High platelet turnover and reactivity in renal transplant recipients patients

High platelet turnover and reactivity in renal transplant recipients patients

Francesca Cesari[1]; Rossella Marcucci[1]; Anna Maria Gori[2]; Roberto Caporale[3]; Alessandra Fanelli[3]; Rita Paniccia[1]; Maria Zanazzi[4]; Elisabetta Bertoni[4]; Aida Larti[4]; Maurizio Salvadori[4]; Gian Franco Gensini[2]; Rosanna Abbate[1]

[1]Department of Medical and Surgical Critical Care, Thrombosis Centre; Center for the Study at Molecular and Clinical Level of Chronic, Degenerative and Neoplastic Diseases to Develop Novel Therapies, University of Florence, Italy; [2]Centro S. Maria agli Ulivi, Fondazione Don Carlo Gnocchi Onlus IRCCS, Impruneta, Florence, Italy; [3]Central Laboratory, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [4]Renal Unit, Careggi University Hospital, Florence, Italy

Summary

Renal transplant recipients (RTRs) patients are at increased risk of cardiovascular morbidity and mortality. We aimed this study to assess reticulated platelets (RP), platelet reactivity and von Willebrand factor (vWF) levels in RTRs patients. In 150 RTRs patients [84 (56%) not on acetylsalicylic acid (ASA) treatment, group A; 66 (44%) on ASA 100 mg treatment, group B] and in 60 healthy control subjects, RP were measured by a Sysmex XE-2100 and were expressed as the percentage of RP of the total optical platelet count (immature platelet fraction; IPF), as the percentage of RP highly fluorescent (H-IPF) and as the absolute number of RP (IPF#). Platelet function was assessed by optical aggregometry (PA) induced by 1 mmol arachidonic acid (AA-PA), 2 and 10 μM ADP (ADP2-PA and ADP10-PA) and 2 μg/ml collagen (Coll-PA). vWF levels were measured by using a miniVidas analyser. Group A and group B showed significant higher values of RP than controls. At a multiple linear regression analysis IPF and IPF# were significantly and positively related to collagen-PA. By analysing group B according to residual platelet reactivity (RPR), we observed a significant higher number of RP among patients with RPR by collagen. Moreover at a multiple logistic regression analysis, IPF# significantly affected the risk of having a RPR by collagen. With regard to vWF, RTRs patients showed higher levels than control subjects. We documented a higher platelet turn-over in both groups of RTRs patients and increased platelet reactivity in RTRs patients not on ASA therapy than controls.

Keywords

Reticulated platelets, platelet reactivity, renal transplantation

Correspondence to:
Francesca Cesari, MSc
Department of Medical and Surgical Critical Care
Thrombosis Centre, University of Florence
Azienda Ospedaliero-Universitaria Careggi
Viale Morgagni 85, 50134 Florence, Italy
Tel.: +39 055 7949420, Fax: +39 055 7949418
E-mail: francesca.cesari@gmail.com

Received: February 18, 2010
Accepted after major revision: June 18, 2010
Prepublished online: August 5, 2010
doi:10.1160/TH10-02-0124
Thromb Haemost 2010; 104: 804–810

Introduction

Despite great improvement in patient and graft survival, the long-term morbidity and mortality in renal transplant recipients (RTRs) are still significant, with a high incidence of cardiovascular disease-related deaths compared with age-matched control populations (1). A number of studies have shown the importance of some classical risk factors such as hyperlipidaemia and hypertension in determining cardiovascular disease in RTRs patients, but the presence of risk factors is not able to completely predict the patient survival (2–3). Various mechanisms can be responsible for the cardiovascular complications in RTRs patients. Disturbances in haemostasis and endothelial dysfunction are common complications of kidney disease, as RTRs patients show an increase of the endothelial cell injury and an activation of coagulation with respect to healthy subjects (4–7). von Willebrand factor (vWF), a multimeric protein which regulates platelet adhesion to the subendothelium, may give a good indication of the grade of endothelial perturbation showing a significant increase among RTRs patients with respect to healthy controls (6). Moreover, platelets from RTRs patients showed an enhanced thromboxane biosynthesis (6) and some immunosuppressive agents commonly used in RTRs patients such as cyclosporine have been found to be associated with increased platelet procoagulant activity (8–9).

To date, epidemiological studies demonstrated that low-dose ASA therapy can substantially improve renal allograft function and survival so reducing the incidence of cardiovascular complications in RTRs patients (10). Nevertheless, it has been recently reported that the magnitude of antiplatelet effects may be depressed in RTRs patients by the presence of an "aspirin resistance" measured by a point-of-care method (11). The presence of an enhanced platelet turnover, as documented by high levels of reticulated platelets (RP) expressed as immature platelet fraction (IPF), was initially reported in populations of patients with idiopathic thrombocytopenic purpura (ITP) (12–13).

