**Nephrology
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Original Article

Impaired expression of glycoproteins on resting and stimulated platelets in uraemic patients

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Abstract

Background. The increased bleeding tendency of chronic renal failure (CRF) patients has been attributed to platelet dysfunction. However, reports on various platelet functions in uraemic patients have been conflicting. The present study sought to analyse platelet function by examining their surface glycoproteins in well-identified populations of CRF patients.

Methods. Three groups were studied: 22 chronic haemodialysis (HD) patients, 25 conservatively treated patients with CRF and 30 controls. Bleeding tendency was assessed by measuring bleeding time and by recording current haemorrhagic symptoms. We measured the fibrinogen receptor GPIIbIIIa, the von Willebrand receptor GPIb, and P-selectin levels on the platelet surface using flow cytometry.

Results. Forty percent of CRF and 45% of HD patients had bleeding. The bleeding time was similar in the HD and CRF groups, but was longer in both groups than in controls. In resting platelets, GPIb expression was lower in CRF patients than in controls. In stimulated platelets (i) GPIb expression was higher in HD patients than in both controls and CRF patients; and (ii) GPIIbIIIa and P-selectin expression were lower in CRF and HD patients than in controls.

Conclusions. These findings indicate that uraemic platelets are hyporesponsive to stimulation.

Keywords: bleeding; flow cytometry; glycoproteins; platelets; uraemia

Introduction

The bleeding tendency of patients with uraemia is characterized by haemorrhagic symptoms and by abnormal prolongation of bleeding time. This bleeding tendency has been attributed classically to abnormalities of platelet function that include impaired adhesion [1] and decreased aggregation [1,2]. In turn, the pathogenesis of the platelet dysfunction is multifactorial and includes intrinsic platelet defects [3,4], anaemia [1], uraemic toxins [1,5], von Willebrand factor [4] and vessel abnormalities [4,6]. Recently, platelet dysfunction was addressed specifically by analyses of platelet surface glycoproteins (GPs). The main platelet glycoproteins are glycoprotein Ib (GPIb) and glycoprotein IIbIIIa (GPIIbIIIa). The total platelet GP content is divided between the membrane and intracellular compartments. GPIb, which is also the von Willebrand factor receptor, is involved in platelet adhesion to the endothelium. The GPIIbIIIa complex is a calcium-dependent heterodimer, composed of one molecule of GPIIb and one molecule of GPIIIa. This complex, also identified as the fibrinogen receptor, is involved in platelet aggregation. In uraemic patients, total platelet GPIb content generally is decreased [2]. However, platelet membrane GPIb levels have been reported to be normal [2,7], decreased [8–10] or increased [11]. Similarly, GPIIbIIIa expression has been described as normal [10,12], decreased [7,13] or increased [8,9]. Moreover, the total content of GPIIb and GPIIIa was normal in uraemic platelets [4,12].

This disparity in results was due to differences in assessment methods and to heterogeneous subject populations that included haemodialysis (HD) patients, peritoneal dialysis patients and undialysed uraemic patients. Moreover, patients with diseases influencing platelet function were not always excluded.

Accordingly, the purpose of this study was, first to assess bleeding tendency in well-identified populations,

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matched for sex and age, that excluded patients with confounding factors that may influence platelet function, and secondly to analyse platelet surface GPs and platelet aggregation in these populations.

Patients and methods

Patients

The principal characteristics of the three subject groups are presented in Table 1. The first two groups were composed of uraemic patients from the out-patient unit of Sainte-Marguerite Hospital. Twenty-five patients with chronic renal failure (CRF) were placed in the first group. They had serum creatinine $> 200\,\mu$mol/l and did not require dialysis.

Twenty-two uraemic patients on chronic maintenance HD for > 6 months were placed in the second group. Mean time on haemodialysis treatment was 48 ± 59 months. The HD procedure was the same for all patients. Patients underwent HD for 4–5 h thrice weekly with cellulose diacetate hollow fibre dialysers and heparin anticoagulation. HD was performed using standard machines with bicarbonate dialysate. Blood flow was maintained at 250–300 ml/min and dialysate flow was set at 500 ml/min. Of the drugs taken by HD and CRF patients, phosphate binders, calcitriol, cholecalciferol, iron and folate were especially prominent. Antihypertensive therapies included calcium blockers, β-blockers and angiotensin-converting enzyme inhibitors.

