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Evaluation of acquired platelet dysfunctions in uremic and cirrhotic patients using the platelet function analyzer (PFA-100™): influence of hematocrit elevation

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Abstract

Background and Objective. **Patients with end-stage renal disease or advanced cirrhosis develop bleeding disorders characterized by defective interaction of platelets with damaged subendothelium. The anemia associated with both clinical entities has a negative influence on hemostasis. We evaluated alterations of platelet function in patients suffering from end-stage renal disease (n=21) or hepatic cirrhosis (n=20) using standard aggregometric techniques and the recently developed platelet function analyzer (PFA-100™). The impact of low hematocrit was also analyzed.**

Design and Methods. **The hemostatic capacity of platelets was tested in the PFA-100™ using citrated blood and standard cartridges containing collagen-ADP (COL-ADP) or collagen-epinephrine (COL-Epi). The hemodynamic influence of hematocrit was also evaluated in blood aliquots in which hematocrit was experimentally increased by adding red blood cells from the same patient.**

Results. **Aggregation studies demonstrated abnormal responses to several agonists in both group of patients. Closure times obtained by the PFA-100™ for control blood samples were 87±3 sec for COL-ADP and 113±5 sec with COL-EPi cartridges. Closure times in uremic and cirrhotic patients with average hematocrits of 0.26 and 0.27 respectively were significantly prolonged (139±12 and 125±14 sec, respectively with COL-ADP and 194±29 and 151±15 sec with COL-Epi cartridges). A 5% increase in the hematocrit caused a reduction in the closure time to 111±7 sec (COL-ADP) and 143±14 sec (COL-Epi) in the uremic group and to 86±4 sec (COL-ADP) and 115±16 sec (COL-Epi) in the cirrhotic group. Our studies confirm the platelet dysfunction in uremic and cirrhotic patients.**

Interpretation and Conclusions. **The PFA-100™ device proved to be useful for testing alterations of primary hemostasis in these acquired disorders and was sensitive enough to detect modifications in hemostasis caused by elevations in hematocrit. Conventional aggregometric tests were able to identify the intrinsic platelet abnormality in uremic and cirrhotic conditions, while the PFA-100™ seemed more sensitive in detecting the negative influence of the hematocrit reduction.**

Key words: bleeding, platelets, hematocrit, uremia, cirrhosis

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Excessive bleeding is a common complication of patients with end stage renal or hepatic diseases. The existence of a platelet dysfunction has been implicated in the development of bleeding symptoms in both uremia[1-3] and cirrhosis.[4-7] A platelet adhesion defect, more evident in studies under flow conditions, has been described in uremic[8] and cirrhotic[9] patients. The nature of the acquired platelet dysfunction in both clinical entities is not yet fully understood. The most plausible explanation is that both defects have a multifactorial origin.[2,6,10,11]

A complex defect of platelet function has been described in cirrhotic patients; impaired TxA_2 synthesis,[12-14] defective signal transduction,[15] presence of a storage pool disease[16] and quantitative defects in platelet GPIb[17] among others, have been implicated in the cirrhotic thrombocytopathy. Alterations in coagulation mechanisms associated with cirrhosis can further impair the overall process of hemostasis in these patients.

The platelet functional defect in uremia shares common features with those described for cirrhosis. Altered arachidonic acid metabolism,[18] defective cytoskeletal assembly,[19] presence of a storage pool defect[17,20] and quantitative or qualitative defects in platelet glycoproteins[21,22] have also been said to be involved in the pathophysiology of uremic platelet dysfunction. A variable degree of thrombocytopenia and anemia is usually present in uremic and cirrhotic patients. The rheological effects of erythrocytes are

of critical importance for platelet function,[23] a factor often disregarded in conventional laboratory tests used for the assessment of primary hemostasis.

