < 0.05 between control subjects and CHF patients.  
CHF = congestive heart failure.

heart failure who did not have a platelet-modifying state. The platelet abnormalities were independent of whether the underlying etiology for the congestive heart failure was coronary artery disease or cardiomyopathy (Table 3).

### Discussion

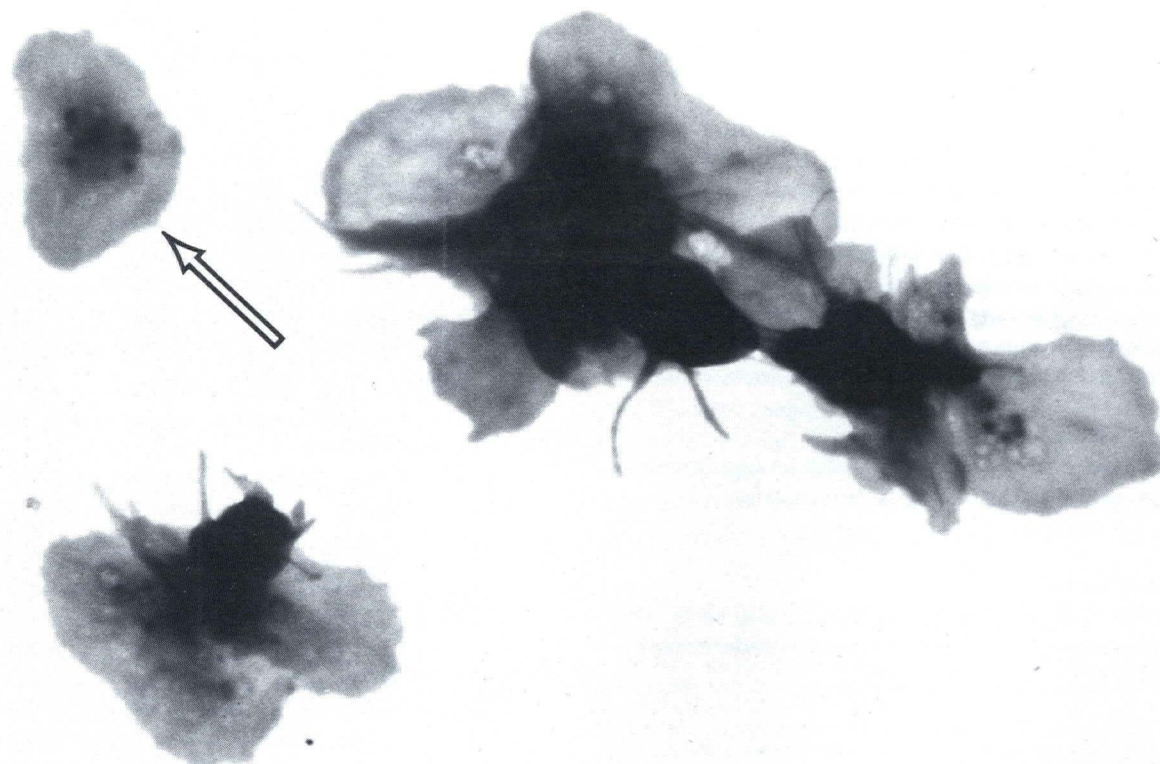
Clinically recognized thromboembolism in patients with congestive heart failure reportedly occurs at a frequency of 15% to 20% (1,2). Evidence of either arterial or pulmonary emboli was found in 22% of patients with dilated cardiomyopathy at autopsy and in 18% by clinical examination of the survivors. Enlarged cardiac size and atrial fibrillation correlated with a higher incidence of thromboembolic episodes (2).

**Table 2**  
**Platelet Aggregation and Release Factors**

	Controls	CHF
Aggregometry		
ADP (%)	50 $\pm$ 8	57 $\pm$ 30
Epinephrine (%)	50 $\pm$ 8	51 $\pm$ 48
Collagen (%)	50 $\pm$ 8	52 $\pm$ 44
Arachidonic acid (%)	50 $\pm$ 8	52 $\pm$ 36
Release factors		
Platelet factor (ng/mL)	7.51 $\pm$ 5.72	21.06* $\pm$ 22.48
Beta-thromboglobulin (ng/mL)	42.93 $\pm$ 34.64	126.97* $\pm$ 83.22

\*p < 0.05 between control subjects and CHF patients.  
CHF = congestive heart failure.