Recently we demonstrated a high rate of platelet turnover as a new mechanism associated with enhanced platelet reactivity in high risk coronary artery disease (CAD) patients on dual antipla-

telet therapy (14). However, to the best of our knowledge data on the presence of RP with particular regard to platelet reactivity and endothelial dysfunction in RTRs patients are not available. Hence, aim of this study was to contemporary assess, in a population of RTRs patients treated or not with acetylsalicylic acid (ASA) and in a population of healthy controls, reticulated platelets, vWF and platelet aggregation.

Methods

Study population

The study population comprised 150 RTRs patients (98male, 52 female) followed according to American Society of Nephrology guidelines (15) with a median age of 50 (17–75) years [group A: 84/150 (56%) not on ASA treatment: group B: 66/150 (44%) on ASA treatment] admitted to the Renal Unit of the Azienda Ospedaliero-Universitaria Careggi at least six months after the renal transplantation. There was no clinical evidence of acute renal graft rejection or proteinuria higher than 1g/24 hours. At the time of the venous blood withdrawal the presence of cytomegalovirus (CMV)-viraemia was investigated using a semiquantitative PCR assay on peripheral blood. There was no clinical or laboratory evidence of acute CMV infection in any patient.

The control population consisted of 60 (41 male, 19 female) healthy subjects with a median age of 52 (21–72) years free from vascular and renal disease selected to be comparable for age and gender with the patients. At the time of blood collection controls were free from the use of ASA or anti-inflammatory agents known to influence platelet function at least from two weeks. In RTRs patients, ASA treatment was given to patients with previous vascular events or in the presence of a documented peripheral vascular disease and/or a renal artery stenosis treated with percutaneous angioplasty and stenting and to patients with the concomitant presence of more than one cardiovascular risk factor.

Current smoking status was determined at the time of blood collection. The subjects were classified as having hypertension according to the guidelines of European Society of Hypertension/ European Society of Cardiology (16) or if they reported taking antihypertensive medications, as verified by the physician. Diabetic subjects were defined in agreement with the American Diabetes Association or on the basis of self-report data (if confirmed by medication or chart review) (17). Dyslipidaemia was defined according to the Third report of the National Cholesterol Education Program (NCEP-III) or if they reported taking anti-dyslipidaemic drugs, as verified by the physician (18). A positive family history was defined as the presence of at least one first-degree relative who had developed CAD before the age of 55 years for men and 65 years for women. All subjects gave informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee.

Blood collection

Venous blood samples anticoagulated by 0.129 M sodium citrate for platelet aggregometry and determination of vWF antigen levels (vWF:Ag) and by EDTA 0.17 M for the evaluation of RP were taken from each patients in the morning, after an overnight fasting.

For vWF:Ag determination, whole blood was immediately centrifuged at 3,000 x g at 4°C for 10 minutes (min) and samples were frozen at –80°C until analysis.

Light transmission aggregometry

Turbidimetric platelet aggregation was used to measure agonist-induced platelet aggregation. Whole blood samples were centrifuged for 10 min at 250 x *g* to obtain platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained on the remaining specimen by further centrifugation at 3,000 x g for 3 min. A platelet count was measured on the PRP and was adjusted between 180 $x10^3/\mu l$ and 300 $x10^3/\mu l$ with PPP. PRP was stimulated with 2 and 10 μM adenosine diphosphate (ADP) (Milan, Italy), with 1 mmol arachidonic acid (AA) (Sigma-Aldrich, Milan, Italy) and with 2 μg/ml collagen (Mascia Brunelli, Milan, Italy) using a APACT 4 aggregometer (Helena Laboratories Italia S.P.A, Milan, Italy).

Platelet aggregation (PA) according to Born's method was evaluated considering the maximal percentage of platelet aggregation in response to different stimuli (ADP2-PA, ADP10-PA, Coll-PA and AA-PA) after 10 min. The intra-assay coefficients of variations (CV) were 4.7% for ADP-PA, 4.4% for AA-PA, and 2.3% for Collagen-PA [19].

We defined patients with residual platelet reactivity (RPR) those with platelet aggregation by AA ≥20% and /or Collagen ≥56% according to the literature (20) and studies from our group (21, 22).

Reticulated platelets

Reticulated platelets were measured as previously described (14) by using the Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan). RP were expressed as a percentage of RP of the total optical platelet count (immature platelet fraction; IPF), as the percentage of RP highly fluorescent (H-IPF) and as the absolute number of RP (IPF#). The intra-assay CV for IPF, H-IPF and IPF# were 10.6%, 18.8% and 10.9% respectively (14).