The third group (controls) included 30 healthy volunteers with normal haematocrit and renal function. Controls were chosen from health centres, and fit the criteria for subjects in good health (Centre d'Examens de Santé DORIA, Centre d'Investigations Cliniques in Marseilles). Sex and age characteristics were the same in the three groups.

None of the subjects had diabetes mellitus, immunological disease, cancer, acute or chronic infection, nephrotic syndrome, primary haemostasis disorder unrelated to uraemia, or a transplanted organ. None had received drugs that interfere with haemostasis (except erythropoietin). The subjects had stopped taking antiplatelet agents such as aspirin or nonsteroidal anti-inflammatory drugs 14 days before study onset.

Table 1. Patient characteristics

	Controls	CRF	HD
No. of subjects	30	25	22
Sex (male/female)	17/13	19/6	14/8
Age (years)	50 ± 14	53 ± 17	44 ± 14
Serum creatinine (μmol/l)	91 ± 14	428 ± 280	
Creatinine clearance (ml/min)[a]	77 ± 21	21 ± 2	
Nephropathies			
Glomerular		7	5
Vascular		1	3
Hereditary		4	3
Interstitial		0	1
Undetermined		13	8
Drugs			
Erythropoietin	0	0	8
Calcium blockers	0	4	12
β-blockers	1	7	2

[a]Creatinine clearance was calculated using the Gault and Cockcroft formula.

The patients were not taking steroids and none had been transfused 6 months before the study. Platelet count, varying from 150–400 Giga/l, was normal in all subjects. The protocol was approved by the ethics committee, and both patient and control volunteers gave written informed consent to participate in the study.

Haemorrhagic symptoms

Haemorrhagic symptoms occuring during the 14 days before the investigation were defined as current symptoms and were collected in the three groups. They included epistaxis, haematuria, ecchymosis, purpura, bleeding from the gums, gingival bleeding, genital bleeding, haemoptysis, telangiectasis, haemarthrosis and petecchiae.

Bleeding time

Bleeding time was measured using the Ivy incision technique on the antecubital face of the forearm under a counterpressure of 4 cm of Hg. This procedure was performed using a commercial device (Simplate R, Organon Teknika, Durham, NC). All bleeding time measurements were performed by the same investigator, before venipuncture and HD and before administration of heparin. For in-patients with vascular access, bleeding time was measured on the opposite forearm.

Blood sampling

Blood samples were obtained from fasting subjects. A 30 ml aliquot of blood was collected into siliconized tubes (Vacutainer®, Beckton Dickinson, San Jose, CA) from a forearm venipuncture using 21 gauge needles in CRF and control subjects. In HD patients, blood was drawn from the access needle (17 or 18 gauge) before a dialysis session. The first 2 ml of blood was collected into tubes containing 0.11 M EDTA to determine platelet count and haematocrit. A 6 ml aliquot of blood was collected into dry tubes to measure serum creatinine in CRF and control subjects. A 7 ml aliquot of blood was collected into a second dry tube to measure serum parathyroid hormone (PTH). A final 15 ml aliquot was collected into four tubes, containing 0.129 M sodium citrate in a ratio of 9 vols blood to 1 vol. of anticoagulant, to study haemostatic parameters (platelet aggregation, plasma fibrinogen) and to quantify membrane platelet glycoproteins.

Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at room temperature for 10 min at 180 g. Platelet-poor plasma (PPP) was obtained by centrifugation of whole blood at room temperature for 10 min at 2500 g.

Flow cytometric analysis of surface antigen expression

Membrane expression of glycoproteins GPIIbIIIa, GPIb, GPIIIa and P-selectin was analysed in whole blood samples during the 3 h following the blood collection. Stimulated platelet phenotype was determined after incubation of 50 μl of whole blood with 450 μl of 200 μM ADP (BioData Corporation, Hatboro, PA) or 450 μl of 50 μM thrombin receptor-activating peptide (TRAP) [thrombin receptor agonist TR (42–55), Human, Neosystem Laboratoire, Strasbourg, France] for 10 min at room temperature.