Limited information is currently available regarding platelet function in patients with congestive heart failure (4). Our investigation included tests that measured both the early stage (adhesion and surface activation) and later stage (aggregation and release of platelet-specific proteins) of the platelet response. Abnormalities of platelet function shown in our study were not reported previously in these patients. Using electron microscopy, we found hyperactive platelets expressed as an abnormally increased number of the spread type platelet which is an early feature of platelet activation. The cohesive capacity of the platelets, demonstrated by their ability to form aggregates, was also evaluated in the procedure which utilized the transmission electron microscope. The average number of aggregates counted during the classification of 100 single platelets was sig-



Figure—A spread type platelet (arrow) and two platelet aggregates seen during a differential count on a patient with congestive heart failure (X5,600).



nificantly increased in the differential counts of the patients with congestive heart failure. Advantages of our platelet electron microscopic survey over routine platelet aggregometry include direct evaluation of the early stages of platelet activation and assessment of the overall aggregability of a platelet population. During the procedure the platelets are maintained in a compatible environment of anticoagulated whole blood and are not subjected to extensive manipulation such as the shear forces of centrifugation.

Routine aggregometry which incorporates a centrifugation step, however, also showed abnormalities of the platelet function in blood samples removed from these patients. In 27% of the congestive heart failure patients (four of 15 patients), the prepared platelet-rich plasma aggregated spontaneously without an additional *in vitro* chemical inducer. The remaining patients showed either a normal amount of aggregation (seven of 15 patients) or diminished aggregation, which was probably related to recent ingestion of agents known to inhibit platelet function (four of 15 patients).

Plasma levels of platelet release factors, platelet factor 4 and beta-thromboglobulin, measures of *in vivo* platelet activation, were also abnormally increased (8,9).

We did not find circulating platelet aggregates in the blood of these patients. Our results conflict with an earlier study in which platelet aggregates were demonstrated in the circulation of some patients with congestive heart failure (4). We suspect that this discrepancy may be explained by differences in the group of control subjects used to establish the normal values in the two investigations. Our control group consisted of healthy subjects of similar ages, while the previous study used young volunteers (mean age of 32 years). The mean value for our platelet aggregate ratio was 0.74 rather than 0.91 which was reported in the other study (4). The lower ratio thus changed the interpretation of whether circulating aggregates were present.

Our findings show evidence of altered platelet function in patients with severe congestive heart failure. While the exact mechanisms responsible for these abnormalities require further clarification, changes secondary to hemodynamic and neurohormonal alterations could be important. Activation of the circulating platelets might occur in relation to the following hemodynamic alterations: 1) stasis due to a low cardiac output state, or turbulence from valvular insufficiency because of dilated cardiac chambers; or 2) release of vasoactive substances because of increased peripheral vascular resistance. Levels of norepinephrine (10), renin-angiotensin (11), and prostaglandin I<sub>2</sub> and E<sub>2</sub> (12) are frequently increased in heart failure patients. Elevated catecholamine levels previously have been associated with platelet activation and hyperaggregation in a variety of conditions such as acute myocardial infarction (13) and mitral valve prolapse (14).

Our study demonstrates that the platelet response is frequently abnormal in patients with congestive heart failure. The altered platelet function is independent of associated platelet-modifying diseases and is consistent whether the etiology of the congestive heart failure is coronary artery disease or cardiomyopathy. The increased platelet reactivity may directly contribute to the high incidence of thromboembolism seen clinically in these patients.

**Table 3**  
**Subgroup Analysis of CHF Patients**

	ETIOLOGY OF CHF		ANTI-PLATELET DRUGS	
	CAD	CM	Yes	No
Platelet count ( $\times 1000$ )	247	277	238	279
Surface activation				
% Spread type	52	20	51	31
Total aggregates	65	61	72	54
Aggregometry				
ADP	59	52	48	67
Epinephrine	54	45	37	67
Collagen	58	40	39	66
Arachidonic acid	56	45	44	62
Release factors				
Platelet factor 4	21.22	20.74	20.15	22.10
Beta-thromboglobulin	129.66	121.60	135.65	117.06
Platelet aggregate ratio	0.83	0.96	0.79	0.97
No. of patients	10	5	6	9

CAD = coronary artery disease.

CM = cardiomyopathy.

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