VWF:Ag levels

VWF:Ag was measured by using a miniVidas analyser (BioMerieux, Lyon, France) by following manufacturer's instructions. The intra- and inter-assay CV are 3.4 % and 7.7%, respectively.

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Windows (Version 13.0). Values are presented as median and range.

The Mann-Whitney test for unpaired data was used for comparison between two groups, while the Kruskal-Wallis test for unpaired data was used for comparison among different groups. A correlation analysis for non-parametric data (Spearman's Test) was performed to establish relationships between IPF, H-IPF, IPF#, agonist-induced platelet aggregation and vWF. After logarithmic transformation, a multivariate linear regression model adjusted for cardiovascular risk factors, previous myocardial infarction, haematocrit, platelet count, ASA, cyclosporine, rapamycin, mycophenolate, tacrolimus, azathioprine and erythropoietin treatment, and glomerular filtration rate (GFR) was used to test the independent associations of RP with platelet reactivity. $P<0.05$ was considered to be statistically significant.

Post-hoc sample size calculation indicated that the number of 84 RTRs patients not on ASA treatment and of 60 controls subjects has a sufficient statistical power (Beta=0.80) to detect a significant difference in platelet aggregation, with an alpha coefficient of 0.05.

Results

Reticulated platelets and platelet aggregation

Demographic, clinical, and laboratory characteristics of the study population are presented in ▶Table 1.

Gender was significantly related to the IPF# parameter; females had significantly higher levels of RP than males [median (range): IPF#: 8300 (3500–20200) vs. 6850 (1700–22900) platelets/μl, p=0.007].

RP were positively related with mean platelet volume (MPV) [IPF and MPV r =0.58 p<0.0001; H-IPF and MPV r=0.59 p<0.0001; IPF# and MPV r=0.66 p<0.0001] and negatively related with platelet count [IPF and platelet count r=-0.18 p=0. 03; H-IPF and platelet count r=-0.27 p=0.001].

By analysing RP values in the three groups, group A showed significant higher values of RP with respect to group B and with respect to controls. Group B showed a significant higher percentage of RP with respect to control subjects (▶Fig. 1).

Notably, a significant difference in platelet aggregation induced by all agonists between group A and controls was observed (▶Table 2). Among cardiovascular risk factors, only hypertension significantly affected AA-PA in group A [median (range): AA-PA 90 (10–100) vs. 85 (19–93) p=0.04].

In the whole patient population, significant positive correlations between reticulated platelets and PA induced by collagen, AA and ADP were found [IPF and Coll-PA: r=0.28, p=0.001; H-IPF and Coll-PA: r=0.26, p=0.001, IPF# and Coll-PA: r= 0.22, p=0.007; IPF and AA-PA: r=0.22, p=0.009; H-IPF and AA-PA: r=0.17, p=0.04; ADP2-PA and IPF: r=0.18, p=0.028; ADP10-PA and IPF: r=0.16, p=0.04].

In order to evaluate the influence of reticulated platelets on platelet aggregation we performed a linear regression analysis. At the univariate model IPF was significantly and positively related with platelet aggregation by AA [$\beta \pm$ SE: lnAA and lnIPF 0.44 ± 0.17, p=0.01] but at the multivariate model after adjusting for age, gender, hypertension, dyslipidaemia, diabetes, smoking habit, previous myocardial infarction, haematocrit, platelet count, ASA, cyclosporine, rapamycin, mycophenolate, tacrolimus, azathioprine and erythropoietin treatment and GFR this association lost its significance.

As regarding RP and collagen-induced platelet aggregation, at the univariate model IPF, H-IPH and IPF# were positively and significantly related with Coll-PA (▶Table 3). At a multiple linear regression analysis adjusted for all the confounding factors these associations remained significant for IPF and IPF# (Table 3).

In patients on ASA, RPR by collagen was observed in 39/66 (59.0%) while RPR by AA was observed in 22/66 (33.3%).

Furthermore, by analysing the group B patients according to the presence or not of RPR, we observed a significant higher number of RP in patients with RPR by collagen (▶Table 4).

At a logistic regression analysis IPF and IPF# significantly affected the risk of having a RPR by collagen at the univariate [odds ratio [OR] (95% confidence interval [CI]): Coll and IPF 1.74 (1.13–2.68) p=0.01; Coll and IPF# 4.07 (1.26–13.08) p=0.02] and at the multivariate model [OR (95%CI): Coll and IPF# 4.70 (1.03–21.39) p=0.04].

Relationship between von Willebrand factor, reticulated platelets and platelet aggregation

RTRs patients showed higher levels of vWF with respect to the control population [vWF:Ag: 195 (71–333)% vs. 108 (70–180)% p<0.0001].

