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Determinants of aspirin resistance in patients with type 2 diabetes

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ABSTRACT

Background. – Cardiovascular disease is a leading cause of mortality among patients with type 2 diabetes mellitus (T2DM). Numerous patients with T2DM show resistance to aspirin treatment, which may explain the higher rate of major adverse cardiovascular events observed compared with non-diabetes patients, and it has recently been shown that aspirin resistance is mainly related to accelerated platelet turnover with persistent high platelet reactivity (HPR) 24 h after last aspirin intake. The mechanism behind HPR is unknown. The aim of this study was to investigate the precise rate and mechanisms associated with HPR in a population of T2DM patients treated with aspirin.

Methods. – Included were 116 consecutive stable T2DM patients who had attended our hospital for their yearly check-up. HPR was assessed 24 h after aspirin intake using light transmission aggregometry (LTA) with arachidonic acid (AA) and serum thromboxane B2 (TXB2) measurement. Its relationship with diabetes status, insulin resistance, inflammatory markers and coronary artery disease (CAD) severity, using calcium scores, were investigated.

Results. – Using LTA, HPR was found in 27 (23%) patients. There was no significant difference in mean age, gender ratio or cardiovascular risk factors in patients with or without HPR. HPR was significantly related to duration of diabetes and higher fasting glucose levels (but not consistently with HbA1c), and strongly related to all markers of insulin resistance, especially waist circumference, HOMA-IR, QUICKI and leptin. There was no association between HPR and thrombopoietin or inflammatory markers (IL-6, IL-10, indoleamine 2,3-dioxygenase activity, TNF- α , C-reactive protein), whereas HPR was associated with more severe CAD. Similar results were found with TXB2.

Conclusion. – Our results reveal that ‘aspirin resistance’ is frequently found in T2DM, and is strongly related to insulin resistance and severity of CAD, but weakly related to HbA1c and not at all to inflammatory parameters. This may help to identify those T2DM patients who might benefit from alternative antiplatelet treatments such as twice-daily aspirin and thienopyridines.

Keywords: Aspirin; Aspirin resistance; Coronary artery disease; Diabetes

INTRODUCTION

Cardiovascular disease is a leading cause of mortality among patients with type 2 diabetes mellitus (T2DM). T2DM is associated with a higher risk of atherosclerosis, recurrence of ischaemic events and stent thrombosis after acute coronary syndrome [1]. Therefore, in addition to therapies aimed at reducing hyperglycaemia and associated risk factors, aspirin has become a cornerstone treatment in patients with diabetes. Given the increased risk of major adverse cardiovascular events (MACE) in patients with T2DM, aspirin treatment has been proposed in high-risk diabetes patients even when there is no evidence of atherosclerotic disease [2–4]. Despite this, in such patients, aspirin treatment has shown poor efficacy in the primary prevention of cardiovascular disease [3]. Similarly, when aspirin is used in secondary prevention, it was found to reduce the risk of cardiovascular events in T2DM patients by only < 10% compared with a > 20% reduction in non-diabetes patients [5]. However, exactly why the effect of aspirin on cardiovascular disease risk is weaker in patients with diabetes compared with the general population remains unclear.

It has been demonstrated that in particular populations, such as patients with T2DM, accelerated platelet turnover could decrease the duration of the effect of aspirin and lead to persistent high platelet reactivity (HPR) 24 h after aspirin intake [6–8]. In such situations, increasing aspirin intake to twice a day was shown to induce more stable platelet inhibition [8]. HPR with aspirin treatment linked to accelerated platelet turnover has been found in 20–30% of diabetes patients [9]. Yet, the main mechanisms behind aspirin resistance in T2DM remain poorly understood. Many explanations have been proposed, such as low-grade inflammation, increased thrombopoietin (TPO) synthesis [10], atheroma, hyperglycaemia [11] and insulin resistance [12]. However, these determinants have been explored separately and showed divergent conclusions.

Thus, the objective of the present study was to explore the potential determinants of HPR simultaneously in a well-characterized population of patients with T2DM.

