Correlation Between Albuminuria and Spontaneous Platelet Microaggregate Formation in Type 2 Diabetic Patients

SHIN-ICHI ARAKI, MD, PHD[1]
HIROYUKI MATSUNO, PHD[2]
MASAKAZU HANEDA, MD, PHD[3]
DAISUKE KOYA, MD, PHD[4]
YOSUKE KANNO, PHD[2]
JUNKO ITHO[2]

AKIO KISHI, MD, PHD[1]
KEIJI ISSHIKI, MD, PHD[1]
TOSHIRO SUGIMOTO, MD, PHD[1]
HIROSHI MAEGAWA, MD, PHD[1]
ATSUNORI KASHIWAGI, MD, PHD[1]
TAKASHI UZU, MD, PHD[1]

OBJECTIVE — Albuminuria in type 2 diabetic patients is a risk factor for cardiovascular disease. We investigated the correlation between albuminuria and spontaneous microaggregation of platelets (SMAP) formed under shear stress.

RESEARCH DESIGN AND METHODS — The study subjects were 401 type 2 diabetic individuals (252 with normoalbuminuria and 149 with albuminuria) who were examined for SMAP under conditions of shear stress only (no agonist stimulation) and the reversibility of platelet microaggregation after stimulation with 1 μmol/l ADP, measured by a laser light-cattering method. Active glycoprotein IIb/IIIa (GPIIb/IIIa) and P-selectin expression levels on platelets as an index of platelet activation were measured by whole-blood flow cytometry.

RESULTS — SMAP formation was noted in 53% of diabetic patients. All patients with SMAP showed an irreversible pattern of platelet microaggregates by a low dose of ADP. SMAP was observed in 75% of diabetic subjects with albuminuria and in 39% of those with normoalbuminuria. Multivariate logistic regression analysis identified urinary albumin excretion rate and brachial-ankle pulse-wave velocity as independent factors associated with SMAP. The degree of SMAP correlated with active GPIIb/IIIa ($\gamma = 0.59$, $P < 0.001$) and P-selectin ($\gamma = 0.55$, $P < 0.001$) expression levels. These early-activated platelet profiles were significantly inhibited in albuminuric patients with aspirin intake, although the effect was incomplete.

CONCLUSIONS — Our study demonstrated an independent association between albuminuria and early changes in activated platelet profiles of type 2 diabetic patients. Further follow-up and intervention studies are needed to establish whether the inhibition of SMAP affects the course of cardiovascular disease in type 2 diabetic patients.

T here is growing evidence that increased urinary albumin excretion rate (AER) in type 2 diabetic patients is both a predictor of progression to chronic renal failure and an independent risk factor for cardiovascular disease (1). The U.K. Prospective Diabetes Study (UKPDS) group demonstrated that type 2 diabetic patients with microalbuminuria or overt proteinuria have a two- to threefold greater risk of cardiovascular death, compared with those patients with normoalbuminuria (2). Furthermore, our group and others recently reported that a reduction of AER in type 2 diabetic patients was associated with a decreased occurrence of cardiovascular complications (3,4). Thus, albuminuria is considered an important therapeutic target for cardiovascular protection. However, the pathophysiological factors underlying this cardiorenal interaction remain unknown. Identifying such factors and developing tools to identify patients at higher risk of both conditions might allow the design of new therapeutic strategies to improve outcomes in type 2 diabetic patients.

Platelet activation and aggregation are fundamental processes in the development of atherosclerosis and thrombosis, both of which contribute to cardiovascular risk (5,6). Platelets aggregate when activated, to form microaggregates of only a few cells as the initial response to various stimuli but large, tighter platelet aggregates appear with prolonged stimulation (7). In diabetic patients, platelets tend to hyperaggregate (8), although previous platelet aggregation measurements by optical density (9) or impedance methods (10) generally reflected the formation of large platelet aggregates in response to exogenous stimulation with various agonists. These conventional methods do not measure platelet microaggregate formation, which occurs at the initial process of platelet activation. Recently, a particle-counting method based on laser light scattering was developed to effectively detect platelet microaggregate formation (11). This new method also measures spontaneous microaggregation of platelets (SMAP), which occurs with a stirring force only and has no requirement for exogenous agonist stimulation. Using this new method, Matsuno et al. (12) reported SMAP as the predominant platelet aggregation event in type 2 diabetic patients. However, this study did not address the association between SMAP and albuminuria.

