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ORIGINAL ARTICLE

Assessment of Platelet Adhesiveness and Aggregation in Mild Acute Pancreatitis Using the PFA-100™ System

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ABSTRACT

Context Acute pancreatitis constitutes a systemic inflammatory process which is often accompanied by thrombosis and bleeding disorders. The role of platelets in the pathophysiology of the disease remains to be elucidated.

Objective In the present study, we evaluated the alterations of platelet function in patients suffering from acute edematous pancreatitis using the recently developed platelet function analyzer PFA-100™.

Design A cohort study with one end-point (difference between patients with acute edematous pancreatitis and normal controls concerning at least one PFA-100™ closure time at the P<0.01 level of statistical significance).

Main outcome measure The hemostatic capacity of platelets was tested in citrated blood and standard cartridges containing collagen-ADP or collagen-epinephrine.

Patients We studied 16 patients (6 women and 10 men, mean age 62.1 years) with acute edematous pancreatitis, who had been admitted to our Internal Medicine Department, along with 32 normal controls of similar age and having the same woman-man ratio.

Results The mean closure time using collagen-ADP cartridges was 69.6 s (95% CI: 60.4-78.7 s) in patients and 96.1 s (95% CI: 93.0-99.3 s) in normal controls (t-value: 7.2; P<0.001). The mean closure time using collagen-epinephrine cartridges was 110.7 s (95% CI: 100.1-121.3 s) in patients and 119.7 s (95% CI: 114.6-124.8 s) in normal controls (t-value: 1.8; P=0.078). The hematocrit in all patients was less than the upper reference value.

Conclusions The PFA-100™ represents a simple and easy to use test for investigating primary hemostasis. Although the method has been widely used in hemorrhagic conditions, this is the first time it has been applied in the prethrombotic model of acute pancreatitis, suggesting increased platelet adhesiveness and aggregation.

INTRODUCTION

Acute pancreatitis constitutes a systemic inflammatory process which, according to one of the main hypotheses, originates from oxidative stress and free radical production inside the gland. The subsequent tissue

damage and the release of inflammatory mediators give rise to a more generalized event. The platelet activation factor is thought to be the most important mediator which is involved in the process and triggers platelet activation [1]. Experimental pancreatitis caused in dogs has been found to affect platelet function and size [2].

The platelet activation pathway has three steps. 1) Activation of the membrane integrin glycoprotein IIb/IIIa, which undergoes conformational changes to create functional membrane receptors for fibrinogen. Calcium-dependent linkage formation between activated receptors and bivalent fibrinogen follows, resulting in platelet aggregation. 2) Secretion of proaggregatory mediators such as ADP, ATP, platelet factor 4 (PF4), beta thromboglobulin (beta-TG), thromboxane A_2 (TXA_2), fibrinogen and thrombospondin which are followed by the translocation of the alpha-granule membrane protein P-selectin (CD 62) to the platelet surface. 3) Exposure of phosphatidylserine, which is the starting point for the coagulation cascade [3].

Alterations in platelet function have been previously described in models of systemic inflammation such as ulcerative colitis and Crohn's disease [4, 5, 6]. A recent study revealed platelet activation followed by impairment of platelet indexes during acute pancreatitis [7].

Platelet activation can be estimated through flow cytometry, measurement of platelet-specific proteins such as beta-TG, PF4 and soluble P-selectin in plasma and TXA_2 metabolites in both plasma and urine dose-response and aggregometry [8]. The PFA-100™ constitutes a recently developed platelet function analyzer, which measures primary hemostasis *in vitro* by simulating *in vivo* platelet adhesion and aggregation under high shear stress [9]. Whole, citrated venous blood is aspirated through a capillary towards an aperture of a collagen-coated membrane containing either ADP or EPI (epinephrine). The time until plug formation is measured in seconds and is called closure time. Two different cartridges containing the agonists ADP (ADP cartridge) and epinephrine (EPI cartridge), are used for the PFA-100™ measurement.

The proposal of the present study was to evaluate primary hemostasis during acute pancreatitis using the PFA-100™ and to estimate the prethrombotic potential carried over during the disease process.

METHODS

Study Design

The present study is a cohort study with a single end-point: the difference between patients with acute edematous pancreatitis and normal controls concerning at least one PFA-100™ closure time using either ADP or EPI cartridges at the $P<0.01$ level of statistical significance.

Patients

Sixteen patients with edematous acute pancreatitis, which were admitted to our Internal Medicine Department between January and December 2003 were enrolled in the study. There were 6 females (37.5%) and 10 males (62.5%), and the mean±SD age was 62.1±17.7 years (95% CI: 52.7-71.6; range: 35-87 years).