The PFA-100™ test system is a microprocessor controlled instrument which emulates *in vitro* the platelet dependent phase of primary hemostasis.[24,25] It can be used to detect abnormalities of primary hemostasis in small samples of anti-coagulated whole blood. The present study was designed to investigate alterations of primary hemostasis in uremic and cirrhotic patients, while delineating the role of the rheological alteration caused by the low hematocrit often present in both clinical entities. The platelet hemostatic capacity (PHC) of patients with uremia or cirrhosis was evaluated with the PFA-100™. Standard turbidimetric procedures were used to control the platelet aggregometric abnormalities in these disorders. The influence of an *in vitro* elevation of hematocrit on PHC in these patients was also assessed using the PFA-100™ instrument.

Design and Methods

Patients

The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki. After approval of the study protocol by the Ethical Committee of the Hospital Clinic, informed consent was obtained from all participants. Sixty-one subjects (20 healthy controls, 21 uremic and 20 cirrhotic patients) were enrolled in the study. The diagnosis of the original disease was established by history, physical examination, laboratory findings and kidney or liver biopsy when not contraindicated. Presence of malignancies, recent gastrointestinal bleeding, hepatic encephalopathy, or evidence of active disseminated intravascular coagulation were conditions for exclusion.

All the uremic patients included in our study had been subjected to maintenance hemodialysis for more then one year. Cirrhotic patients had been classified as Child-Pugh A and B following criteria already established.[26] A group of 20 normal blood donors was used as controls. Descriptive information about the different study groups is provided in Table 1. All subjects included in our study were carefully interviewed to exclude any possible intake of drugs known to affect platelet function in the 10 days preceding our investigations.

Blood sampling and experimental design

Whole blood was obtained by venipuncture and anticoagulated with 3.8% buffered citrate (129 mM/L; Becton Dickinson Systems Eur. Meylan Cedex, France). Blood from uremic patients was always collected immediately before the hemodialysis session. Blood was divided into two aliquots. The first aliquot was used for baseline PFA-100™ testing and for hematologic determinations in a Coulter MDII Series Analyzer (Coulter Corporation, Miami, FL, USA). The second aliquot was centrifuged to produce platelet rich plasma (PRP) employed for routine aggregometric procedures. A portion of the pellet obtained after PRP separation was added to the first aliquot to raise the hematocrit by 5% and the blood tested again in the PFA-100™.

Table 1. Characteristics of the different study groups.

	Control subjects	*Uremic patients*	*Cirrhotic patients*
Number of subjects	20	21	20
Sex (male/female)	11/9	14/7	15/5
Age (years)	45±9	51±10	49±12
Platelets (x10^9/L)	193±44	163±54*	126±57°$^{\#}$
Hematocrit (L/L)	0.387±0.031	0.27±0.036**	0.267±0.025°

*Values expressed as mean ± standard deviation. *p< 0.05 and °p< 0.01 vs control values;. #p<0.05 vs uremic group.*

Platelet aggregation

PRP was obtained by centrifugation of citrated blood for 20 min at 100× g. Platelet poor plasma (PPP) was separated by centrifugation at higher speed (1,200× g, 20 min). Aggregation studies were carried out in a Hitachi Aggrecorder (Kyoto Dai-Ichi Kagaku Co. Ltd, Kyoto, Japan). All samples of PRP were adjusted to 150-180,000 plts/µL and placed in 6-mm wide siliconized cuvettes. The minimum and maximum amplitudes of the aggregometer were adjusted with PRP (0% transmission) and PPP (100% transmission). The ability of PRP to aggregate was tested with: 1.4 mmol/L arachidonic acid (Menarini Diagnostics, Florence, Italy), 1 µmol/L thromboxane mimetic U46619 (Upjohn Co., Kalamazoo, MI, USA), 1-4 µmol/L ADP (Menarini Diagnostics, Barcelona, Spain), 2.5 µg/mL collagen (Menarini Diagnostics, Barcelona, Spain), and 0.8-1 mg/mL ristocetin (Sigma Chemical Co., St Louis, MO, USA). Fifty microliters of the activating agents were added to 450 µL of PRP, under stirring. Results were expressed as percentages of maximal aggregation obtained after 5 min of stimulation.