We therefore investigated the association between changes in early-activated platelet profiles and increased albuminuria in type 2 diabetic patients. The incidence of SMAP was investigated in relation to albuminuria status. To further evaluate the active state of SMAP, we simultaneously measured the cell surface expression levels of two markers of platelet activation: active fibrinogen receptor glycoprotein IIb/IIIa (GPIIb/IIIa), an

• •

important player in platelet adhesion, and P-selectin (CD62p), a transmembrane protein present in the α- (or dense) granules.

RESEARCH DESIGN AND METHODS

The diabetic subjects were recruited from type 2 diabetic patients who regularly visited the outpatient clinic of the Department of Medicine, Shiga University of Medical Science, in 2006–2007. Patients were clinically diagnosed with type 2 diabetes in accordance with the criteria of the World Health Organization. Patients were excluded based on the following criteria: complicating cancer, liver disease, infectious disease, collagen disease, nondiabetic kidney disease confirmed by renal biopsy, intake of nonsteroidal anti-inflammatory drugs within the previous 2 weeks, and intake of antiplatelet drugs with the exception of aspirin. Eligible patients were informed of the study protocol in oral and written forms. A total of 401 patients were finally enrolled in this study. Thirty healthy volunteers were also enrolled as the healthy control group to confirm the results of our previous study (17 male and 13 female, aged 60 ± 7 years [mean $\pm$ SD], BMI 23.1 ± 2.9 kg/m^2, systolic blood pressure 131 ± 9 mmHg, total cholesterol 199 ± 20 mg/dl, triglycerides 109.8 ± 26.4 mg/dl, and taking no medication). Each individual provided a blood sample for biochemical analysis and detection of platelet microaggregation under fasting conditions and underwent standard physical examinations; brachial-ankle pulse-wave velocity (baPWV) was also measured. AER was determined by an immunoturbidimetry assay (Hitachi 7070E; Hitachi High-Technologies, Tokyo, Japan) in a single 24-h urine sample collected on the same day as the blood sample was collected. AER <20 μg/min was considered normoalbuminuria, and AER ≥ 20 was classified as albuminuria. The group with albuminuria comprised 120 patients with microalbuminuria (20 μg/min $\leq$ AER < 200 μg/min) and 29 patients with overt proteinuria (200 μg/min $\leq$ AER). aPWV was measured by an automatic device (BP-203RPE; Colin, Komaki, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the simplified prediction equation proposed by the Japanese Society of Nephrology: eGFR (milliliters per minute per 1.73 m^2) $= 194 \times$ (age [years])$^{-0.287} \times$ (serum creatinine [milligrams per deciliter])$^{-1.094} \times 0.739$ (if female).

The study protocol and informed consent procedure were approved by the Ethics Committee of Shiga University of Medical Science. All participants provided written informed consent.