DESIGN AND METHODS

Settings

This prospective single-centre study enrolled consecutive patients who had come for their yearly check-up to the University Centre for the Study of Diabetes and its Complications at Lariboisière University Hospital between 1 April and 31 July 2016.

Study population

Patients were eligible if they had presented with T2DM for > 1 year and been treated with 75-mg of non-enteric coated aspirin once daily as single antiplatelet therapy. Exclusion criteria were anticoagulation, dual antiplatelet therapy or non-steroidal anti-inflammatory drugs (NSAIDs), platelet counts < 100,000/mm³ or > 500,000/mm³, or known inflammatory disease, coagulopathy or pregnancy. The study protocol was approved by the local ethics committee, and all patients gave their written informed consent to participate.

Study design

Patients' blood samples were obtained from the antecubital vein upon hospital admission (between 0800–0900 h in the morning), 24 h after the last aspirin intake and before any other treatment was taken. Samples were delivered to the laboratory within 1 h and processed within 2 h. Compliance was systematically assessed before blood sampling by face-to-face interviews concerning aspirin compliance during the past week, precise time of last intake and concomitant intake of NSAIDs.

Clinical data for all patients were collected, including weight, height, body mass index (BMI), waist circumference, blood pressure (after resting for 5 min), history of chest pain, history of dyspnoea, duration of diabetes and insulin therapy, and known diabetes complications. Evaluation of peripheral artery disease was assessed using continuous Doppler (Dopplex D900, Huntleigh Diagnostics Ltd., Cardiff, Wales, UK) to obtain the ankle-brachial index (ABI). Hypertension was considered values of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, as defined by the current recommendations [13].

Diabetes parameters

Fasting blood glucose and microalbumin levels were measured by spectrophotometry (ARCHITECT c8000, Abbott Laboratories, Chicago, IL, USA). Waist index was determined by dividing waist circumference by 80 cm in women or 94 cm in men. Insulin and C-peptide levels were measured by chemiluminescence (ARCHITECT i2000, Abbott). Glycated haemoglobin (HbA1c) was quantified by high-performance liquid chromatography (HPLC) with 415-nm detection (G8 HPLC Analyzer, Tosoh Bioscience, Inc., San Francisco, CA, USA). Homoeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: $\text{insulin level} \times \text{fasting glucose (mmol/L)} / 22.5$ (only in patients not on insulin treatment). The quantitative insulin sensitivity check index (QUICKI) was calculated as follows: $1 / (\log [\text{insulin level } (\mu\text{IU/mL})] + \log [\text{fasting glucose (mg/dL)}])$ (only in patients not on insulin treatment). Leptin concentration was measured in duplicate for each patient by enzyme-linked immunosorbent assay (ELISA; Human Leptin DuoSet ELISA, R&D Systems, Inc., Minneapolis, MN, USA).

Platelet aggregation tests and inflammatory markers

Aspirin resistance was assessed by light transmission aggregometry (LTA) triggered by 0.5 mg/mL of arachidonic acid (AA) and by serum thromboxane B2 (TXB2) concentration, as described previously elsewhere [6]. To assess TPO, von Willebrand factor (vWf) and inflammatory markers [interleukins (ILs) and tumour necrosis factor (TNF)- α], the techniques used are detailed in the [Appendix \(see supplementary materials associated with this article online\)](#). Patients were considered biologically aspirin-resistant when platelets were reactive to AA with a maximum aggregation intensity $\geq 20\%$ [6].

Indoleamine 2,3-dioxygenase (IDO) activity

IDO activity reflects the overall inflammatory pattern, and is defined as the ratio of kynurenine to tryptophan plasma concentrations. Blood samples were collected in lithium heparin tubes containing a separating gel. Plasma concentrations of kynurenine and tryptophan were measured by HPLC as previously described [15].

Coronary artery disease (CAD) evaluation

CAD was defined as the antecedent of myocardial infarction or acute coronary syndrome, or a previous need for angioplasty or coronary bypass grafting. For patients with no history of CAD, scoring of coronary artery calcium (Agatston score) was systematically performed to precisely assess the coronary atherosclerotic burden.