Hemostatic capacity in the Platelet Function Analyzer

Platelet function was also analyzed using the PFA-100™ system.[24] Basically, the system monitors platelet interaction on collagen-ADP (COL-ADP) or collagen-epinephrine (COL-Epi) coated membranes. Samples of citrated blood are aspirated under controlled flow conditions through a 150 µm aperture cut into the membrane. The platelet hemostatic capacity (PHC) of the blood sample is indicated by the time required for the platelet plug to occlude the aperture (closure time) expressed in seconds.[25]

Statistical analysis

Student's t test was used for statistical comparisons. A *p* level of 0.05 was considered as statistically significant.

Results

Descriptive data of the patient groups

Numbers of subjects included and descriptive data of the different study groups expressed as mean ± standard deviation are provided in Table 1. Platelet counts were below control levels (193±44$\times 10^9$/L, mean±SD) in the different groups, being 163±54 $\times 10^9$/L in the uremic patients and 126±57 $\times 10^9$/L in the cirrhotic group. Platelet counts in the cirrhotic groups were statistically lower ($p<0.05$) with respect to the uremic group. Hematocrit levels were significantly decreased in both group of patients compared with control subjects. No statistical differences were observed between the hematocrits in the uremic (0.27±0.036 L/L) or cirrhotic groups (0.267±0.025 L/L).

Platelet aggregation studies

Standard turbidimetric studies demonstrated consistent abnormalities in the responses to aggregating agents in both groups of patients (Table 2). The abnormalities affected almost all agents tested.

Hemostatic capacity

The PHC of each sample was assessed as a function of the time required for the platelet plug to occlude the aperture (closure time). Closure times obtained with the PFA-100™ in control blood samples were 87±4 sec (mean±SEM) for COL-ADP and 113±5 sec with COL-EPi cartridges. Two different levels of confidence could be determined arithmetically by adding 1 or 2 standard deviations to the mean values. For the COL-ADP cartridge (SD=18) the 105 sec interval included 68% of the observations, and the 123 sec interval included 95% of the observations. Similarly, two levels could be calculated for the COL-EPi cartridges (SD=24), being respectively 137 sec and 161 sec.

Closures times in uremic (Figure 1) and cirrhotic patients (Figure 2) with average hematocrits of 26 and 27% respectively, were significantly prolonged (139±12 and 125±14 sec, respectively with COL-ADP and 194±29 and 151±15 sec with COL-Epi cartridges; mean±SEM). Elevation of the hematocrit levels (up to an average 31%) resulted in shortening of the closure time to 111±7 sec (COL-ADP) and 143±14 sec (COL-Epi) in the uremic group and to 86±4 sec (COL-ADP) and 115±16 sec (COL-Epi) in the cirrhotic group.

Discussion

Results obtained in the present study confirm the existence of platelet dysfunction in uremic and cirrhotic patients. The PFA-100™ device proved to be useful for testing alterations of primary hemostasis in these acquired disorders and was sensitive enough to detect modifications in hemostasis caused by elevation of the hematocrit level.

In our study, both groups of patients analyzed

Table 2. Platelet aggregation studies.

	Control subjects	*Uremic patients*	*Cirrhotic patients*
Arachidonic acid (1.4 mM)	93±6	58±33°	52±26°
U46619 (1 µM)	91±7	65±22°	52±22°
ADP (4 µM)	87±9	69±21°	57±25°
ADP (2 µM)	85±10	61±21°	48±21°
ADP (1 µM)	54±26	46±19*	37±21*
Collagen (2.5 µg/mL)	89±9	64±29°	53±22°
Epinephrine (20 µM)	82±19	56±26°	39±27°
Ristocetin (1 mg/mL)	64±14	49±21*	37±23°
Ristocetin (0.8 mg/mL)	48±13	37±17*	26±12°