The diagnosis of acute pancreatitis was established using medical records, physical examination and laboratory tests (elevated serum amylase more than 3 times the upper reference value) and was confirmed by a spiral CT-scan of the abdomen. Patients with hemorrhagic/necrotizing forms of the disease were excluded. The etiology of acute pancreatitis was found to be alcoholic in 5 (31.3%) patients and biliary in 11 (68.8%) patients. The APACHE II score was calculated for each patient (mean±SD: 3.00±2.61; 95% CI: 1.61-4.39; range 0-8). The mean CRP was 6.13±1.86 mg/dL (95% CI: 5.14-7.11 mg/dL; range 4-11 mg/dL; upper reference value: 0.80 mg/dL). The mean hematocrit was 39.8±3.4% (95% CI: 38.0-41.6%; range 34-45%; reference range of men: 42-52%; reference range of women: 36-48%); it was within the reference range in

8 (50.0%) patients while, in the remaining 8 (50.0%) patients, it was below the lower reference value. The mean interval between the onset of symptoms and admission was less than 12 hours (mean±SD: 9.9±5.8 h; 95% CI: 6.8-13.0, range 2-24 h). None of the patients were treated with acetylsalicylic acid, anti-inflammatory drugs or oral anticoagulants for at least 10 days prior to admission.

Thirty-two normal controls (16 females, 50.0%; 16 males, 50.0%; mean±SD age 53.1±15.5 years; 95% CI: 47.5-58.7; range 19-84 years) were also studied; 25 of them were recruited from the medical and nursing staff and the other 7 - who were older - were recruited from relatives of the staff. All controls were in good general health, were not taking any medication and had no signs of any disease. The mean age and woman-man ratio were not significantly different between the patients and the controls (P=0.077 and P=0.542, respectively).

Blood Sampling

A whole blood sample was obtained at admission by venipuncture from the antecubital vein with a 21-gauge needle using citrated BD Vacutainer® blood sampling tubes (BD Vacutainer® Systems, Belliver Industrial Estate, Plymouth, UK) for each patient and control. The blood samples were stored at room temperature for approximately 1 hour. After the incubation period, duplicate measurements using PFA-100^{TM} were performed.

The Platelet Function Analyzer PFA-100^{TM}

The PFA-100^{TM} (Dade Behring, Düdingen, Switzerland) is composed of a microprocessor-controlled device and single-use test cartridges. The test cartridges consist of a sample reservoir, a capillary and a membrane coated with two 2 mg equine type I collagen and either 10 mg epinephrine bitartrate (EPI cartridge) or 50 mg adenosine 5'diphosphate (ADP cartridge). Blood is pipetted into the reservoir and aspirated through a capillary with a diameter of 200 μm with constant negative pressure resulting in high shear forces (5,000-6,000 s^{-1}). The capillary ends in a membrane aperture with a diameter of 150 μm. Platelets adhere at the aperture where they are activated by the collagen and then aggregate. The two agonists (epinephrine and ADP) enhance aggregation. Finally, a platelet plug occludes the aperture and blood flow stops. The time measured in seconds from the beginning of the test until formation of an occluding platelet plug is called closure time. If an occluding platelet formed a plug after 300 seconds, the analysis stopped. All subjects were tested with both reagents (epinephrine and ADP) and double measurements were performed each time [10].

ETHICS

The study was performed in accordance with the ethical guidelines of the Helsinki Declaration. After approval of the study protocol by the Ethical Committee of our Hospital, oral informed consent was obtained from all patients and controls.

STATISTICS

Frequencies, mean, SD, 95% confidence intervals (95% CI) of the mean and ranges were used as descriptive statistics. The Kolmogorov-Smirnov test was used to confirm the normal distribution of continuous variables. The Fisher's exact test and the Student's t-test for independent variables were used to compare gender and closure times between patients and controls, respectively. Linear regression method based on least squares estimation was used for the evaluation of possible correlations between the continuous variables.

Two-tailed P values were evaluated. Statistical significance was set as P values less than 0.01. Analysis was performed with the use of the SPSS 8.0 for Windows statistical software package.

RESULTS

Results are summarized in Table 1. Mean closure times obtained by the PFA-100™ were significantly different between patients and controls in the case of the collagen-ADP cartridges (P<0.001) but not in the case of the collagen-EPI cartridges (P=0.078).
A statistically significant correlation (r=0.696; P=0.003) was observed between PFA-100™ closure times with collagen-ADP cartridges and PFA-100™ closure times with collagen-EPI cartridges in patients with pancreatitis (Figure 1). In these patients, gender (P=0.390 and P=0.457), age (P=0.515 and P=0.968), etiology (P=0.511 and P=0.805), APACHE II score (P=0.700 and P=0.832), CRP (P=0.848 and P=0.993), and hematocrit (P=0.665 and P=0.178) were not significantly correlated with either collagen-ADP or collagen-EPI closure times, respectively.