A significant difference in vWF factor levels was showed between group A and group B patients [vWF:Ag: 208 (82–311)% vs. 175 (71–333)% p=0.003] and between group B and control subjects [vWF:Ag: 175 (71–333)% vs. 108 (70–180)% p<0.0001]. Moreover, a significant positive correlations were present between vWF:Ag and reticulated platelets [IPF and vWF:Ag r=0.27 p=0.001; H-IPF and vWF:Ag r=0.20 p=0.016] and between vWF:Ag and AA-PA and Coll-PA [AA-PA and vWF:Ag r=0.26 p=0.002; Coll-PA and vWF:Ag r=0.24 p=0.003] in the whole patients population.

Moreover, by analysing the group B patients according to the presence or not of RPR, we observed higher albeit not significant levels of vWF:Ag in patients with RPR by both AA and collagen [RPR by AA 190(71–274) % vs. 164 (71–333)% p=0.35; RPR by collagen 186 (101–333)% vs. 164 (71–274) % p=0.084].

Table 1: Demographic, clinical and laboratory characteristics of the study population. *Values are expressed as median and range. DVT, deep-vein thrombosis; CHD, coronary heart disease; PAD, peripheral arterial disease; ASA, acetylsalicylic acid ; GFR, glomerular filtration rate ; § p<0.005 between group A and group B. # p<0.01 between controls and group A. ° p<0.0005 between controls and group B.

Variable	Group A N=84	Group B N=66	Controls N=60
Age (years) * § #	48 (17–74)	52 (22–75)	52 (21–72)
Males, n (%)	54 (64.3)	44 (66.6)	41 (68.3)
GFR, ml/min*	65 (24–168)	64 (24–152)	
Cardiovascular risk factors			
Hypertension, n (%)# °	65 (77.3)	58 (87.9)	15 (25)
Smoking habit, n (%)	2 (2.4)	1 (1.5)	5 (8.3)
Hypercholesterolaemia, n (%)# °	45 (53.6)	25 (37.9)	7 (11.7)
Diabetes, n(%)§ °	7(8.3)	17 (25.7)	1 (1.6)
Comorbidities			
Previous DVT, n (%)	8 (9.5)	4 (4.8)	
Neoplastic disease, n (%)	12 (14.2)	9 (13.6)	
CHD, n (%)§	0 (0)	10 (15.1)	
PAD, n (%)§	0(0)	6 (9.0)	
Renal artery stenosis, n (%)	4 (4.8)	2 (3)	
Liver disease, n (%)	5 (5.9)	6 (9.1)	
Medications			
Statins, n (%)	27 (32.1)	19 (28.8)	
Antihypertensive agents, n (%)§	48 (57.1)	54 (81.8)	
Cyclosporine, n (%)	68 (80.9)	58 (87.9)	
Mycophenolate, n(%)	47 (55.9)	42 (63.6)	
Corticosteroids, n (%)	79 (94)	59 (89.3)	
Tacrolimus, n (%)	10 (11.9)	8 (12.1)	
Everolimus, n (%)	14 (16.6)	8 (12.1)	
Sirolimus, n (%)	5 (5.9)	0 (0)	
Azathioprine, n (%)	4 (4.8)	4 (6.1)	
Erythropoietin, n (%)	7 (8.3)	7 (10.6)	
Laboratory parameters			
Leucocyte number (x10^3/μl)* #	7.6 (3.3–14.1)	7.0 (2.9–15.7)	6.5 (4.3–11.3)
Red blood cells (x10^6/μl)* #°	4.38 (3.0–7.05)	4.37 (3.14–6.29)	4.91 (3.82–5.60)
Haemoglobin (g/dl)* #°	13.3 (9.9–17.3)	13.3 (10.1–17.4)	14.1 (12.1–16.4)
Haematocrit (%)*#	38.8 (29.8–54.5)	39.8 (30.8–54.7)	41.8 (35.7–45.6)
Platelets (x10^3/μl)*	220 (147–475)	223 (139–449)	212 (154–317)

Discussion

This paper provides new information about the presence of a high platelet turn-over in RTRs patients and increased platelet reactivity in RTRs patients not on anti-aggregating therapy and the relationship between this hyperreactive state and the presence of RP. In addition, RTRs patients not on ASA therapy showed significantly higher levels of vWF with respect to RTRs patients on ASA therapy, and a positive relationship was observed between RP, platelet aggregation by AA and collagen and vWF levels.