Resting platelet phenotype was determined after incubation of 50 μl of whole blood with 450 μl of buffer alone for 10 min at room temperature. For each condition, a 50 μl sample of final solution was then labelled with 10 μl of monoclonal antibodies (mAbs) used under saturating conditions for 10 min at room temperature. The mAbs were anti-GPIIbIIIa (anti-CD41, P2 clone, isotype IgG1, Immunotech, Marseilles, France), anti-GPIIIa (anti-CD61, SZ21 clone, isotype IgG1, Immunotech), anti-GPIb (anti-CD42b, SZ2 clone, isotype IgG1, Immunotech), anti-P-selectin (anti-CD62P, AK6 clone, Cymbus, UK) and irrelevant control mAb (ST40 clone, isotype IgG1, Immunotech). The samples were then incubated for 10 min at room temperature with fluorescein isothiocyanate (FITC)-conjugated sheep polyclonal anti-mouse Ig (DDAF, Silenus, Melbourne, Australia). Finally, blood samples were fixed with 1% paraformaldehyde for 10 min and diluted in buffer for flow cytometric analysis.

Samples were analysed by flow cytometry on an Epics XL (Coultronics, Margency, France). This analysis was focused on platelets that were identified by their forward and right angle scatter features. The mean fluorescence intensity was quantified on the whole population and collected by using logarithmic amplification. Corrected fluorescence intensity was obtained by subtracting irrelevant control fluorescence from the mean fluorescence intensity measured for each mAb. These values were then converted into mAb-bindings site per cell through a quantitative indirect immunofluorescence (QIFI) method.

QIFI method

The QIFI method is based on the relationship between fluorescence intensity measured by flow cytometry and antigenic expression. The coefficient of variation of the QIFI method was 4% for replicates analysed on the same day. The reliability of the method was assessed by comparison with direct radiobinding studies. The sensitivity threshold of quantification is nearly 500 antigens per platelet.

We used a kit for assessment of the main platelet GPs using quantitative flow cytometry (CytoQuant™ Gp, Diagnostica Stago, Asnières, France). Briefly, calibrated beads having a size similar to platelets with known amounts of murine IgG were labelled with the second layer FITC-conjugated polyclonal antibody. These labelled beads were used as standards to construct a regression curve between fluorescence intensity and the number of mAb-binding sites per platelet. When mAbs are used under saturating conditions, the number of binding sites of mAb molecules corresponds to the number of available antigenic sites.

Aggregation studies

Platelet aggregation studies were carried out in a four-channel aggregometer (Platelet aggregation profiler model PAP-4, BioData Corporation, Hatboro, PA) while stirring PRP aliquots at 37°C. ADP (10 μM), collagen (1.9 μg/ml), sodium arachidonate (500 μg/l) and ristocetin (1.5 mg/ml) were used as inducers (BioData Corporation, Hatboro, PA). Platelet aggregation with the four platelet agonists was assessed by determining the maximal platelet aggregation percentage. Collagen aggregation was also assessed by the latent period between addition of the aggregating agent and the onset of the aggregation phase, expressed in minutes.

Blood cell count, fibrinogen, serum creatinine and serum PTH

Blood cell count, fibrinogen and serum creatinine assays were performed using automated instruments. PTH measurement was performed with an immunoradiometric assay.

Statistical analysis

Data analysis was performed with SPSS software, version 8.0 (SPSS Inc Chicago, IL). All results were expressed as mean ± SD. A *P*-value < 0.05 was considered statistically significant. Qualitative unpaired clinical data were compared between two or three groups by Fisher tests or χ^2 tests. Quantitative data with normal distributions were compared by parametric tests, and data with abnormal distributions or with poor numbers ($n < 16$) were analysed by non-parametric tests. Multiple groups were analysed by analysis of variance (ANOVA) or by Kruskall–Wallis tests. In the case of significant main effects, *post hoc* analysis was performed using Student's *t*-tests for the ANOVA, or the Wilcoxon test for the Kruskall–Wallis tests. Comparisons between two groups were performed by Student's *t*-test or Mann–Whitney *U*-tests. Univariate analysis was performed using Pearson's correlation.

Results

Assessment of haemorrhagic symptoms

Haemorrhagic symptoms in CRF and HD patients included ecchymosis, gingival and genital bleeding, epistaxis, petecchiae and telangiectasis. Bleeding was found in 10 patients in the CRF group (40%), in 10 patients in the HD group (45%) and in three controls (17%). The percentage of subjects with bleeding was significantly higher in both the CRF and HD groups than in controls ($P = 0.009$ and $P = 0.003$, respectively).

Nineteen haemorrhagic symptoms were observed in the 25 CRF patients, 15 in the 22 HD patients, and three in the 30 controls. Ecchymosis, the most frequent haemorrhagic symptom, was found in 32% of the CRF group, in 41% of the HD group, and in 6% of the control group. Ecchymoses were significantly more frequent in the CRF group ($P = 0.014$) and in the HD group ($P = 0.004$) than in controls.