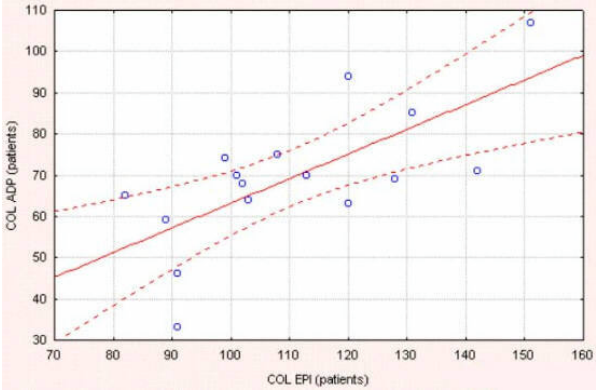

Figure 1. Correlation between collagen-EPI and collagen-ADP closure times (r=0.696; P=0.003). The solid line represents the basic line evaluated by the linear regression method (collagen-ADP closure time = 3.293 + 0.599 * collagen-EPI closure time). The dotted lines represent the ±95% confidence interval.

DISCUSSION

It has been already stated that platelets are directly involved in the systemic inflammatory process of acute pancreatitis which leads to consumption compensated by an immediate bone marrow response [7]. In this study, clinical interest is focused on the fact that the onset of acute pancreatitis is characterized by an elevated activation platelet ratio, as has been shown by flow cytometry, which is inversely correlated with age and is more pronounced in alcoholic disease, while remission of the disease is accompanied by the elevation of all platelet indices, especially in alcoholic disease and in episodes of augmented severity [7].
Taking into consideration the fact that acute pancreatitis is accompanied by thrombosis of the pancreatic microvasculature and potent microangiopathy, the demonstration of increased platelet adhesiveness and aggregation may provide further explanation for the pathophysiology of the process. Moreover, it has been shown that stimuli which activate platelets lower their size both experimentally [11] and clinically in case of acute pancreatitis [7]. The latter is not well-explained, though it could be attributed to circulating platelet microparticles. These platelet microparticles, which constitute fragments of intact platelets, are mainly formed in autoimmune diseases or situations affecting microvasculature, have a substantially smaller size and are characterized by high thrombogenicity due to the exposure of new phospholipids on their surface [12].
In the present study, measurements carried out by PFA-100™ showed a statistically

Table 1. Comparison of closure times obtained by the PFA-100™ between patients and controls. Mean±SD and 95% confidence intervals are shown.

	Patients (n=16)	Controls (n=32)	t- value	P value
Collagen-ADP closure time (s)	69.6±17.1 (60.4-78.7)	96.1±8.8 (93.0-99.3)	7.2	<0.001
Collagen-EPI closure time (s)	110.7±19.9 (100.1-121.3)	119.7±14.1 (114.6-124.8)	1.8	0.078

significant shortening of the collagen-ADP closure time, but not of that of the collagen-EPI, in patients versus controls, although collagen-EPI has been found to strongly correlate with collagen-ADP in acute pancreatitis patients. This confirms increased platelet adhesiveness and aggregation [13] in the early stages of the inflammatory process of acute pancreatitis, which may underline the prethrombotic potential of the disease. The reason why EPI-dependent platelet activation gives no significant results might depend on the milder effects of this activator on platelet aggregation.

Although the use of PFA-100^{TM} closure time is common practice in investigating hemorrhagic diathesis, we hereby propose its utility in the opposite case, namely the prethrombotic state following acute pancreatitis. Our study correlates, for the first time, the shortening of PFA-100^{TM} closure time values with augmented thrombotic tendency. This also emphasizes the usefulness of PFA-100^{TM} in the evaluation of thrombotic states.

Since a balance of 15% in reproducibility has been reported [10], we performed duplicated analysis for routine use of PFA-100^{TM}. Moreover, the sensitivity of the PFA-100^{TM} method in detecting borderline alterations of the platelet function seems largely dependent on the establishment of an in-house normal range [14]. We therefore used a control group consisting of 32 normal volunteers (equal to the double of the size of the patient sample), which allowed us to delineate sufficiently accurate reference values for our laboratory.