Cardiovascular disease is the major cause of death among RTRs and various factors can contribute to induce its susceptibility (23, 24). The presence of cardiovascular risk factors, infections, and direct effects of immunosuppressive agents are able to induce endothelial cells activation (25, 26) with an up-regulation of endothelial adhesion molecules and subsequent adhesion and activation of platelets and leukocytes (27, 28). In our study, among cardiovascular risk factors, hypertension is significantly related to the presence of an increased platelet reactivity induced by AA. In hypertensive patients, a reduction of nitric oxide synthetase (NOS) activity associated with an activation of platelet aggregability has been documented (29) and it is likely that diminished NO bioavailability contributes to an increase platelet activation (30). Interestingly, the presence of diabetes did not influence RP, platelet aggregation or vWF:Ag. This effect is probably due to the low

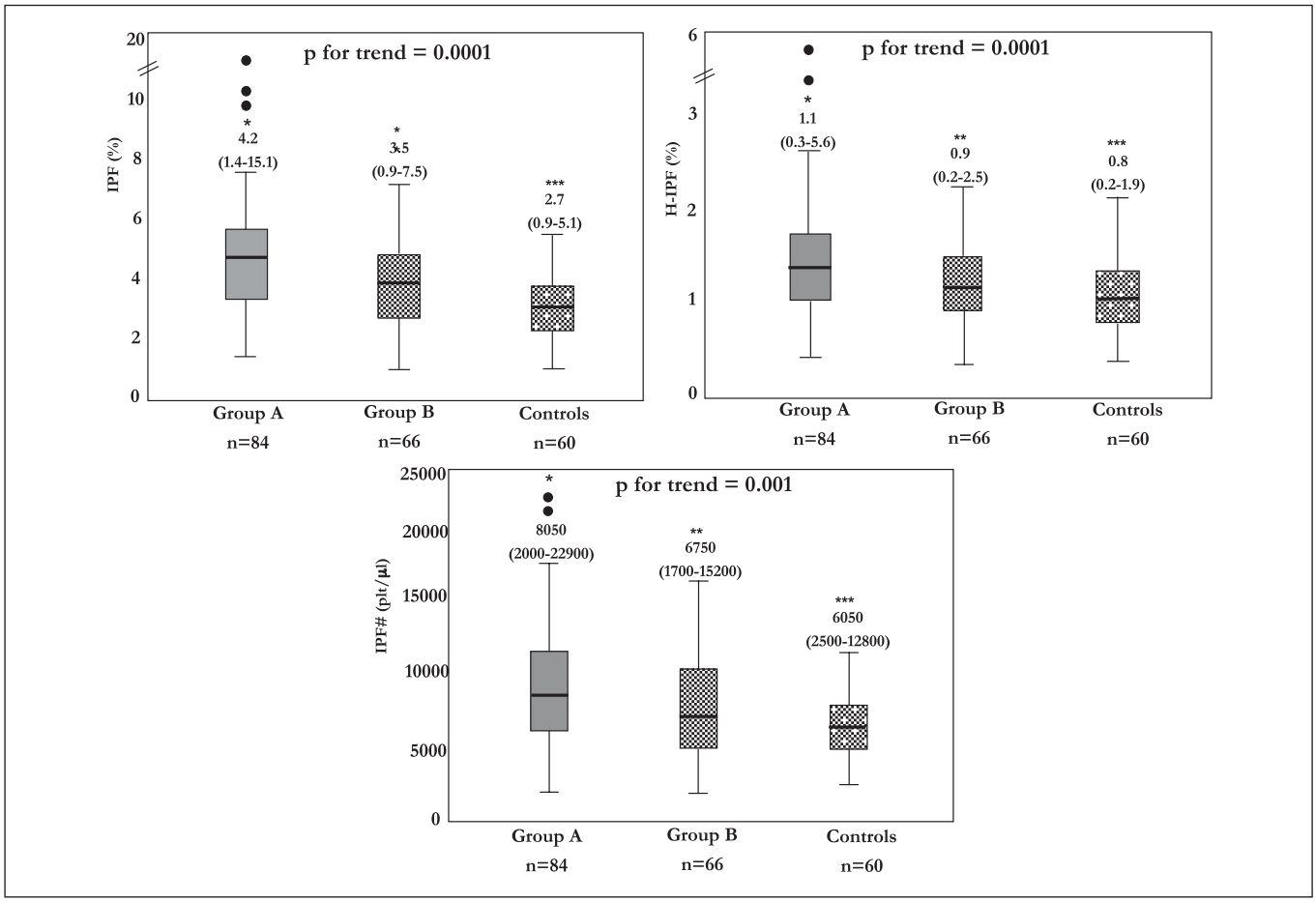

Figure 1: IPF, H-IPF and IPF# in RTRs patients not on ASA treatment (group A), on ASA treatment (group B) and in control subjects. * group A vs. controls: IPF p<0.0001: H-IPF p<0.0001; IPF# p<0.0001. ** group A vs. group B: IPF p=0.003; H-IPF p=0.006; IPF# p=0.017. *** group B vs. controls: IPF p=0.004; H-IPF p=0.092; IPF# p=0.17.

number of diabetic patients or to the predominant effect of other cardiovascular risk factors in RTRs patients.