*Values expressed as % of maximal aggregation (mean ± standard deviation). *p< 0.05 and °p< 0.01 vs control values*

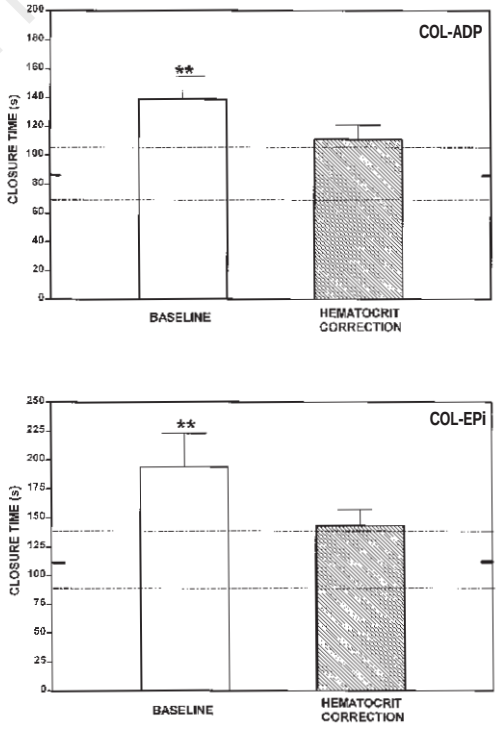

Figure 1. Bar diagrams represent closure times detected in the group of uremic patients with the two cartridges tested (COL-ADP and COL-EPi). Each bar represents average values ± SEM. Ticks denote average values in the control group while the discontinuous lines delineate 1SD range of normality. ($p<0.01$ vs control group).**

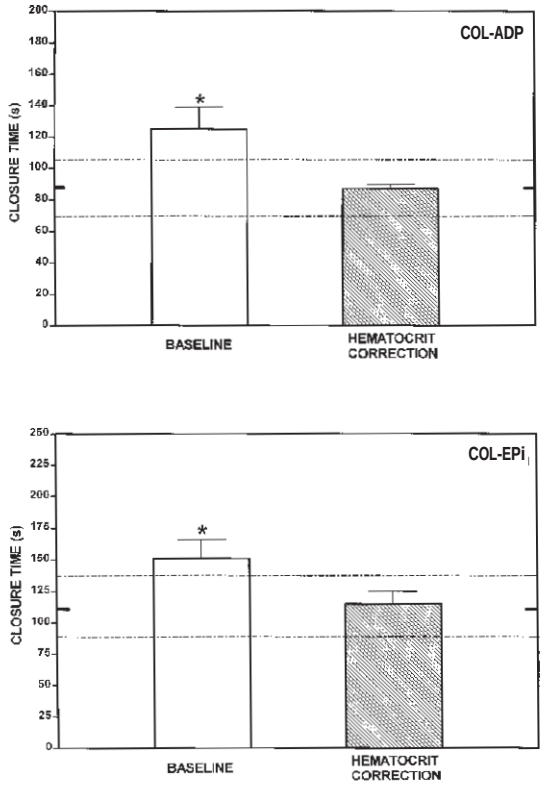

Figure 2. Bar diagrams represent closure times detected in the group of cirrhotic patients with the two cartridges tested (COL-ADP and COL-EPi). Each bar represents average values ± SEM. Ticks denote average values in the control group while the discontinuous lines delineate 1SD range of normality. *p<0.05 vs control group.

showed altered patterns of platelet aggregation which were consistent with abnormally prolonged closure times as measured by the PFA-100™. The PFA-100™ was thus sensitive enough to detect an alteration of primary hemostasis concurrent with the uremic and cirrhotic conditions.[3,6] Although abnormal closure times were detected with both cartridges, the sensitivity of the COL-Epi seemed slightly better than that of COL-ADP especially in the uremic group of patients. Despite platelet counts in this group of patients being greater than those of cirrhotic patients, the PHC seemed more affected in the uremic group. The *in vitro* results of our present study suggest that the intrinsic alteration of primary hemostasis detected using the PFA-100™ could be more severe in the uremic patients.