Detection of platelet microaggregation

Platelet-rich plasma was obtained from blood collected into sodium citrate (14 μmol/l) by immediate centrifugation at 155g for 12 min at room temperature. The platelet-rich plasma was removed and centrifuged at 1,750g for 10 min at room temperature in the presence of prostaglandin I$_2$ (20 μmol/l). The resultant platelets were washed with HEPES-Tyrode buffer and resuspended to 4×10^8 cells/ml. In this platelet suspension, platelets did not form the microaggregates without stirring force or any exogenous agonists. Platelet microaggregation was determined by measuring the light-scattering intensity (13) on a PA-200 aggregometer (Kowa, Tokyo, Japan). SMAP was observed under low shear stress conditions at a stirring speed of 1,000 rpm (26 dyn/cm^2) without stimulation with an exogenous agonist. This degree of low shear stress is considered to be the same degree of shear stress occurring in the arterial stream, suggesting that this system provides a condition that mimicked the state of platelets in the artery. The reversibility of platelet microaggregation in response to a low dose of ADP (1 μmol/l) was also investigated. The data were recorded as a two-dimensional graph showing the change in total light intensity over time, expressed as a cumulative summation at 10-s intervals of scattered light intensity (Ii) and the number of particles corresponding to that intensity (Ni) in terms of particle size (intensity) (ΣIiNi) (volts × counts per second). Particles with an intensity of 25–400 mV represented small aggregates (9–25 μm). The degree of SMAP was described by measurement of the area under the curve (AUC) of each detection line for 5 min. AUC data were expressed as $\times 10^5$ particles. Each measurement was performed twice. All individuals showed identical results as to whether SMAP was formed. The mean values of two AUC measurements as the degree of SMAP in each individual were used for the analysis to minimize the variability.

Quantification of GPIIb/IIIa and P-selectin expression levels

The platelet surface expression levels of GPIIb/IIIa and P-selectin were assessed by whole-blood flow cytometry using a fluorescein isothiocyanate-conjugated PAC-1 antibody and a phycoerythrin-conjugated CD62p antibody, as described previously (14,15). In brief, a saturating antibody concentration in 50 μl was added to 50 μl of whole blood. Negative control samples contained prepared IgG and IgM. The samples were incubated for 15 min at room temperature and then were analyzed within 2 h by flow cytometry. The platelets were identified based on particle size and complexity using a peridimin-chlorophyll-conjugated CD61 antibody. Fluorescence data from 10,000 platelet events were collected. The expression levels were expressed as a percentage of the positively identified platelets. All antibodies were purchased from BD Biosciences (San Jose, CA).

Statistical analysis

Categorical variables were compared between the two groups using χ^2 tests, an unpaired Student t test was used for normally distributed variables, and the Mann-Whitney U test was used for nonnormally distributed variables. For the nonparametric comparison among four groups, a Kruskal-Wallis test was first performed to find a measure of the aggregate degree to which the group means differed and then the Mann-Whitney U test with Bonferroni correction was used to compare the differences between groups. A Spearman correlation coefficient was used to analyze the association between two variables. A multivariate logistic regression model was applied to evaluate the independence of factors that were significantly different between the groups, using logarithmic transformed values of nonnormally distributed variables. All data were analyzed using the SPSS software package (version 11; SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Platelet microaggregation in diabetic patients

SMAP was detected in 211 (52.6%) of 401 patients with type 2 diabetes (Fig. 1*A*) but not in any of the 30 healthy volunteers tested here (Fig. 1*B*) or in our previous study (12). The diabetic patients with SMAP showed an abnormal and irrevers-
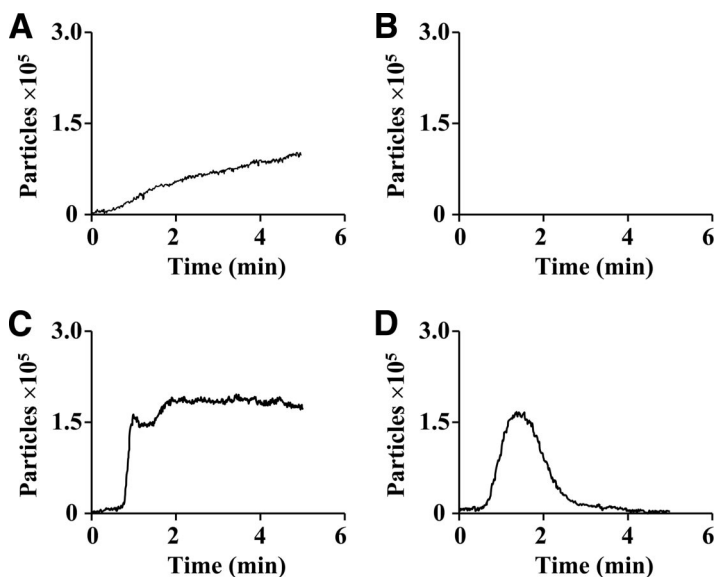
Figure 1—*Typical patterns of SMAP and reversibility of platelet microaggregation by low-dose ADP, measured by a laser light-scattering system. The spontaneous formation of microaggregated platelets under low shear stress alone without any exogenous agonists was observed in 52.6% of diabetic patients (A), whereas it was not observed in 47.4% of diabetic patients and in any healthy volunteers (B). Diabetic patients with SMAP showed irreversible platelet microaggregation in response to ADP (1 μmol/l) (C), whereas the others showed the typical reversible pattern of platelet microaggregation within 5 min (D).*

