

# Platelet contractile force (PCF) and clot elastic modulus (CEM) are elevated in diabetic patients with chest pain

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Accepted 7 May 2002

## Abstract

**Aims** Platelet function and clot structure may be altered in diabetes. We have noted increased platelet contractile force (PCF) and clot elastic modulus (CEM) in patients presenting to the emergency department with chest pain. Twenty-six of the chest pain patients were diabetic. Here, we compare the PCF, CEM and platelet aggregation in diabetic chest pain patients, non-diabetic patients with chest pain and asymptomatic controls.

**Patients and methods** PCF, CEM and collagen whole blood aggregations were measured in 100 chest pain patients and 25 asymptomatic controls.

**Results** Platelet concentrations for diabetic patients, non-diabetic patients and controls were identical. PCF was significantly ( $P < 0.05$ ) elevated in diabetic chest pain patients ( $9.42 \pm 0.59$  kdynes) vs. controls ( $7.40 \pm 0.32$  kdynes). CEM in diabetic patients ( $29.96 \pm 2.19$  kdynes/cm<sup>2</sup>) was significantly elevated relative to that in non-diabetic chest pain patients ( $25.22 \pm 0.84$  kdynes/cm<sup>2</sup>) and normal controls ( $23.18 \pm 0.74$  kdynes/cm<sup>2</sup>). Collagen-induced whole blood aggregation was decreased ( $P < 0.05$ ) in diabetic chest pain patients vs. controls. PCF values ( $10.23 \pm 0.76$  kdynes) in diabetic patients with haemoglobin A<sub>1c</sub> > 7% were higher than in any other group.

**Conclusion** PCF and CEM are elevated in diabetic chest pain patients. The significance of these laboratory findings awaits additional clinical studies.

Diabet. Med. 19, 862-866 (2002)

**Keywords** clot retraction, coronary artery disease, chest pain, diabetes mellitus, platelet function

## Introduction

Patients with diabetes are at increased risk of thrombosis [1]. Eighty percent of Type 2 diabetic patients will die a thrombotic death [2]. Of these deaths, 75 % will result from cardiovascular events. Other deaths result as complications of peripheral vascular and cerebrovascular disease [3].

Although platelet counts are normal in diabetic patients [4], platelet activity is increased in this patient population. Platelets from patients with both types of diabetes have a heightened response to ADP [5,6]. Up to one-third of those with Type 2

diabetes have increased circulating platelet aggregates [7]. Several studies have found high levels of platelet release products such as thromboxane B<sub>2</sub> [5,8], R-thromboglobulin [4,6-8], platelet factor 4 [4], and fibronectin [5] in patients with diabetes.

We have noted increased platelet contractile force (PCF) in patients with coronary artery disease (CAD) [9] and thromboangiitis obliterans [10]. Since these disorders involve abnormal endothelial function, a subsequent study investigated PCF and clot elastic modulus (CEM) in 100 patients presenting to the emergency department with chest pain [11]. Twenty-six of these had diabetes. We compared the PCF, CEM and platelet aggregation values in the diabetic and non-diabetic patients and asymptomatic controls.

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Table 1 Demographics of study participants

	Normal controls	Diabetics with chest pain	Non-diabetics with chest pain
Number	25	26	74
Age	39-66	41-87	23-85
Sex			
Females	18 (72%)	14 (54%)	41 (55%)
Males	7 (28%)	12 (46%)	33 (45%)
Race			
Black	4 (16%)	20 (77%)	51 (69%)
White	19 (76%)	5 (19%)	23 (31%)
Other	2 (8%)	1 (4%)	0
Smokers	1 (4%)	8 (31%)	31 (42%)
Hypertension	0	17 (65%)	24 (32%)
Hypercholesterolaemia	0	8 (31%)	20 (27%)
Aspirin use	0	12 (46%)	13 (18%)
NSAID use	0	6 (23%)	13 (18%)
Oral hyperglycaemic	0	13 (50%)	0
Insulin	0	8 (31%)	0
Previous MI	0	6 (23%)	15 (20%)
Previous PTCA	0	6 (23%)	8 (11%)
Previous CABG	0	4 (15%)	7 (9%)
Previous catheterization	0	6 (23%)	19 (26%)

## Methods

### Subjects

Under a protocol approved by the Institutional Review Board for Human Studies of Virginia Commonwealth University, written, informed consent was obtained from all patient and control subjects. One hundred patients presenting to the emergency room with chest pain and 25 asymptomatic volunteers were enrolled.