We preferred to use an external control group rather than examining samples from the same patient at a later stage, as there was no safe process which would abolish the misleading effect of secondary, confusing parameters interfering with the platelet function analyzer results during the disease process (disseminated intravascular coagulation and primary fibrinolysis as well as plasma and blood transfusions and low molecular weight heparin administration). Furthermore, the use of a control group of similar age and gender distribution, remains a very valuable tool. The use of an external control group is emphasized in cases of unpredictable biological processes (a platelet function analyzer has never been used before to evaluate augmented platelet adhesiveness/aggregation and prethrombotic state and the case of pancreatitis is the first application on that field).

Moreover, PFA-100^{TM} closure time is affected by the hematocrit, platelet count and platelet function [15]. In the present study, all patients had a hematocrit less than the upper reference value and, in a previous study [7], the platelet count was shown to be comparable between patients and controls. However, platelet function is markedly increased in the onset of acute pancreatitis [7].

In summary, PFA-100^{TM} represents a simple and easy-to-use test for investigation of primary hemostasis. Using this method, we demonstrated that, in the early stages of acute pancreatitis, platelet adhesiveness and aggregation is increased, contributing to the prethrombotic potential of the disease. Furthermore, we demonstrated the potential usefulness of PFA-100^{TM} in studying thrombotic states.

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Abbreviations beta-TG: beta thrombo-globulin; EPI: epinephrine; PFA: platelet function analyzer; PF4: platelet factor 4; TXA_2: thromboxane A_2

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References

1. Kerekes L, Arkossy P, Altorjay I, Huszka M, Kappelmayer J, Toth P, et al. Evaluation of hemostatic changes and blood antioxidant capacity in acute and chronic pancreatitis. Hepatogastroenterology 2001;48:1746-9. [PMID 11813615]

2. Lukaszyk A, Bodzenta-Lukaszyk A, Gabryelewicz A, Bielawiec M. Does acute experimental pancreatitis affect blood platelet function? Thromb Res 1989;53:319-25. [PMID 2524119]

3. Musselman DL, Marzec UM, Manatunga A, Penna S, Reemsnyder A, Knight BT, et al. Platelet reactivity in depressed patients treated with paroxetine. Arch Gen Psychiatry 2000;57:875-82. [PMID 10986551]

4. Collins CE, Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. Gastroenterology 1994;106:840-5. [PMID 8143990]

5. Danese S, de la Motte C, Sturm A, Vogel JD, West GA, Strong SA, et al. Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. Gastroenterology 2003;124:1249-64. [PMID 12730866]

6. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroumbakis IE, et al. Mean platelet volume: a useful marker of inflammatory bowel disease activity. Am J Gastroenterol 2001;96:776-81. [PMID 11280550]

7. Mimidis K, Papadopoulos V, Kotsianidis J, Filippou D, Spanoudakis E, Bourikas G, et al. Alterations of platelet function, number and indexes during acute pancreatitis. Pancreatology 2004;4:22-7. [PMID 14988655]

8. Michelson AD, Furman MI. Laboratory markers of platelet activation and their clinical significance. Curr Opin Hematol 1999;6:342-8. [PMID 10468151]

9. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer-PFA-100. Semin Thromb Hemost 1995;21:106-12. [PMID 7660150]

10. Wuillemin WA, Gasser KM, Zeerleder SS, Lämmle B. Evaluation of a Platelet Function Analyser (PFA-100®) in patients with a bleeding tendency. Swiss Med Wkly 2002;132:443-8. [PMID 12457302]

11. Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation: methodological issues. Platelets 2002;13:301-6. [PMID 12189016]

12. Ault KA. The clinical utility of flow cytometry in the study of platelets. Semin Hematol 2001;38:160-8. [PMID 11309697]

13. Gosselin RC, Paglieroni T, Owings J, Utter G, Jacoby R, Larkin EC. Decreased PFA-100 values is associated with increased platelet activation. In: 7th Annual Meeting of the European Hematology Association. Florence, Italy, 6-9 June 2002. Bologna, Italy: Monduzzi Editore, International Proceedings Division, 2002. [ISBN: Book 88-323-2606-X - CD-ROM 88-323-2607-9]

14. Mammen EF, Comp PC, Gosselin R, Greenberg C, Hoots WK, Kessler CM, et al. A new method of assessment of platelet dysfunction. Semin Thromb Hemost 1998;24:195-202. [PMID 9579642]

15. Escolar G, Cases A, Vinas M, Pino M, Calls J, Cirera I, et al. Evaluation of acquired platelet dysfunctions in uremic and cirrhotic patients using the platelet function analyzer (PFA-100TM): influence of hematocrit elevation. Haematologica 1999;84:614-9. [PMID 10406903]