ible pattern of platelet microaggregation, even after stimulation by a low dose of ADP (Fig. 1*C*), whereas a normal reversible pattern was observed in patients without SMAP and in all healthy volunteers (Fig. 1*D*). Table 1 provides details of the clinical characteristics of the diabetic subgroups according to the formation of SMAP. Waist-to-hip ratios, the frequency of taking renin-angiotensin system inhibitors, urinary AER, eGFR, and baPWV were significantly different between diabetic patients with SMAP and those without such events. Multiple logistic regression analysis including these factors identified the levels of AER (odds ratio 3.4 [95% CI 2.2–5.3], $P < 0.001$) and baPWV (15.3 [1.1–227.5], $P < 0.05$) as independent factors associated with SMAP detection.

Correlation between platelet microaggregation and albuminuria

Next, the frequency and degree of SMAP were analyzed according to albuminuria status (>20 μg/min). The frequency of the formation of SMAP in diabetic patients with albuminuria was significantly higher than in those with normoalbuminuria (75% of 149 patients with albuminuria vs. 39% of 252 patients with normoalbuminuria, $P < 0.001$). Similarly, the degree of SMAP evaluated by

AUC was significantly higher in those with albuminuria than in those with normoalbuminuria (0.52 [interquartile range

0.01–1.46] vs. 0.00 [0.00–0.16], $P < 0.001$). In addition, the degree of SMAP was significantly correlated with the levels of urinary AER as continuous variables (Spearman $\gamma = 0.43$, $P < 0.001$). Regardless of the status of albuminuria, the platelet microaggregates in all patients with SMAP showed the irreversible pattern after stimulation by a low dose of ADP, and those in the patients without SMAP were reversible.

Effect of aspirin medication on platelet microaggregation

We evaluated the effect of aspirin medication on the frequency and degree of SMAP. Of the diabetic patients, 163 (41%) were prescribed 100 mg/day aspirin. Patients with albuminuria and aspirin intake had a significantly lower frequency of SMAP than those without it (50 of 76 patients taking aspirin vs. 62 of 73 patients not given aspirin), whereas the SMAP rates were similar in the two subgroups in patients with normoalbuminuria (37 of 87 patients taking aspirin vs. 62 of 165 patients without aspirin intake). The degree of SMAP among the patients with albuminuria was similarly significantly lower with than without aspirin (0.33 [0.00–1.27] vs. 0.88 [0.17–1.76]), whereas patients with nor-

Table 1—*Clinical characteristics of the study subjects according to the formation of SMAP*