### Reagents

Human thrombin, free of plasmin and plasminogen, was purchased as a lyophilized powder (Sigma Chemical Co., St Louis, MO, USA), dissolved in water, diluted with 0.10 M NaCl, divided into 50-100 µl lots and frozen at -80°C. Nanopure water was used in the preparation of all solutions.

### Sample preparation

Blood was collected via aseptic venipuncture into evacuated tubes containing 3.2% sodium citrate. Clotting was initiated by adding thrombin (1 NIH unit/ml) and calcium chloride (10 mM) to 700 µl of citrated whole blood. Force development was measured for 900 s and recorded in kilodynes (kdynes). Clot elastic modulus was measured concurrently and reported in kilodynes per cm<sup>2</sup> (kdynes/cm<sup>2</sup>). Measurements of PCF, CEM and collagen-induced whole blood platelet aggregation were run in duplicate.

### Measurement of force development during clot retraction

The Hemodyne® RM-2 haemostasis analyser (Hemodyne, Inc., Richmond, VA, USA) measures forces generated by platelets

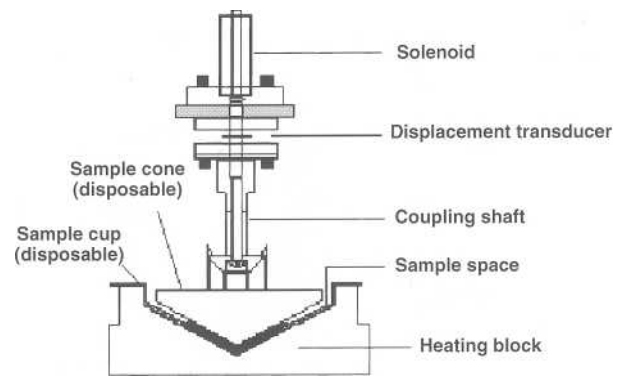


Figure 1 Side view schematic of the Hemodyne RM-2 device used to measure platelet contractile force and clot elastic modulus.

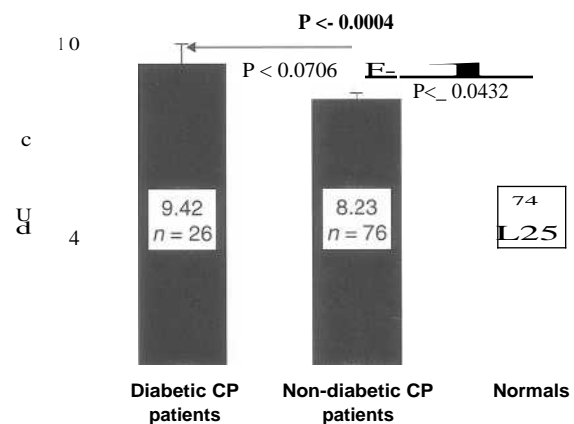


Figure 2 Platelet contractile force in diabetic and non-diabetic patients with chest pain versus asymptomatic controls.

within a clot formed between a thermostated cup and a parallel upper surface [12,13]. As soon as a fibrin network is formed, platelets within the network pull fibrin strands inward, transmitting force through the network. Force measurement is accomplished utilizing a displacement transducer coupled to the upper surface which produces a voltage proportional to the amount of force generated.

### Measurement of clot elastic modulus

CEM was measured simultaneously with PCF. The ratio of applied force (stress) to measured displacement (strain) is used to calculate the elastic modulus. Stress equals the applied force ( $F_{\text{applied}}$ ) divided by the area of application, and strain is the degree of deformation induced by the applied force. The strain induced by  $F_{\text{applied}}$  is measured as the change in clot thickness and is recorded as the ratio of the change in gap distance to the original gap distance.