Assessment of primary haemostasis

Bleeding time. Bleeding time was 10.6 ± 6.1 min in the HD group, 8.4 ± 3.4 min in the CRF group and 4.7 ± 0.9 min in the control group. These bleeding times were similar in the HD and CRF groups, which were both significantly longer than in controls ($P = 0.0001$ for the two tests) (Figure 1).

Surface antigen expression in the basal state. Although control platelets showed more GPIIbIIIa than those of uraemic platelets, this difference was not statistically significant. GPIIIa did not differ between the three groups. The expression of GPIb was lower on
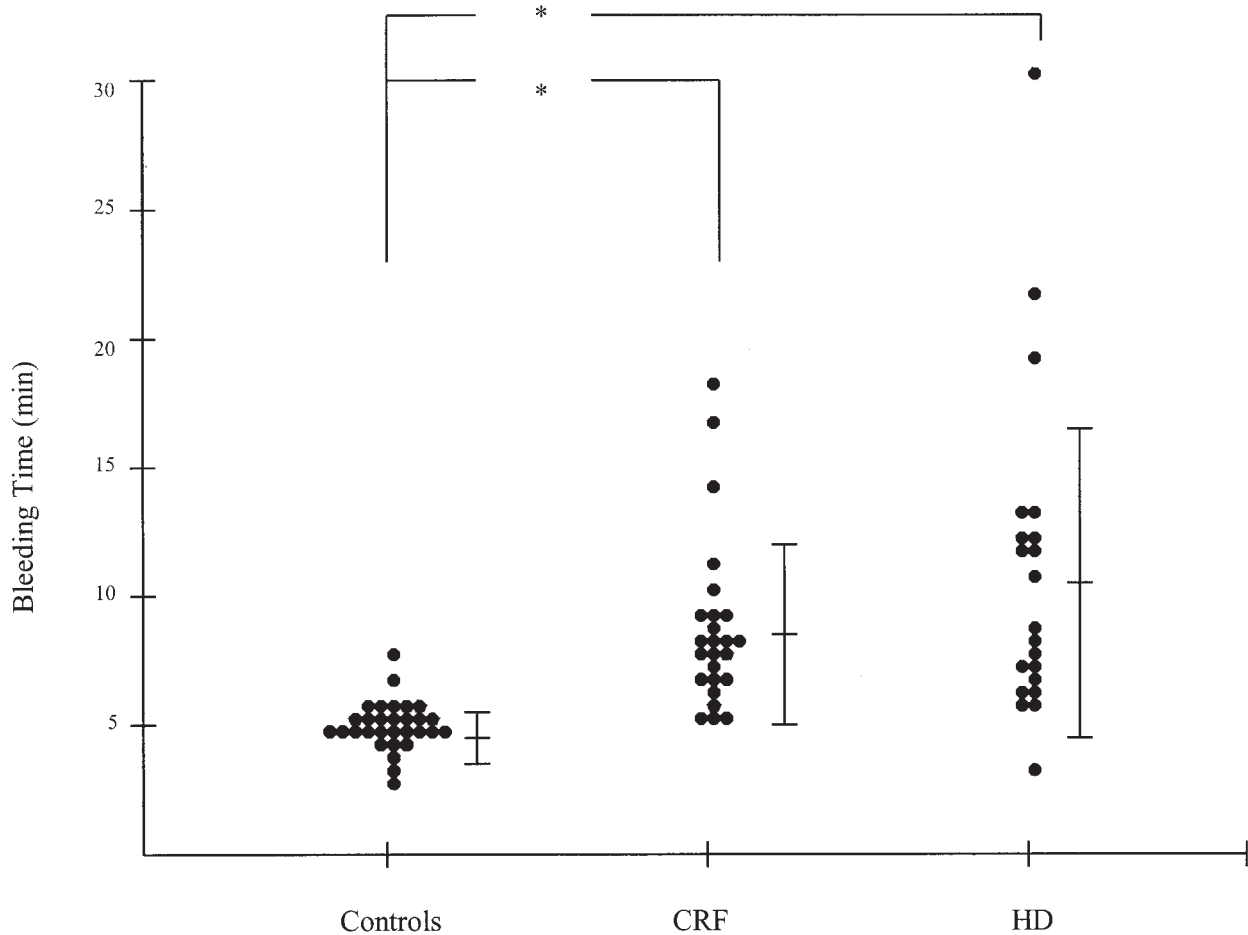
Fig. 1. Bleeding time. Significance was estimated using Kruskall–Wallis tests. CRF, chronic renal failure patients; HD, haemodialysed patients. $*P = 0.0001$ compared with controls.

platelet membranes in CRF patients than in controls [$29 \pm 6.5 \times 10^3$ binding site antibodies per platelet (BSAP) in CRF vs $33.5 \pm 8 \times 10^3$ BSAP in controls, $P = 0.02$]. The expression of GPIb was lower on platelet membranes in HD patients ($30 \pm 6.5 \times 10^3$ BSAP) than in controls, but this difference was not statistically significant ($P = 0.07$). P-selectin was not detectable on platelet membranes in any of the three groups (Figure 2).