Statistical analysis

Mean biological values were used for all statistical analyses. Continuous variables were tested for non-normality by Shapiro–Wilk test, and presented as means \pm standard deviation (SD) if

normally distributed or, if otherwise, as medians with interquartile range [IQR]. Categorical data were presented as numbers (n) and proportions (%).

Differences between our two study groups (HPR vs no HPR) were tested with a two-sided *t* test if normally distributed or, if otherwise, by Mann–Whitney test. Proportions in the two groups were tested using Fisher’s exact test. Correlations were determined using Spearman’s rank correlation coefficient. *P* values < 0.05 was considered statistically significant.

All statistical analyses were performed using SPSS Statistics version 23 software (IBM Corp., Armonk, NY, USA). Also, it was decided to include all patients until the number of HPR cases was > 25 patients. There were no missing data.

RESULTS

Population characteristics

A total of 116 consecutive patients (mean age 65 ± 9 years, 69% male) were included between 1 April and 31 July 2016. Nearly 50% of them had a history of CAD or cerebrovascular complications. Median duration of diabetes was 15 years [IQR 11–24 years] and 50% of patients were being treated with insulin. This patient population also had a high rate of associated risk factors, as typically seen in T2DM: 85% had dyslipidaemia; 77% had hypertension; and 16% were currently smokers. In addition, these patients were overweight (median BMI 28 kg/m^2 [IQR 24–32 kg/m^2]) with android obesity (waist circumference: 103 ± 13 cm). The standard biological test results [total haemoglobin levels, microalbumin concentrations, creatinine levels, glomerular filtration rate (GFR)] were normal.

Platelet aggregation and turnover

Our study found 27 patients (23%) with HPR as measured by LTA. Median LTA and TXB2 concentrations were significantly higher in patients with HPR (Table I). All patients with HPR as measured by LTA had TXB2 concentrations > 2.45 ng/mL (Table I) and, in this HPR group, mean platelet volume (MPV) was significantly higher, suggesting accelerated platelet turnover in these patients. Platelet counts were similar in both groups (Table I).

Patients' characteristics according to HPR status

Table II shows the general characteristics of patients (excluding diabetes status) in each subgroup (HPR or no HPR). There were no differences concerning age and gender ratios (Table II). Also, the main associated risk factors were similar in the two groups except for hypertension, which was more frequent in the HPR group (Table II). The proportion of patients treated with a statin did not significantly differ between the two groups (Table II).

Diabetes and insulin resistance according to HPR status

As shown in Table III, diabetes duration was longer in the HPR group, as was duration of insulin treatment, although the number of patients treated with insulin was similar. There was no significant difference in mean HbA1c values, whereas fasting glucose levels were significantly higher in the HPR group (Table III). In addition, all parameters associated with the metabolic syndrome and insulin resistance were significantly higher in the HPR group (waist circumference, waist index, history of hypertension, HOMA-IR, QUICKI) except for high-density lipoprotein (HDL) cholesterol levels (Table III). In patients treated with insulin, the daily dose of long-acting insulin was significantly higher in HPR patients (Table III).

CAD according to HPR status

There were no significant differences concerning the history of CAD in our two groups (Table II), whereas median coronary artery calcium scores were significantly higher in the HPR patients (Table IV). Likewise, the rate of low coronary artery calcium scores (< 10), which demonstrates low atheroma, was highly significantly lower in the HPR group (Table IV). Concerning cardiovascular and atherosclerotic parameters, no differences were found in vascular stiffness: pulse pressure and ABI were similar in both groups (Table II).

Thrombopoietin and systemic inflammation according to HPR status

No significant differences in TPO levels were found between patients with and without HPR (Table V), and all inflammatory markers tested were also similar in both groups (Table V). WhileIDO activity (a marker of systemic inflammation) was high in our study population, there was no association with HPR (Table V). There was also no correlation between TPO and inflammatory markers in the overall population. In addition, concentrations of vWf and fibrinogen were similar in each of our subgroups (Table I).