### Platelet aggregation

Platelet aggregation was measured using a Chrono-Log® whole blood lumi-aggregometer. Citrated whole blood (450 µl) was

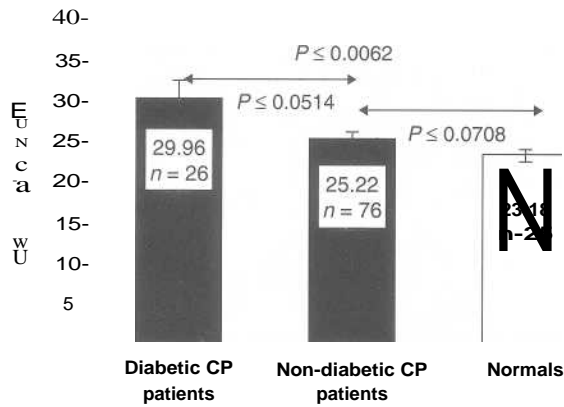


Figure 3 Clot elastic modulus in diabetic and non-diabetic patients with chest pain versus asymptomatic controls.

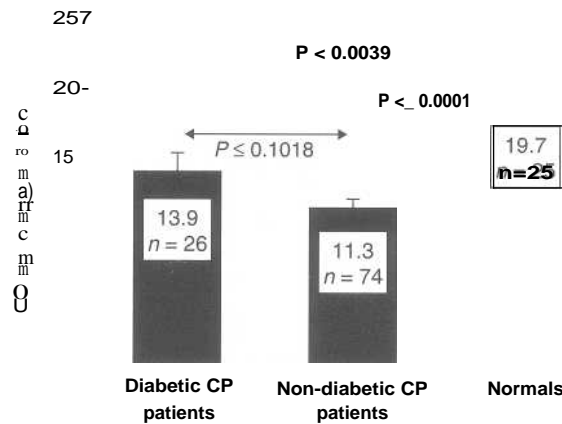


Figure 4 Extent of collagen-induced platelet aggregation in diabetic and non-diabetic patients with chest pain versus asymptomatic controls.

mixed with 450 µl of saline and placed in an aggregometer cuvette equipped with a stirring bar. Platelet aggregation was induced by the addition of collagen (3 µg/ml; Chronolog, Havertown, PA, USA) and the change in impedance was monitored for 6 min.

**Statistical analysis**

Results were analysed using Sigma Plot Software (SPSS Science, Chicago, IL, USA).

One-way analysis of variance (ANOVA) was used to test each measured parameter for overall differences between groups. Associations between group pairs were examined using Duncan's multiple comparison tests. All values were expressed as mean ± srm, and P < 0.05 was considered significant.

**Results**

**Population demographics**

The patient population was predominantly African American (20 of 26 for diabetic patients and 51 of 74 for non-diabetic patients), while the asymptomatic controls were predominantly

white (18 of 25). However, previous studies have documented no effect of race on the PCF parameter [11]. The mean age for the patient population was 53 years and the mean age for the controls was 47 years (Table 1).

**Platelet concentrations**

Platelet concentrations for the diabetic patients ( $248 \pm 16.5 \times 10^3/\mu\text{l}$ ), non-diabetic patients ( $255 \pm 12.37 \times 10^3/\mu\text{l}$ ) and normal controls ( $266 \pm 13.82 \times 10^3/\mu\text{l}$ ) were very similar.

**Platelet contractile force**

PCF was significantly (P < 0.05) elevated in diabetic chest pain patients ( $9.42 \pm 0.59$  kdynes) relative to non-diabetic patients ( $8.23 \pm 0.25$  kdynes) and to controls ( $7.40 \pm 0.32$  kdynes) (Table 2).

**Clot elastic modulus**

CEM in diabetic patients ( $29.96 \pm 2.19$  kdynes/cm<sup>2</sup>) was significantly elevated relative to that in non-diabetic chest pain patients ( $25.22 \pm 0.84$  kdynes/cm<sup>2</sup>; P < 0.05) and to controls ( $23.18 \pm 0.74$  kdynes/cm<sup>2</sup>; P < 0.05) (Table 2).