Anemia is a frequent complication in both uremic and cirrhotic patients. Red blood cells play an important role in facilitating the interaction of platelets with subendothelial surfaces.[23,27] There is abundant information concerning the beneficial effect of hematocrit elevation on primary hemostasis in uremic patients.[28-30] The information on this approach in cirrhotic patients is scarce. Our present study indicates that the elevation of hematocrit might be of clinical benefit in both clinical conditions.

The fact that PHC in the PFA-100™ were almost normalized in both group of patients after elevation of the hematocrit suggests that this device is sensitive enough to detect the negative influence of a reduced hematocrit in hemostasis.[27] Although the rise in hematocrit improved the platelet hemostatic capacity in both uremic and cirrhotic patients, the improvement in hemostasis seemed more evident in the cirrhotic group. To us, the differential response to the increase of hematocrit levels observed in uremic or cirrhotic patients provides indirect evidence of the different pathophysiological mechanisms involved in the platelet dysfunction in the two conditions.

In fact, the favorable effect on the PFA-100™ closure time after elevation of the hematocrit in the cirrhotic group is partially in conflict with previous results from our group. In a recent study, we were able to detect abnormal platelet adhesion in cirrhotic patients when their blood was maintained under flow conditions through annular chambers containing denuded arterial segments.[9] Interestingly, in those studies the experimental increase of hematocrit did not improve platelet-subendothelial interactions in the cirrhotic patients. Possibly, variations in shear rates in the different experimental settings may account for the discrepancies. While our previous studies in perfusion devices were performed at 800 sec^{-1}, the shear rate obtained in the PFA-100™ system ranges from 5000 to 6,000 sec^{-1}.[24] Such variations in shear rate may have a dramatic impact on platelet interactions with subendothelial components, and the consecutive aggregation process associated with formation of the occlusive plug.[31]

The sensitivity of the new instrument in detecting borderline alterations of platelet function seems largely dependent on the establishment of an in-house normal range.[32] In our study, the range of normality was determined concomitantly in a group of 20 normal donors who were first investigated to exclude underlying abnormalities of primary hemostasis. Analysis of the data allowed us to delineate two intervals of confidence at 1 and 2 SD which by definition include 68% or 95% of the observations respectively.

The simplicity of the PFA-100™ system would facilitate its use as a primary screening test for congenital or acquired defects of primary hemostasis, once the limits of normality have been calculated. If the closure times fall within +1SD, no further test should be required. If closures times are within 1SD and 2SD, repetition of the test and additional studies are required.[33] Closure times above 2SD unequivocally identify the existence of a congenital or acquired

abnormality of primary hemostasis that requires exhaustive investigation. It must be emphasized that the PFA-100™ offers global information on primary hemostasis, that cannot replace the qualitative information provided by conventional aggregometry.

In summary, our studies confirm the existence of a platelet dysfunction in uremic and cirrhotic patients. Conventional aggregometric tests were able to identify the intrinsic platelet abnormality in uremic and cirrhotic conditions. The PFA-100™ device proved to be useful for testing alterations of primary hemostasis in these acquired disorders and appeared more sensitive in detecting modifications in hemostasis caused by elevations in hematocrit. The PFA-100™ system may become an useful tool for an overall evaluation of primary hemostasis.

Contributions and Acknowledgments

GE was primarily responsible for the conception of this investigation and production of the article. AC, JC and IC provided the clinical assessment of the patients, and participated in the design of the study. MV and MP performed the experiments and collaborated in the analysis of data. AO participated in the discussion of the results.

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Disclosures

Conflict on interest: Dade Behring provided the PFA-100™ instrument and the test cartridges free of charge.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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