Relationship between TXB2 and clinical or biological characteristics

As shown in Table VI, plasma TXB2 concentrations 24 h after last aspirin intake correlated with fasting glucose and HbA1c levels. In addition, all parameters of insulin resistance (HOMA-IR, QUICKI, leptin, BMI, waist circumference, waist index, long-acting insulin dose) were significantly related to TXB2 concentrations. However, there was no relationship between TXB2 and plasma cytokines or TPO except for high-sensitivity C-reactive protein (hsCRP).

DISCUSSION

Our present study has shown that nearly 25% of patients with T2DM present with HPR during aspirin treatment. However, in these patients, HPR was not associated with any particular inflammatory pattern. On the other hand, HPR was strongly associated with all parameters of insulin resistance as well as a history of diabetes and an increase in atherosclerotic burden, but not with diabetes equilibrium.

Loss of aspirin efficacy in patients with T2DM

Aspirin is less effective for primary and secondary prevention of cardiovascular disease in diabetes patients, the very population at higher risk of such diseases [5]. In the Platelet Inhibition and Patient Outcomes (PLATO) study, patients with diabetes were found to have 1.66-fold more MACE compared with the general population after acute coronary syndrome, despite optimal antiplatelet treatment with ticagrelor and aspirin [16]. It has been suggested that the increased rate of MACE in this population could at least be partly explained by a decrease in aspirin efficacy.

The leading explanation for loss of aspirin efficacy in diabetes is accelerated platelet turnover [8,16]. Under normal conditions, approximately 10–15% of circulating platelets are replaced daily; in cases of accelerated turnover, an increased percentage of newly formed platelets (> 20%) is synthesizing thromboxane A₂ (TXA₂) and triggering platelet aggregation, thereby reducing aspirin efficacy [6,18]. The study by Martin et al. [19] was one of the first to show accelerated platelet turnover in diabetes by demonstrating that such patients had an increased number of megakaryocytes (ploidy) whereas, in an earlier study, Mortensen et al. [7] had shown that the biological half-life of platelets (radiolabelled with indium-111) was significantly shorter in diabetes. More recently, accelerated platelet turnover was found in 20–30% of diabetes patients, thereby suggesting that HPR might represent the main cause of the

decreased aspirin efficacy described in this population. Indeed, a recent meta-analysis found that 21% of diabetes patients are aspirin-resistant [9]. Similarly, 23% of our present study patients showed HPR, which was confirmed by elevated plasma TXB2 levels. MPV was also significantly higher in our patients with HPR, which suggests the presence of younger platelets in the blood circulation. Similarly, previous studies have shown an association between MPV and thrombotic risk in patients with diabetes [21].

It is important to remember that HPR is usually associated with negative outcomes in cardiovascular patients. Gum et al. [14] had previously demonstrated, in patients with stable cardiovascular disease, that HPR measured by LTA was strongly associated with MACE. In the same way, monitoring HPR during aspirin treatment, and adapting the antiplatelet therapy in response, could help to reduce ischaemic events in CAD patients [20–22]. Several studies and guidelines have proposed that aspirin administered twice daily may improve treatment efficacy and lower the rate of HPR in diabetes patients [23,24]. An alternative treatment for patients with clinical aspirin resistance (recurrent ischaemic events while on aspirin treatment) might also be to use thienopyridines [a class of adenosine diphosphate (ADP) receptors/P2Y12 inhibitors] [8,16].

The mechanism usually proposed to explain accelerated platelet turnover in diabetes is the presence of an inflammatory syndrome, with an increase in megakaryocyte production linked to inflammation [8,19].

HPR with aspirin treatment is not associated with inflammation

Diabetes is considered a low-grade inflammatory disease [25]. Most of the clinical and experimental data demonstrates that adipose tissue, liver and muscles are able to induce systemic inflammation in patients with obesity and T2DM [26]. This production of

proinflammatory markers (including IL-6, IL-1 β and TNF- α) is thought to promote insulin resistance and macrovascular complications in diabetes patients [27].