In summary, basal state platelets in HD patients had the same phenotype as in the control group. In contrast, platelets in the CRF group expressed significantly fewer GPIb antigens on their membranes than those of the controls.

Surface antigen expression after platelet stimulation with ADP. Expression of GPIIbIIIa was lower on platelet membranes in the CRF ($P = 0.023$) and HD ($P = 0.01$) groups than in controls ($69 \pm 11.5 \times 10^3$ BSAP in CRF, $67.5 \pm 16.5 \times 10^3$ BSAP in HD, and $77.5 \pm 12 \times 10^3$ BSAP in controls). The CRF and HD groups did not differ from each other. GPIIIa and GPIb expression did not differ between the three groups. In all groups, stimulation with ADP led to the appearance of membrane platelet P-selectin

expression, causing an increase from the basal state. P-selectin expression did not differ between the three groups (Figure 2).

In summary, stimulation of platelets with ADP in CRF and HD patients caused significantly less GPIIbIIIa membrane expression than in control platelets.

Surface antigen expression after platelet stimulation with TRAP. Expression of GPIIbIIIa was lower on platelet membranes in the CRF ($P = 0.016$) and HD ($P = 0.002$) groups than in controls ($78 \pm 16.5 \times 10^3$ BSAP in CRF, $77 \pm 17 \times 10^3$ BSAP in HD vs $88.5 \pm 11.5 \times 10^3$ BSAP in controls). There was no difference between the CRF and HD groups. GPIIIa expression did not differ significantly between the three groups. Expression of GPIb was higher on platelet membranes in the HD group than in both the CRF and control groups ($17.5 \pm 4.5 \times 10^3$ BSAP in HD group vs $15 \pm 4.5 \times 10^3$ BSAP in CRF, $P = 0.049$; and $12.5 \pm 4 \times 10^3$ BSAP in the control group, $P = 0.0004$). There was no difference between the CRF and control groups. In all three groups, stimulation with TRAP led to the appearance of membrane platelet P-selectin expression, causing an
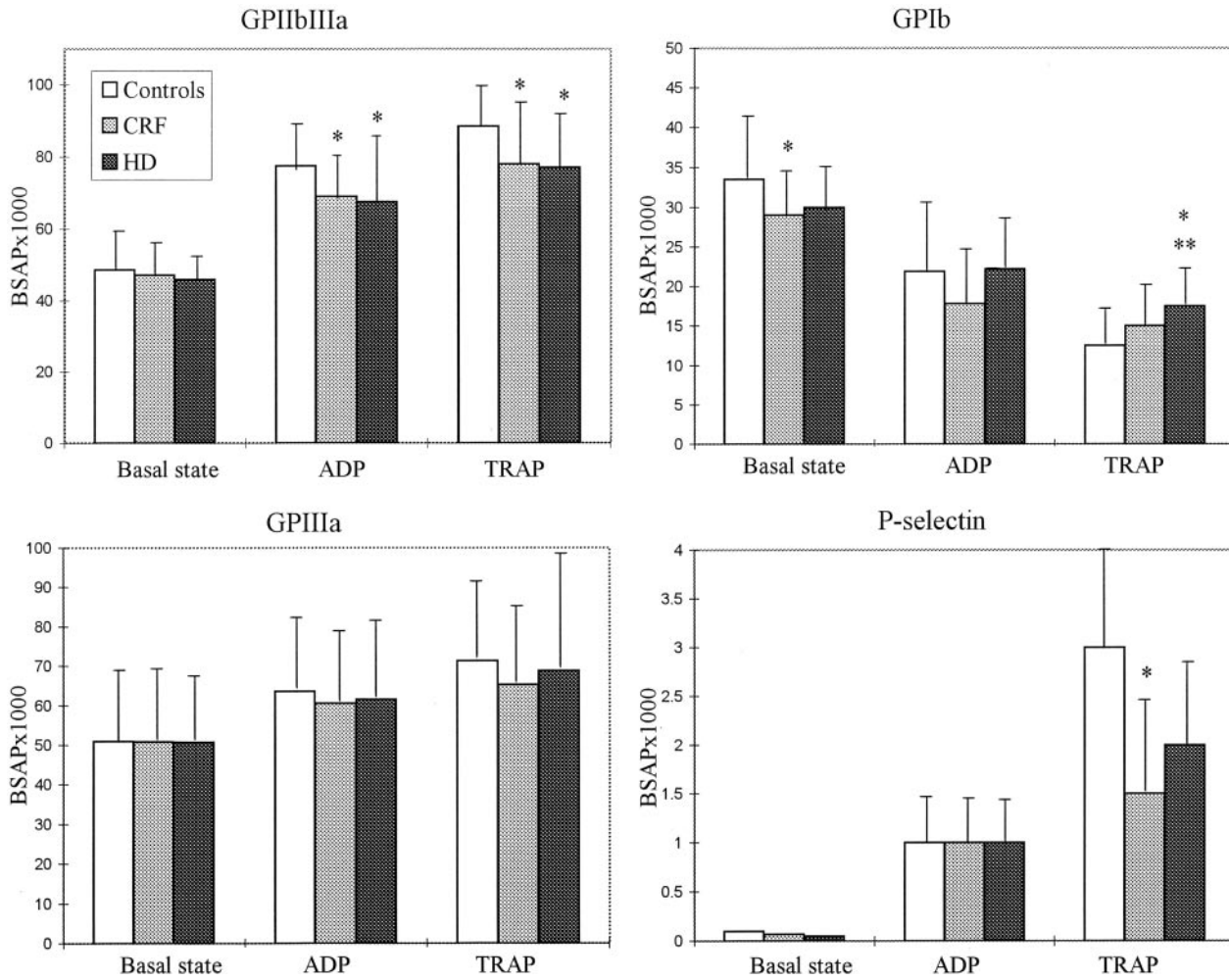
Fig. 2. Expression of platelet membrane glycoproteins in the basal state and in response to ADP and TRAP in controls, CRF (chronic renal failure) and HD (haemodialysed) patients. Quantities of platelet glycoproteins are expressed as the number of binding site antibodies per platelet (BSAP). Significance for GPIIbIIIa on ADP- and TRAP-stimulated platelets and for GPIb and P-selectin on TRAP-stimulated platelets was estimated using Wilcoxon's tests. Significance for GPIb on resting platelets was estimated with Student's *t*-tests. *$P < 0.05$ compared with controls; **$P < 0.05$ compared with CRF group.