In the whole patients populations and in particular in RTRs patients not on ASA treatment, we documented a high degree of endothelial perturbation, showed by the increased levels of vWF, which can induce enhanced platelet adhesion to the subendothelium, and consequently increased platelet activation and consumption.

RP are immature platelets with a greater mass and a greater prothrombotic potential with respect to smaller platelets. Indeed, they aggregate more rapidly in response to collagen, have higher levels of intracellular thromboxane A2 as well as increased levels of procoagulant surface protein like P-selectin and GpIIb/IIIa (31–33). Moreover, it has been recently demonstrated that RP are associated with diminished antiplatelet effects of ASA and increased aspirin resistance, possibly due to the increased reactivity and the presence of uninhibited cyclooxygenase (COX)-1 and COX-2 activity (34).

With regard to this issue, we recently reported that a high rate of platelet turnover, as documented by the presence of RP, represents a new mechanism associated with enhanced platelet reactivity in high-risk CAD patients on dual antiplatelet therapy (14).

Similarly, the high rate of platelet turnover in RTRs patients

	RTRs not on aspirin (group A) n=84	RTRs on aspirin (group B) n=66	Controls n=60
Coll-PA*# (%)	86 (41–95)	64 (5–100)	75 (10–91)
AA-PA*# (%)	88 (10–100)	17 (5–92)	80 (10–123)
ADP2-PA*# (%)	82 (9–100)	64 (21–89)	67 (17–96)
ADP10-PA*# (%)	88 (33–100)	80 (34–94)	74 (46–94)

Table 2: Differences on platelet aggregation between RTRs patients not on ASA treatment (group A) and controls. *Values are expressed as median and range. # p<0.0001 group A vs. controls. RTRs, renal transplant recipients.

Table 3: Univariate and multivariate linear regression analysis between reticulated platelets (IPF; H-IPF and IPF#) and collagen-induced platelet aggregation (Coll-PA). *Adjusted for age, gender, hypertension, dyslipidaemia, diabetes, smoking habit, haematocrit, ASA, cyclosporine and erythropoietin treatment, and glomerular filtration rate (GFR).

	Univariate		Multivariate*	
	β ± SE	P	β ± SE	P
LogIPF and LogColl-PA	0.34 ± 0.079	<0.0001	0.22 ± 0.074	0.003
LogH-IPF and LogColl-PA	0.19 ± 0.069	0.007	0.17 ± 0.11	0.145
LogIPF# and LogColl-PA	0.27 ± 0.070	<0.0001	0.20 ± 0.067	0.008

Table 4: Differences in IPF, H-IPF and IPF# according to RPR.

	Without RPR by collagen n=27	With RPR by collagen n=39	P	Without RPR by AA n=44	With RPR by AA n=22	P
IPF (%)	2.9 (0.9–6.2)	3.9 (1.8–7.5)	0.009	3.5 (0.9–6.3)	3.3 (1.0–7.5)	0.88
IPF# Platelets/μl	5600 (1700–10600)	7300 (3100–15200)	0.03	7150 (2100–11100)	6500 (1700–15200)	0.62
H-IPF (%)	0.8 (0.2–1.9)	1.0 (0.3–2.5)	0.12	1.0 (0.3–2.5)	0.8(0.2–2.2)	0.30

documented in the present paper is independently associated with the risk of a collagen-mediated residual platelet reactivity, despite of ASA treatment. These results are in keeping with a recent observation of "aspirin resistance" documented in 40 RTRs patients with a percentage (27.5%) similar to our finding (33%) (11).

The number of newly produced circulating platelets depends on the stimulation of the thrombopoietic capacity of bone marrow as well as on the platelet removal from the bloodstream through an activation-dependent mechanism that enhances platelet turnover. In RTRs patients both mechanisms can be evoked in determining the number of reticulated platelets. In particular, the use of medications such erythropoietin can promote a release of RP from the bone marrow (35, 36); however, in our patients population only a minor percentage received this drug, and at the multivariate model adjusted also for this confounding factor, the associations between RP, platelet reactivity and RPR remained significant.

The presence of a "sticky" endothelium in RTRs patients has been documented in various previous studies, by reporting high levels of intercellular and vascular cell adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, as well as augmented levels of thrombomodulin in RTRs patients (4, 7). Taken together, all these aspects reflect a status of endothelial injury and activation that can play a role in determining an enhanced platelet adhesion and activation, with a consequent consumption of platelets and a compensatory production from the bone-marrow of larger reticulated platelets.

Furthermore, RTRs patients showed increased levels of thrombospondin-1 (TSP1), with respect to a control population (37). TSP1 is abundantly present in platelet alpha-granules, from where it is released upon platelet activation by reinforcing and stabilising platelet aggregates (38). TSP1, locally released by platelets, competes also with ADAMTS13 during VWF proteolysis and controls the degree of VWF multimer processing (39). The higher levels of TSP1 can inhibit the activity of ADAMTS13 and determine higher levels of VWF by supporting the platelet hyper-function.