Nevertheless, our study found no relationship between inflammation and HPR. Likewise, previous studies have indicated a lack of relationship between HPR and inflammation in diabetes when looking at CRP, fibrinogen and vWf [12]. Our study also found no link between TPO (a marker of platelet production) and HPR, although the mean TPO level was slightly increased, as found by other researchers [28]. Finally, mean IDO activity (a marker of systemic inflammation) was clearly elevated in our study population, which was probably related to obesity and diabetes but, again, not associated with HPR [29]. On the other hand, Larsen et al. [10] found a correlation between platelet turnover, TPO and IL-6. However, the relationship was weak, their study population was not restricted to diabetes patients and the mean TPO level was very low.

HPR linked to hyperglycaemia and insulin resistance

Our present study found a very strong relationship between HPR and clinical or biological parameters linked to insulin resistance, but a less powerful relationship with hyperglycaemia. In addition, only a weak correlation was found between HPR and HbA1c levels, but a stronger correlation with fasting glucose. One explanation for these different results between fasting glucose and HbA1c could be glycaemic variability or glycaemic excursions, which could have deleterious effects on vascular endothelium [30]. Several other explanations have already been proposed linking HPR and hyperglycaemia [11,31]. For example, aspirin-mediated non-specific acetylation could be competing with non-enzymatic modifications, such as protein glycosylation and oxidative damage due to the production of free radicals [32].

A relationship between HPR and insulin resistance has also been suggested in previous studies [17,33]. BMI was the most frequent factor found in relation to HPR. However, these studies did not use both LTA and TXB2 measurement, which are considered the best methods for assessing HPR [34]. Moreover, in those studies, blood samples were taken only a few hours after aspirin intake whereas, because HPR is a time-dependent phenomenon, it is probably better to perform platelet studies 24 h after the last aspirin intake (just before the next intake) [6,33]. In any case, the relationship between insulin resistance and accelerated platelet turnover needs to be investigated. Atherosclerosis could be the link, as a strong association has been found between insulin resistance and extent of atherosclerosis in diabetes patients [31].

HPR and atherosclerosis

One explanation for the link between HPR and insulin resistance could be that it is mediated through endothelial dysfunction and increased peripheral platelet consumption. Winocour et al. [35] demonstrated that accelerated platelet turnover in diabetic rats is related to platelet activation and consumption along with alteration of blood vessel walls, a finding supported by other studies [36]. Furthermore, Neergaard-Petersen et al. [37] recently found increased platelet activation in diabetes patients with CAD, insulin-resistance-induced endothelial dysfunction and vascular smooth muscle cell disorders, which could lead to vascular alterations. Moreover, it is important to bear in mind that our present study is the first to assess coronary atherosclerotic burden using coronary calcium scores, and that cardiovascular complications in patients with T2DM start at the stage of insulin resistance, before glucose levels increase [38].

Study limitations

This was a single-centre prospective study involving only a limited number of patients. However, these patients were fully explored, and a significant rate of HPR with a range similar to that previously described [6] was found using two methods (LTA and TBX2 concentration). Ultimately, it was decided to use LTA to classify patients for HPR, as TBX2 can be produced from sources other than platelets and the cut-off value is more difficult to identify. Nevertheless, the clear relationship found between HPR and several parameters of insulin resistance suggests that our study had sufficient statistical power. On the other hand, in our study, although insulin resistance was approached only through indirect methods, other studies have previously shown that QUICKI and HOMA-IR are strongly correlated with euglycaemic–hyperinsulinaemic clamp results, considered the reference test for exploring insulin resistance [39].

CONCLUSION

Aspirin resistance is frequently seen in patients with T2DM and appears to be strongly related to insulin resistance and atherosclerotic extent, but poorly associated with hyperglycaemia and with no association with low-grade inflammation. These results suggest that, in diabetes patients, HPR is related more to peripheral platelet consumption than to medullar hyperproduction. However, the mechanisms involved still require further study, as such knowledge could help us to detect those diabetes patients at high risk of HPR and to better understand the high rate of MACE found in such patients with aspirin treatment. In any case, our present findings can help to identify the patient population for whom an alternative antiplatelet treatment may be proposed.