increase from the basal state. P-selectin expression was lower on platelet membranes in CRF patients ($P = 0.0008$) than in controls ($1.5 \pm 1 \times 10^3$ BSAP in CRF *vs* $3 \pm 1 \times 10^3$ BSAP in controls). Although expression of P-selectin was lower on platelet membranes in HD patients ($2 \pm 1.5 \times 10^3$ BSAP) than in controls, this difference was not statistically significant ($P = 0.056$) (Figure 2).

In summary, stimulation of platelets with TRAP in CRF and HD patients caused significantly less GPIIbIIIa antigen membrane expression than in control platelets. The expression of P-selectin was lower in the CRF group than in controls, and the expression of GPIb was higher in the HD group than in both the CRF and control groups.

Platelet aggregation. Haemodialysed patients had a decreased maximum percentage of platelet aggregation caused by ristocetin (Table 2). CRF and HD patients showed an increased latent period for collagen aggregation (1.3 ± 0.47 min in HD, $P < 0.05$ *vs*

Table 2. Platelet aggregation

	Controls	CRF	HD
ADP (%)	77 ± 11	82 ± 9	76 ± 15
Collagen (%)	75 ± 8	78 ± 10	71 ± 11
Sodium arachidonate (%)	64 ± 20	64 ± 15	63 ± 13
Ristocetin (%)	82 ± 8	81 ± 9	$71 \pm 16*$

Data are means $\pm$ SD.
*$P = 0.0007$ compared with controls. Significance was estimated using Student's *t*-tests.

1.58 ± 0.56 min in CRF, $P < 0.05$ *vs* 0.88 ± 0.24 min in controls, as determined by Kruskall–Wallis tests). In the CRF group, the latent period for collagen aggregation correlated with serum creatinine levels ($P = 0.042$, $r = 0.409$).