	Diabetic patients without SMAP	Diabetic patients with SMAP
n	190	211
Sex (male/female)	110/80	119/92
Age (years)	64 ± 11	65 ± 9
Duration of diabetes (years)	17 ± 11	16 ± 9
BMI (kg/m^2)	24.6 ± 4.3	24.7 ± 3.8
Waist-to-hip ratio	0.93 ± 0.07	0.95 ± 0.08*
A1C (%)	7.2 ± 0.8	7.2 ± 1.0
Diabetes treatment (%) (diet/oral agents/insulin)	11/55/34	7/49/44
Systolic blood pressure (mmHg)	133 ± 17	134 ± 14
Diastolic blood pressure (mmHg)	73 ± 10	72 ± 10
Hypertension (%)	66	74
Taking renin-angiotensin system inhibitors (%)	41	59*
Total cholesterol (mg/dl)	194 ± 31	197 ± 29
Triglycerides (mg/dl)	86 (61–116)	92 (62–134)
HDL cholesterol (mg/dl)	54 (46–65)	52 (45–62)
Taking statins (%)	52	48
Urinary albumin excretion rate (μg/min)	9 (6–16)	23 (8–87)†
eGFR (ml/min per 1.73 m^2)	74 ± 23	70 ± 20*
baPWV (cm/s)	1,606 (1,416–1,800)	1,726 (1,521–1,987)†
Taking aspirin (%)	40	41
Current smoking (%)	22	21

Data are means ± SD for normally distributed continuous variables or medians (25th–75th interquartiles) for skewed continuous variables. *$P < 0.05$, †$P < 0.01$ vs. diabetic subjects without SMAP.
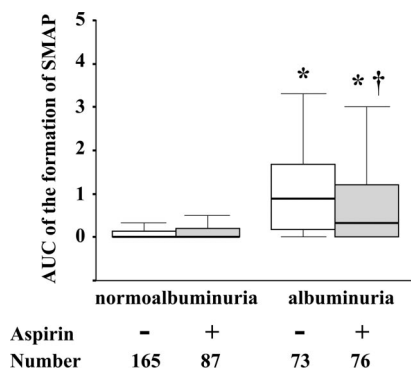
Figure 2—*Box-and-whisker plots of SMAP assessed by AUC in diabetic patients with normoalbuminuria and albuminuria, treated with or without aspirin. In these plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively. Kruskal-Wallis test: P < 0.001, *P < 0.001 vs. normoalbuminuric patients treated with and without aspirin, †P = 0.01 vs. albuminuric patients without aspirin (Mann-Whitney U test).*

moalbuminuria showed no such difference according to aspirin intake (0.00 [0.00–0.14] vs. 0.00 [0.00–0.21]) (Fig.

2). In addition, the strong correlation between the degree of SMAP and the levels of urinary AER as continuous variables was investigated in the patients without aspirin intake ($\gamma = 0.53$, $P < 0.001$), whereas the weak association between them was found in those with aspirin intake ($\gamma = 0.27$, $P < 0.001$).

Correlation between platelet microaggregation and GPIIb/IIIa and P-selectin expression

To further evaluate the active state of SMAP, we quantitated the expression levels of active GPIIb/IIIa and P-selectin on the platelets. The expression levels of active GPIIb/IIIa were higher in diabetic patients with SMAP than in those without SMAP (29.9% [interquartile range 19.3–42.2] vs. 15.5% [11.7–21.9]). Similarly, the expression levels of P-selectin were higher in diabetic patients with detectable SMAP than those without it (8.1% [5.1–16.0] vs. 4.4% [3.2–5.6]). The expression levels of both markers correlated significantly with the degree of SMAP (Spearman $\gamma = 0.59$ for active GPIIb/IIIa and $\gamma = 0.55$ for P-selectin).

Finally, we investigated the relationship between the expression levels of these surface markers and urinary AER in the subgroups based on aspirin intake. In patients not taking aspirin, the expression level of each surface marker correlated significantly with urinary AER (Spearman $\gamma = 0.60$ for active GPIIb/IIIa and $\gamma = 0.57$ for P-selectin) (Fig. 3A and C). Similar correlations were also observed in patients taking aspirin, albeit with a slightly weaker coefficient of correlation (Spearman $\gamma = 0.51$ for active GPIIb/IIIa and $\gamma = 0.34$ for P-selectin, Fig. 3B and D).