REFERENCES

1. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FWA, et al. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in

- patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation* 2008;118:1626–36.
2. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* 2016;37:2315–81.
 3. Kokoska LA, Wilhelm SM, Garwood CL, Berlie HD. Aspirin for primary prevention of cardiovascular disease in patients with diabetes: A meta-analysis. *Diabetes Res Clin Pract* 2016;120:31–9.
 4. Xie M, Shan Z, Zhang Y, Chen S, Yang W, Bao W, et al. Aspirin for primary prevention of cardiovascular events: meta-analysis of randomized controlled trials and subgroup analysis by sex and diabetes status. *PloS One* 2014;9:e90286.
 5. Collaboration AT. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*. 2002;324(7329):71–86.
 6. Henry P, Vermillet A, Boval B, Guyetand C, Petroni T, Dillinger J-G, et al. 24-hour time-dependent aspirin efficacy in patients with stable coronary artery disease. *Thromb Haemost*. 2011;105(2):336–44.
 7. Mortensen SB, Larsen SB, Grove EL, Kristensen SD, Hvas A-M. Reduced platelet response to aspirin in patients with coronary artery disease and type 2 diabetes mellitus. *Thromb Res* 2010;126:e318–22.
 8. Dillinger J-G, Drissa A, Sideris G, Bal dit Sollier C, Voicu S, Manzo Silberman S, et al. Biological efficacy of twice daily aspirin in type 2 diabetic patients with coronary artery disease. *Am Heart J* 2012;16:600–6.e1.
 9. Simpson SH, Abdelmoneim AS, Omran D, Featherstone TR. Prevalence of high on-treatment platelet reactivity in diabetic patients treated with aspirin. *Am J Med* 2014;127:95.e1–9.
 10. Larsen SB, Grove EL, Hvas A-M, Kristensen SD. Platelet turnover in stable coronary artery disease - influence of thrombopoietin and low-grade inflammation. *PloS One* 2014;9:e85566.
 11. Cohen HW, Crandall JP, Hailpern SM, Billett HH. Aspirin resistance associated with HbA1c and obesity in diabetic patients. *J Diabetes Complications* 2008;22:224–8.
 12. Kaplon-Cieslicka A, Postula M, Rosiak M, Peller M, Kondracka A, Serafin A, et al. Younger age, higher body mass index and lower adiponectin concentration predict higher serum thromboxane B2 level in aspirin-treated patients with type 2 diabetes: an observational study. *Cardiovasc Diabetol* 2014;13:112.
 13. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013;34:2159–219.

14. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003;41:961–5.
15. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin Chem* 1997;43:2424–6.
16. James S, Angiolillo DJ, Cornel JH, Erlinge D, Husted S, Kontny F, et al. Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATelet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J* 2010;31:3006–16.
17. Rocca B, Santilli F, Pitocco D, Mucci L, Petrucci G, Vitacolonna E, et al. The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. *J Thromb Haemost* 2012;10:1220–30.
18. Pedersen AK, FitzGerald GA. Dose-related kinetics of aspirin. *N Engl J Med* 1984;311:1206–11.
19. Martin JF, Kristensen SD, Mathur A, Grove EL, Choudry FA. The causal role of megakaryocyte–platelet hyperactivity in acute coronary syndromes. *Nat Rev Cardiol*. 2012;9(11):658–70.
20. Verdoia M, Schaffer A, Barbieri L, Casseti E, Nardin M, Bellomo G, et al. Diabetes, glucose control and mean platelet volume: a single-centre cohort study. *Diabetes Res Clin Pract* 2014 ;104:288–94.
21. Christensen KH, Grove EL, Würtz M, Kristensen SD, Hvas A-M. Reduced antiplatelet effect of aspirin during 24 hours in patients with coronary artery disease and type 2 diabetes. *Platelets* 2015;26:230–5.
22. Cayla G, Cuisset T, Silvain J, Leclercq F, Manzo-Silberman S, Saint-Etienne C, et al. Platelet function monitoring to adjust antiplatelet therapy in elderly patients stented for an acute coronary syndrome (ANTARCTIC): an open-label, blinded-endpoint, randomised controlled superiority trial. *Lancet Lond Engl* 2016 ;388:2015–22.
23. Dillinger J-G, Sideris G, Kchaou I, Bal Dit Sollier C, Manzo Silberman S, Voicu S, et al. Monitoring persistent platelet reactivity in patients with unprotected left main stenting. *J Intervent Cardiol* 2013;26:578–85.
24. Members AF, Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2013;34:3035–87.
25. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract* 2014;105:141–50.
26. Greenfield JR, Campbell LV. Relationship between inflammation, insulin resistance and type 2 diabetes: “cause or effect”? *Curr Diabetes Rev* 2006;2:195–211.
27. Creager MA, Lüscher TF, Of P with the A, Cosentino F, Beckman JA. Diabetes and