Platelet aggregation in the CRF and HD groups was normal in the presence of ADP and sodium arachidonate. In contrast, aggregation was abnormal in the presence of collagen in the CRF and HD groups, and

Table 3. Blood cell count and fibrinogen

	Controls	CRF	HD
Platelet count (10^3/mm^3)	227 ± 68	232 ± 54	223 ± 49
Haematocrit (%)	42 ± 4	32 ± 4*	33 ± 4*
Fibrinogen (g/l)	3.22 ± 0.52	4.5 ± 1.3*,+	3.84 ± 1.39
Leukocyte count (/mm^3)	6623 ± 2200	6296 ± 2757	6214 ± 1400

Data are means ± SD.
*$P = 0.0001$ compared with controls; +$P < 0.05$ compared with HD group. Significance was estimated using Student's *t*-tests for haematocrit and Kruskall–Wallis tests for fibrinogen.
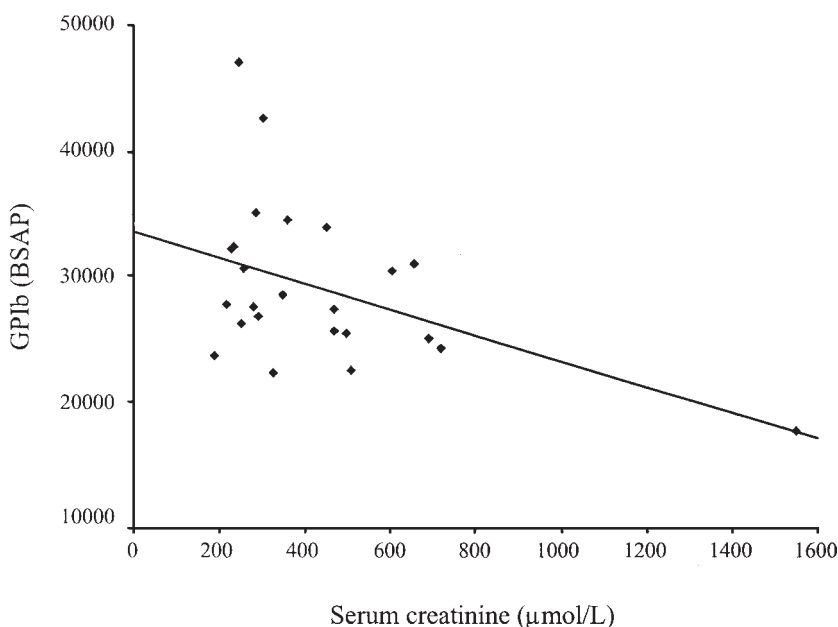

Fig. 3. Simple linear correlation between platelet membrane GPIb levels in the basal state and serum creatinine in the CRF (chronic renal failure) group. The quantity of platelet GPIb is expressed as the number of binding sites of mAb per platelet (BSAP). Correlation was performed by Pearson's analysis. $r = -0.454$ ($P = 0.023$).

was again abnormal in the presence of ristocetin, but only in HD patients.

Blood cell count and fibrinogen. Table 3 shows platelet counts, haematocrit, leukocyte counts and fibrinogen levels in the three groups.

Determinants of platelet glycoprotein and bleeding time abnormalities. In uraemic patients, we analysed the relationship between platelet glycoproteins and both serum creatinine and serum PTH, which are factors reported to influence platelet function.

In the CRF group, GPIb in the basal state significantly correlated with both serum creatinine ($r = -0.454$, $P = 0.023$) (Figure 3) and serum PTH ($r = -0.428$, $P = 0.033$) (data not shown).

To determine the factors responsible for the prolonged bleeding time in patients, correlation coefficients were calculated between bleeding time and the abnormal platelet GP levels.

In the CRF group, after TRAP platelet stimulation, bleeding time was significantly correlated with GPIb

($r = 0.554$, $P = 0.021$) and with P-selectin ($r = -0.515$, $P = 0.034$).

Discussion

The aim of this study was to assess bleeding tendency and to analyse platelet surface GPs in uraemic patients. The main findings were that (i) several subjects in the CRF and HD groups had bleeding symptoms; (ii) the bleeding time was longer in CRF and HD patients than in controls; (iii) GPIb expression on resting platelets was lower in CRF subjects than in controls, and this reduction correlated with severity of renal failure; (iv) GPIIbIIIa expression on resting platelets was similar in CRF, HD and controls; (v) GPIb expression on stimulated platelets was higher in HD than in controls and CRF; and (vi) GPIIbIIIa and P-selectin expression on stimulated platelets was lower in CRF and HD ($P = 0.056$) than in controls.