CONCLUSIONS — The present study provided new evidence that both increased AER and baPWV are independent factors associated with the abnormal formation of SMAP in type 2 diabetic patients. Furthermore, the patients with SMAP showed an irreversible pattern of platelet microaggregation by ADP, and the degree of SMAP correlated with the enhanced expression of active GP IIb/IIIa and P-selectin, indicating that SMAP is pathophysiologically active. These early-activated platelet profiles were signifi-
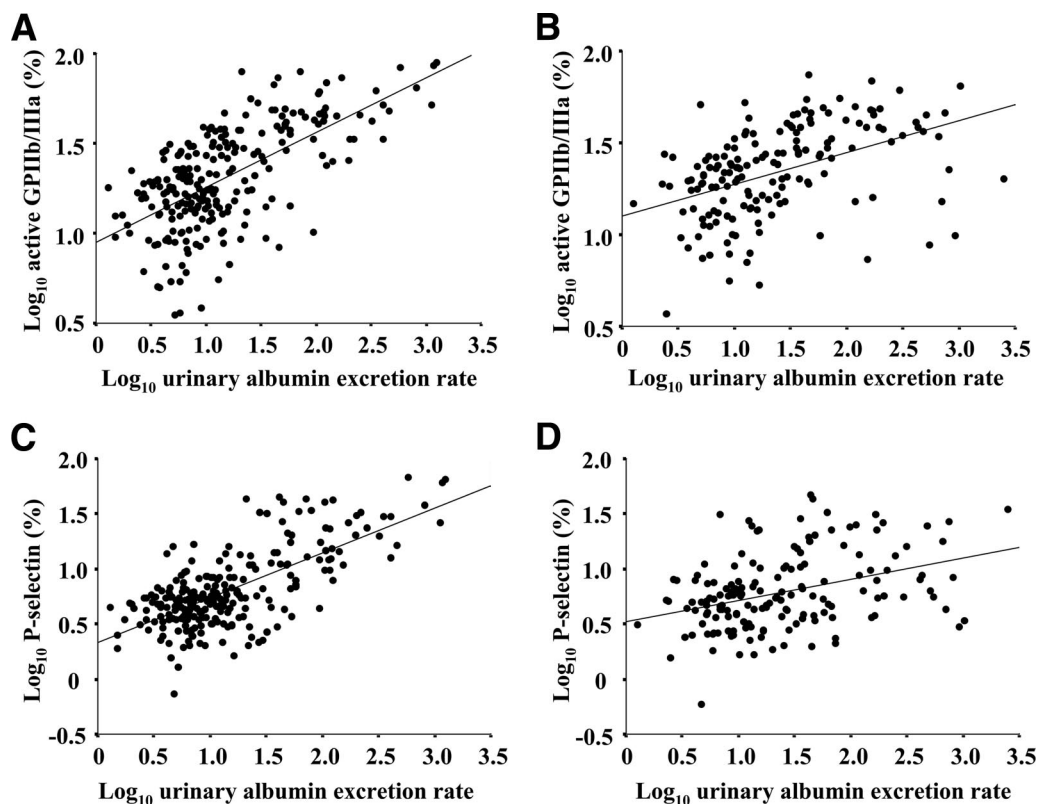

Figure 3—*Correlation between urinary albumin excretion rate and expression of platelet surface markers, active GPIIb/IIIa, and P-selectin. The patients were divided into two subgroups based on aspirin intake: for active GPIIb/IIIa, those not taking aspirin (A) ($\gamma = 0.60$; P < 0.001) and those taking aspirin (B) ($\gamma = 0.51$; P < 0.001), and for P-selectin, those not taking aspirin (C) ($\gamma = 0.57$; P < 0.001) and those taking aspirin (D) ($\gamma = 0.34$; P < 0.001). Log-transformed values were plotted on each figure.*

cantly inhibited in albuminuric patients with aspirin intake, although the effect was incomplete.