Vascular Disease Pathophysiology, Clinical Consequences, and Medical Therapy: Part I. *Circulation* 2003;108:1527–32.

28. Senaran H, Ileri M, Altınbaş A, Koşar A, Yetkin E, Öztürk M, et al. Thrombopoietin and mean platelet volume in coronary artery disease. *Clin Cardiol* 2001;24:405–8.
29. Munipally PK, Agraharm SG, Valavala VK, Gundae S, Turlapati NR. Evaluation of indoleamine 2,3-dioxygenase expression and kynurenine pathway metabolites levels in serum samples of diabetic retinopathy patients. *Arch Physiol Biochem* 2011;117:254–8.
30. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* 2008;57:1349–54.
31. Santilli F, Simeone P, Liani R, Davì G. Platelets and diabetes mellitus. *Prostaglandins Other Lipid Mediat* 2015;120:28–39.
32. Winocour PD, Watala C, Perry DW, Kinlough-Rathbone RL. Decreased platelet membrane fluidity due to glycation or acetylation of membrane proteins. *Thromb Haemost* 1992;68:577–82.
33. Rocca B, Santilli F, Pitocco D, Mucci L, Petrucci G, Vitacolonna E, et al. The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. *J Thromb Haemost*. 2012;10(7):1220–30.
34. Lordkipanidzé M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. *Eur Heart J* 2007;28:1702–8.
35. Winocour PD, Kinlough-Rathbone RL, Mustard JF. Platelet survival in rats with spontaneous diabetes mellitus. *J Lab Clin Med* 1987;109:464–8.
36. Watala C, Pluta J, Golanski J, Rozalski M, Czyz M, Trojanowski Z, et al. Increased protein glycation in diabetes mellitus is associated with decreased aspirin-mediated protein acetylation and reduced sensitivity of blood platelets to aspirin. *J Mol Med Berl Ger* 2005 ;83:148–58.
37. Neergaard-Petersen S, Hvas A-M, Grove EL, Larsen SB, Gregersen S, Kristensen SD. The influence of haemoglobin a1c levels on platelet aggregation and platelet turnover in patients with coronary artery disease treated with aspirin. *PloS One*. 2015;10:e0132629.
38. Haffner SM, Miettinen H. Insulin Resistance Implications for Type II Diabetes Mellitus and Coronary Heart Disease. *Am J Med* 1997;103:152–62.
39. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 2008;294:E15–26.

Table I
Platelet aggregation and haemostasis parameters according to aspirin response

Variable	No HPR n = 89 (77%)	HPR n = 27 (23%)	<i>P</i>^a
<i>Platelet aggregation:</i>			
Median LTA (%)	7 [4–11]	73 [45–81]	< 0.0001
Median TXB2 (ng/mL)	1.2 [7.7–9.4]	27.4 [16.3–85]	< 0.0001
TXB2 > 2.45 ng/mL	21 (24%)	27 (100%)	< 0.0001
<i>Platelet turnover:</i>			
Platelet count (/mm ³)	223,000 [187,000– 259,000]	211,000 [171,000– 249,000]	0.32
Mean platelet volume (fL)	7.9 [7.3–8.6]	8.4 [7.7–9.4]	0.04
von Willebrand factor (IU)	138 [104–165]	150 [117–172]	0.18

Continuous data are presented as medians [interquartile range]; categorical data are presented as numbers (n) and proportions (%);