On the other hand, some cytokines are able to augment megakaryopoiesis quantitatively (40) and in RTRs higher levels of tumour necrosis factor-α, a cytokine which can act in promoting commitment along the megakaryocytic lineage leading to increased thrombopoiesis, have been reported (4).

What is known about this topic?

- Renal transplant recipients (RTRs) patients are at increased risk of cardiovascular morbidity and mortality.
- Epidemiological studies demonstrated that low-dose aspirin therapy can improve renal allograft function and survival so reducing the incidence of cardiovascular complications in RTRs patients.
- Scarce data are available about the platelet function reactivity in RTRs patients, and no data are present about the possible role of reticulated platelets (RP).

What does this paper add?

- We demonstrated a high platelet turnover in RTRs patients and an increased platelet reactivity in RTRs patients not on anti-aggregating therapy.
- RTRs patients not on aspirin therapy showed significantly higher levels of von Willebrand factor (vWF) with respect to RTRs patients on aspirin therapy.
- A positive relationship was observed between RP, platelet aggregation by arachidonic acid and collagen and vWF levels.
- The presence of elevated percentage of RP could be an additional mechanism involved in the increased cardiovascular risk profile of RTRs patients.

Study limitations

Some limitations of our study have to be acknowledged. First, the number of patients investigated is limited. Moreover it remains to be elucidated whether reticulated platelets represent the effect of increased consumption of over stimulated platelets, or the mechanism of platelet hyper-function. Finally, the lack of a follow-up period does not allow us to obtain data on the long-term outcomes and to establish if IPF values could have a role in the cardiovascular prognosis of RTRs patients.

However, this is the first report which investigated reticulated platelets in relation to platelet reactivity in the clinical setting of RTRs patients, so documenting an enhanced platelet turnover associated with increased vWF and platelet function.

The presence of elevated percentage of reticulated platelets could be an additional mechanism involved in the increased cardiovascular risk profile of RTRs patients. If future studies performed in the same clinical setting confirm our data, antiplatelet prophylaxis should be more widely used as a useful tool in reducing the risk of cardiovascular complications in RTRs patients.