Assessment of inappropriate platelet activation is one way to risk-stratify patients who are at high risk of atherosclerosis and atherothrombosis. The microaggregates produced in the early phase of platelet activation are now considered to potentially aggravate thrombus formation (7). Under normal conditions, these platelet microaggregates dissolve within a few minutes, as shown in this study (Fig. 1*D*). In the present study, 53% of type 2 diabetic patients showed SMAP and abnormal irreversibility of the microaggregation after low-dose ADP. This high proportion of diabetic patients with abnormal platelet hyperaggregability is in agreement with the result from a cohort studied previously (12). The SMAP formation was observed under a low-shear stress that mimicked the state of the arterial bloodstream without the stimulation of exogenous agonists. In addition, the degree of SMAP was associated with enhanced expression of active GPIIb/IIIa and P-selectin. Thus, the formation of SMAP is considered to show the abnormal activated state of platelets in diabetic patients, which is enhanced according to the increase of albuminuria.

In the present study, SMAP occurrence was associated with both albuminuria and baPWV. In agreement with these results, other investigators reported that high levels of platelet microaggregation were associated with adverse outcomes in patients with cardiovascular disease (16) and the ankle-brachial index in patients with peripheral arterial disease (17). In addition, diabetic patients with SMAP showed overexpression of platelet surface markers, active GPIIb/IIIa, and P-selectin. Such upregulation of these molecules has also been associated with the development of atherogenesis (18). Taken together, the early platelet activation in type 2 diabetic patients may be a risk factor for cardiovascular disease.

A recent guideline of the American Diabetes Association recommended prophylactic use of antithrombotic agents, with low-dose aspirin, for diabetic subjects, especially those with albuminuria (19). However, Di Minno and Violi (20) indicated that aspirin alone is insufficient to prevent thrombosis in diabetic patients with angiopathy. In the present study, SMAP formation was inhibited in albuminuric patients taking aspirin compared with those not taking it. However, SMAP formation was still associated with albuminuria, even in patients taking aspirin, suggesting that the inhibitory effect of aspirin may be insufficient in diabetic patients with albuminuria. Further follow-up and intervention studies are required to investigate whether the incomplete inhibition of early-activated platelet profiles is a cardiovascular risk.

Several plausible mechanisms underlying the cardiorenal interaction have been proposed. Deckert et al. (21) proposed in the Steno hypothesis that excess leakage of albumin into urine reflects widespread vascular (endothelial) damage. This endothelial dysfunction is considered a common feature of cardiorenal interactions (22); it leads to platelet activation, adhesion, and subsequent platelet aggregate formation. Platelets are also a rich source of chemokines and cytokines, released within seconds of platelet activation (23). Thus, the formation of SMAP in diabetic patients with albuminuria might either cause or reflect systemic vascular endothelial dysfunction. An alternative explanation is that any pathophysiological alteration in a diabetic kidney could directly affect platelet activation. Glomerular hypertension, which induces mechanical shear stress (24), and excess renal production of type IV collagen in the kidney (25), a powerful activator of platelets, might induce activation of platelets that circulate into the kidney.

The present study had some limitations. It was not possible to ascertain whether the early-activated platelet profile was a cause or consequence of the increased urinary albumin excretion because of the nature of the cross-sectional study. The present study also could not address whether the incidence of future cardiovascular disease is higher in patients with SMAP and whether the inhibitory effect of aspirin on the degree of SMAP would be sufficient to prevent the development of cardiovascular disease.

In summary, the majority of type 2 diabetic patients with albuminuria showed an altered profile of early platelet activation including SMAP events. Given the growing concern over cardiovascular consequences in type 2 diabetic patients, further follow-up and intervention studies are needed to establish whether the inhibition of SMAP is a therapeutic target to prevent cardiovascular complications in type 2 diabetic patients.