Table 2 Statistical analysis of study parameters

Parameters	PCF		CEM		Collagen aggregations		
	Mean	stM	Mean	stN4	Mean	sEVi	
	Normal controls (NL)	25	7.40	0.317	23.18	0.738	19.1
Diabetics with chest pain (DMCP)	26	9.42	0.586	29.96	2.187	13.9	1.409
Non-diabetics with chest pain (nDMCP)	74	8.23	0.249	25.22	0.836	11.3	0.717
One-way anova		P = 0.006		P = 0.005		P < 0.001	
Duncan's multiple comparison test		P < 0.05"		P < 0.05"		P < 0.05"	
NL vs. DMCP		P > 0.05		P > 0.05		P < 0.05X	
NL vs. nDMCP		P < 0.05"		P < 0.05'		P > 0.05	

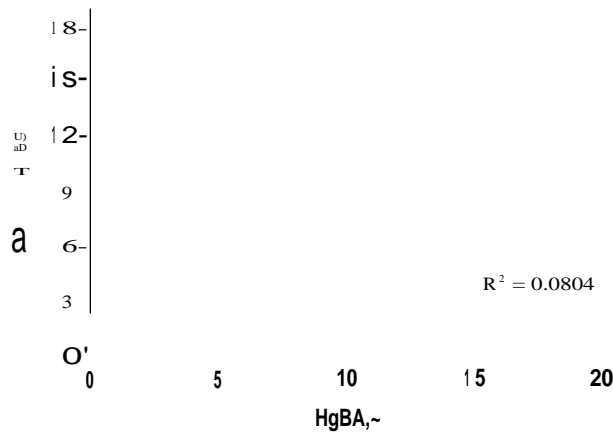


Figure 5 Relationship of hemoglobin  $A_{1c}$  levels to platelet contractile force in diabetic patients presenting with chest pain.

### Platelet aggregation studies

Collagen-induced whole blood aggregation was decreased in diabetic chest pain patients ( $13.9 \pm 1.41$  S2;  $P < 0.05$ ) and in non-diabetic chest pain patients ( $11.26 \pm 0.72$  S2;  $P < 0.05$ ) vs. asymptomatic controls ( $19.1 \pm 0.91$  S2). Many of the patients were on aspirin, while all of the controls were free of medication (Table 2).

### Correlation of haemoglobin $A_{1c}$ with PCF

In 14 diabetic patients, haemoglobin A<sub>1c</sub> levels were measured. In these patients, PCF increased with A<sub>1c</sub> level ( $R^2 = 0.0804$ ). The PCF values ( $10.23 \pm 0.76$  kdynes) found in diabetics with haemoglobin A<sub>1c</sub> > 7% are the highest thus far reported.

### Discussion

As with previous studies [4], platelet counts in our patients with diabetes were not elevated. Despite similar platelet concentrations; PCF was significantly higher in clots formed from diabetic blood. Platelets from diabetic patients produced > 40% more force per platelet. This difference was highly significant ( $P < 0.01$ ). The elevation of PCF occurred despite the fact that many of the diabetic patients were on multiple anti-platelet medications. The decreased platelet aggregation in these patients was consistent with drug effect.

While the origin of the enhanced force is uncertain, similar elevations have been found in patients with coronary artery disease [9,11] and thromboangiitis obliterans [10]. Both conditions are known to involve significant endothelial abnormalities and dysfunction. Chronic low-level platelet activation, as documented by higher levels of release products, may be playing a significant role. Indeed, activation of thrombin, the most profound known agonist of platelets, is known to be elevated in diabetes. Both prothrombin activation fragment 1 + 2 [14,15] and thrombin-anti-thrombin complexes [14,16] concentrations are elevated in diabetes. Baseline low-level

thrombin activation may be of particular import since PCF is a thrombin-dependent process [17,18].

The aetiology of the increased CEM noted in our diabetic patients is probably multifactorial. First, CEM is directly dependent on fibrinogen concentration [19,20], which is elevated in patients with diabetes [4,6,21]. Second, hyperglycaemia of diabetes results in hyperglycosylation of fibrinogen [22,23]. When hyperglycosylated fibrinogen clots, it forms altered fibrin structures composed of small diameter fibrin fibres (unpublished data). Such structures have increased elastic modulus (unpublished data). Third, PCF generated during clot formation puts the clot structure under stress and causes increased elastic modulus [24]. Inhibition of PCF reduces CEM [9,25].

The results of this study provide evidence of enhanced platelet activity (PCF) and clot structure (CEM) in patients with diabetes. The potential clinical significance and utility of these parameters as markers of thrombotic risk await additional clinical study.

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