References

1. Foley RN, Parfrey PS, Samak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998; 32: 112– 119.
2. Kasiske BL, Chakkera HA, Roel J. Explained and unexplained ischemic heart disease risk after renal transplantation. J Am Soc Nephrol 2000; 11: 1735–1743.
3. Raine AE. Hypertension and ischemic heart disease in renal transplant recipients. Nephrol Dial Transplant 1995; 10: 95–100.
4. Cottone S, Palermo A, Vaccaro F, et al. Inflammation and endothelial activation are linked to renal function in long-term kidney transplantation. Transplant Int 2007; 20: 82–87.
5. Marcucci R, Zanazzi M, Bertoni E, et al. Risk factors for cardiovascular disease in renal transplant recipients: new insights. Transpl Int 2000; 13S: 419–424.
6. Averna M, Barbagallo CM, Ganci A, et al. Determinants of enhanced thromboxane biosynthesis in renal transplantation. Kidney Int 2001; 59: 1574–1579.
7. Hryszko T, Malyszko J, Malyszko JS, et al. A possible role of thrombin-activatable fibrinolysis inhibitor in disturbances of fibrinolytic system in renal transplant recipients. Nephrol Dial Transplant 2001; 16: 1692–1696.
8. Malyszko J, Malyszko JS, Takada A, et al. Effects of immunosuppressive drugs on platelet aggregation in vitro. Ann Transplant 2002; 7: 55–68.
9. Tomasiak M, Rusak T, Gacko M, et al. Cyclosporine enhances platelet procoagulant activity. Nephrol Dial Transplant 2007; 22: 1750–1756.
10. Grotz W, Siebig S, Olschewski M, et al. Low-dose aspirin therapy is associated with improved allograft function and prolonged allograft survival after kidney transplantation. Transplantation 2004; 12: 1848–1853.
11. Acikel S, Yildirir A, Aydinalp A, et al. Incidence of aspirin resistance and its relationship with cardiovascular risk factors and graft function in renal transplant recipients. Transplant Proc 2008; 40: 3485–3488.
12. Briggs C, Kunka S, Hart D, et al. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol 2004; 126: 93–99.
13. Abe Y, Wada H, Tomatsu H, et al. A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). Thromb Res 2006; 118: 463–469.
14. Cesari F, Marcucci R, Caporale R, et al. Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. Thromb Haemost 2008; 99: 930–935.
15. Kasiske BL, Vazquez MA, Harmon WE, et al. Recommendations for the outpatient surveillance of renal transplant recipients American Society of Transplantation. J Am Soc Nephrol 2000; 11: S1–86.
16. Practice guidelines for primary care physicians: 2003 ESH/ESC Hypertension guidelines. ESH/ESC hypertension guidelines committee. Hypertension 2003; 21: 1779–1786.
17. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003; 26: 5–20.
18. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adult (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143–3421.
19. Paniccia R, Antonucci E, Maggini N, et al. Assessment of platelet function on whole blood by multiple electrode aggregometry in high-risk patients with coronary artery disease receiving antiplatelet therapy. Am J Clin Pathol 2009; 131: 834–842.
20. Gum PA, Kottke-Marchant K, Welsh PA, et al. A prospective, blinded determination of natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003; 41: 961–965.
21. Marcucci R, Gori AM, Paniccia R, et al. Residual platelet reactivity is associated with clinical and laboratory characteristics in patients with ischemic heart disease undergoing PCI on dual antiplatelet therapy. Atherosclerosis 2007; 195: 217–223.
22. Gori AM, Marcucci R, Paniccia R, et al. Thrombotic events in high risk patients are predicted by evaluating different pathways of platelet function. Thromb Haemost 2008; 100: 1136–1145.
23. Pham PT, Pham PC, Danovitch GM. Cardiovascular disease posttransplant. Semin Nephrol 2007; 27: 430–444.
24. Marcén R. Cardiovascular risk factors in renal transplantation--current controversies. Nephrol Dial Transplant. 2006; 21: 3–8.
25. Davis SF, Yeung AC, Meredith IT, et al. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. Circulation 1996; 93: 457–462.
26. Denton MD, Davis SF, Baum MA, et al. The role of the graft endothelium in transplant rejection: evidence that endothelial activation may serve as a clinical marker for the development of chronic rejection Pediatr Transplant 2000; 4: 252–260.
27. Labarrere CA, Lee JB, Nelson DR, et al. C-reactive protein, arterial endothelial activation, and development of transplant coronary artery disease: a prospective study. Lancet 2002; 360: 1462–1467.
28. Reul RM, Fang JC, Denton MD, et al. CD40 and CD40 ligand (CD154) are coexpressed on microvessels in vivo in human cardiac allograft rejection. Transplantation 1997; 64: 1765–1774.
29. Moss MB, de Siqueira MA, Mann GE, et al. Platelet aggregation in arterial hypertension. Is there a nitric oxide-urea connection? Clin Exp Pharmacol Physiol 2010; 37: 167–172.
30. Fatini C, Sticchi E, Bolli P, et al. Platelet aggregability is modulated by eNOS locus in non-type 2 diabetic patients with acute coronary syndrome. Nutr Metab Cardiovasc Dis 2009; doi:10.1016/j.numecd.2009.07.001.
31. Martin JF, Trowbridge EA, Salmon GL, et al. The biological significance of platelet volume: its relationship to bleeding time, platelet thromboxane B2 production and megakaryocyte nucler DNA concentration. Thromb Res 1983; 32: 443–460.
32. Jakubowski JA, Thompson CB, Vaillancourt R, et al. Arachidonic acid metabolism by platelets of differing size. Br J Haematol 1983; 53: 503–511.
33. Tschoepe D, Roesen P, Kauffmann L, et al. Evidence for abnormal platelet glycoprotein expression in diabetes mellitus. Eur J Clin Invest 1990; 20: 166–170.
34. Guthikonda S, Lev EI, Patel R, et al. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. Journal Thromb Haemost 2007; 5: 490–496.
35. Stohlawetz PJ, Dzirlo L, Hergovich N, et al. Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. Blood 2000; 95: 2983–2989.
36. Tàssies D, Reverter JC, Cases A, et al. Effect of recombinant human erythropoietin treatment on circulating reticulated platelets in uremic patients: association with early improvement in platelet function. Am J Hematol 1998; 59: 105–109.
37. Altun B, Usalan C, Haznedaroglu IC, et al. Thrombopoietin and thrombospondin in renal allograft recipients. Blood Coagul Fibrinolysis 1999; 10: 233–237.
38. Bonnefoy A, Hoylaerts MF. Thrombospondin-1 in von Willebrand factor function. Curr Drug Targets 2008; 9: 822–832.
39. Pimanda JE, Ganderton T, Maekawa A, et al. Role of thrombospondin-1 in control of von Willebrand factor multimer size in mice. J Biol Chem 2004; 279: 21439–21448.
40. Li J, Franco RS, Wang Y, et al. Megakaryocytic differentiation of HIMeg-1 cells induced by interferon gamma and tumour necrosis factor alpha but not by thrombopoietin. Cytokine 1998; 10: 880–889.