References
1. de Zeeuw D, Raz I. Albuminuria: a great risk marker, but an underestimated target in diabetes. Diabetes Care 2008;31(Suppl. 2): S190–S193
2. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, UKPDS Group: Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003;63: 225–232
3. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM. Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. Circulation 2004;110:921–927
4. Araki S, Haneda H, Koya D, Hidaka H, Sugimoto T, Isono M, Isshiki K, Chin-Kanasaki M, Uzu T, Kashiwagi A. Reduction of microalbuminuria as an integrated indicator for renal and cardiovascular risk reduction in patients with type 2 diabetes mellitus. Diabetes 2007;56:1727–1730
5. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. Diabetes Care 2003;26:2181-2188
6. Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. J Thromb Haemost 2004;2:1282–1291
7. Ruggeri ZM. Platelets in atherothrombosis. Nat Med 2002;8:1227–1234
8. Cerbone AM, Macarone-Palmieri N, Saldalamacchia G, Coppola A, Di Minno G, Rivellese AA. Diabetes, vascular complications and antiplatelet therapy: open problems. Acta Diabetol. In press
9. Born GV, Hume M. Effects of the numbers and sizes of platelet aggregates on the optical density of plasma. Nature 1967;215: 1027–1029
10. Cardinal DC, Flower RJ. The study of platelet aggregation in whole blood. Br J Pharmacol 1979;66:94–95
11. Ozaki Y, Satoh K, Yatomi Y, Yamamoto T, Shirasawa Y, Kume S. Detection of platelet aggregates with a particle counting method using light scattering. Anal Biochem 1994;218:284–294
12. Matsuno H, Tokuda H, Ishisaki A, Zhou Y, Kitajima Y, Kozawa O. P2Y12 receptors play a significant role in the development of platelet microaggregation in patients

with diabetes. J Clin Endocrinol Metab 2005;90:920–927

13. Matsuno H, Kozawa O, Nagashima S, Kanamaru M, Uematsu T. Comparative antiplatelet effects of aspirin, vapiprost and GR144053, a GPIIb/IIIa antagonist, with a special reference to the role of platelet microaggregates. Br J Pharmacol 1999; 127:1129–1134

14. Abrams CS, Ellison N, Budzynski AZ, Shattil SJ. Direct detection of activated platelets and platelet-derived microparticles in humans. Blood 1990;75:128–138

15. Fijnheer R, Modderman PW, Veldman H, Ouwehand WH, Nieuwenhuis HK, Roos D, de Korte D. Detection of platelet activation with monoclonal antibodies and flow cytometry: changes during platelet storage. Transfusion 1990;30:20–25

16. Miyamoto S, Kawano H, Sakamoto T, Soejima H, Kajiwara I, Shimomura H, Kojima S, Hokamaki J, Sugiyama S, Hirai N, Yoshimura M, Ozaki Y, Ogawa H. Formation of platelet microaggregates correlates with adverse clinical outcome in patients with coronary artery disease. Thromb Haemost 2003;89:681–686

17. Kudoh T, Sakamoto T, Miyamoto S, Matsui K, Kojima S, Sugiyama S, Yoshimura M, Ozaki Y, Ogawa H. Relation between platelet microaggregates and ankle brachial index in patients with peripheral arterial disease. Thromb Res 2006;117: 263–269

18. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378–3384

19. American Diabetes Association. Standards of medical care in diabetes—2009. Diabetes Care 2009;32(Suppl. 1):S13–S61

20. Di Minno G, Violi F. Aspirin resistance and diabetic angiopathy: back to the future. Thromb Res 2004;113:97–99

21. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage: the Steno hypothesis. Diabetologia 1989;32:219–226

22. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? Diabetologia 2008;51:714–725

23. Gleissner CA, von Hundelshausen P, Ley K. Platelet chemokines in vascular disease. Arterioscler Thromb Vasc Biol 2008; 28:1920–1927

24. Kikkawa R, Koya D, Haneda M. Progression of diabetic nephropathy. Am J Kidney Dis 2003;41(Suppl. 1):S19–S21

25. Haneda M, Kikkawa R, Horide N, Togawa M, Koya D, Kajiwara N, Ooshima A, Shigeta Y. Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. Diabetologia 1991; 34:198–200