Reports on platelet function in uraemic patients have been conflicting. This may be due to the use of heterogeneous populations and to inclusion of patients with treatments or co-morbidities that influence platelet activation. For these reasons, we excluded patients with diabetes mellitus, cancer, infection, immunological disease and those receiving treatment interfering with haemostasis. In addition, we divided patients with uraemia into two groups, according to whether they received conservative or haemodialysis treatment.

Bleeding tendency was assessed by bleeding time and by the occurrence of haemorrhagic symptoms during the 2 weeks preceding the investigation. Haemorrhagic symptoms were 2-fold more frequent in CRF and HD than in controls, and bleeding time was longer in CRF and HD than in controls.

Basal state GPIb expression was lower in CRF than in controls. This finding confirmed previous reports showing reductions in platelet GPIb in CRF [8,9]. According to the literature, this decrease may be explained by four mechanisms. First, a defect in protein synthesis, which is supported by the low total GPIb platelet content reported in CRF [2], may explain the reduced levels. Secondly, loss of membrane protein by proteolytic cleavage may be due to the increased plasmin and thrombin seen in uraemia [2]. Moreover, patients with CRF have increased soluble glycocalicin, the proteolytic cleavage product of the extracellular GPIbα chain [2]. Membrane protein loss may also be due to excessive production of microparticles and loss of membrane fragments exposing GPIb [14], or to an increased proportion of senescent platelets with structural modifications, such as membrane loss and α-granule loss [15]. As the third mechanism, platelet activation after stimulation is usually followed by decreases in GPIb expression because of its translocation to the canalicular system [16]. However, a lack of overexpression of GPIIbIIIa and a lack of P-selectin expression suggest that platelets are not activated spontaneously. Uraemic toxins represent the fourth mechanism. In support of this, we found a relationship between GPIb and serum creatinine; moreover, various substances have been implicated in the impairment of platelet function [13].

Platelet activation was impaired after stimulation with ADP and TRAP in both CRF and HD. This impairment was related to the expression of GPIIbIIIa, P-selectin and GPIb. In normal conditions, platelet stimulation produces increases in GPIIbIIIa [17], the appearance of P-selectin [18], and decreases in GPIb. In the present study, platelet stimulation resulted in lower GPIIbIIIa and P-selectin levels in CRF and HD than in controls, whereas GPIb values were higher in HD than in controls. This is the first report of a simultaneous defect in platelet GPIIbIIIa and P-selectin expression after stimulation in CRF. Previous studies have reported impaired GPIIbIIIa expression after platelet stimulation only in HD [3].

Since both GPIIbIIIa and P-selectin are expressed on the platelet surface following α-granule secretion, the low expression of these glycoproteins after platelet stimulation in CRF and HD may be due to a defect in α-granules. An impaired α-granule release has been reported in uraemia [6]. The expression of GPIIbIIIa may also be decreased because the receptors are occupied by adhesive proteins such as fibrinogen or their fragments [13].

GPIb values after stimulation were higher in HD than in controls. These are the first data reporting GPIb expression after platelet stimulation in HD patients. In addition, the prolongation of bleeding time in CRF and HD was related to GPIb expression after platelet stimulation. This again is the first report of such a relationship. In previous studies, only the influence of spontaneous GPIb expression on bleeding time has been analysed [2]. After platelet stimulation, GPIb expression usually decreases because it translocates to the canalicular system. The elevated GPIb values in HD may be secondary to a defect in GPIb translocation. This defect in turn may be related to an impaired cytoskeleton since GPIb and the membrane skeleton are linked, and since cytoskeleton abnormalities have been reported in HD [19]. These abnormalities have been attributed to repeated platelet stress during HD. Thus, a deleterious effect on the organization of the platelet cytoskeleton would lead to the development of platelet refractoriness [19]. It is also possible that elevated GPIb values are due to the increased proportion of old platelets in HD. This is supported by data showing that the number of reticulated platelets, the youngest platelets in the circulation, is decreased in HD [20]. The authors suggested that HD leads to elimination of young and biologically more active platelets [20].

In conclusion, we found that uraemic platelets are hyporesponsive to stimulation. This hyporesponsiveness occurred in both undialysed patients with CRF and patients treated by maintenance HD, suggesting that HD does not correct platelet abnormalities.

Conflict of interest statement. None declared.

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