^a by Mann–Whitney test for continuous data; by Fisher’s exact test for categorical data;

HPR: high platelet reactivity; LTA: light transmission aggregometry; TXB2: thromboxane B2

Table II
Patients' characteristics according to aspirin response

Variable	No HPR n = 89 (77%)	HPR n = 27 (23%)	P ^a
Age (years)	65 ± 9.5	65 ± 7	0.84
Male (%)	63 (71%)	17 (63%)	0.48
<i>History of:</i>			
Coronary artery disease	37 (42%)	13 (48%)	0.66
Myocardial infarction	9 (10%)	2 (7%)	1
Coronary bypass grafting	6 (7%)	0 (0%)	0.33
Stroke	1 (1%)	1 (4%)	0.41
Transient ischaemic attack	3 (3%)	1 (4%)	1
<i>Associated risk factors:</i>			
Dyslipidaemia (%)	76 (85%)	23 (85%)	1
Hypertension (%)	64 (72%)	25 (93%)	0.04
Smoking history (%)	45 (50%)	12 (45%)	0.90
Current smoking (%)	15 (17%)	4 (15%)	0.77
Familial history of CVD (%)	14 (16%)	4 (15%)	1
Body mass index (kg/m ²)	27.4 [24.4–31.2]	32.3 [26.6–34.6]	0.01
<i>Treatment:</i>			
Beta-blocker	35 (39%)	13 (48%)	0.50
ACEi/angiotensin-II receptor antagonist	58 (65%)	22 (81%)	0.15
Statin	69 (78%)	21 (78%)	1
<i>Cardiovascular and atherosclerotic parameters:</i>			
Systolic blood pressure (mmHg)	131 ± 20	132 ± 22	0.71
Diastolic blood pressure (mmHg)	70 ± 11	71 ± 12	0.81
Pulse pressure (mmHg)	60 [50–69]	59 [50–70]	0.98
Sexual impotence (%)	36 (40%)	15 (55%)	0.03
Ankle–brachial index (ABI) score	1.18 [1.03–1.31]	1.25 [1.1–1.33]	0.17
Abnormal ABI (%)	13 (15%)	1 (4%)	0.13
<i>Biological data:</i>			
Haemoglobin (g/dL)	13.8 [12.5–14.8]	13.2 [12.1–14.6]	0.27
Creatinine (µmol/L)	74 [61–94]	74 [61–85]	0.28
GFR (mL/min/1.73 m ²)	87 [69–100]	87 [76–93]	0.53
GFR < 50 mL/min/1.73 m ²	12 (13%)	0 (0%)	0.07
GFR < 30 mL/min/1.73 m ²	10 (11%)	0 (0%)	0.11
Microalbumin (mg)	13.5 [7–33]	13 [7–37.5]	0.87

Continuous data are presented as medians [interquartile range] or means ± standard deviation; categorical data are presented as numbers (n) and proportions (%);

^a by Mann–Whitney or Student's *t* test, as appropriate, for continuous data; by Fisher's exact test for categorical data;

HPR: high platelet reactivity; CVD: cardiovascular disease; ACEi: angiotensin-converting enzyme inhibitor; GFR: glomerular filtration rate

Table III
Diabetes and insulin resistance according to aspirin response

Variable	No HPR n = 89 (77%)	HPR n = 27 (23%)	P ^a
<i>Diabetes:</i>			
Diabetes duration (years)	14 [9.5–22.5]	19 [13–30]	0.01
Number of oral antidiabetic drugs	2 [1–3]	2 [2–3]	0.13
Insulin treatment (%)	43 (48%)	16 (59%)	0.38
Duration of insulin treatment (years)	8 [3–12]	13 [8–17]	0.01
Mean dose of long-acting insulin (IU/day)	18 [13.5–29.5]	35 [21–57]	0.01
Mean dose of long-acting insulin/body weight (IU/day/kg)	0.24 [0.17–0.36]	0.42 [0.24–0.56]	0.006
Fasting glucose (g/L)	1.51 [1.22–1.79]	1.85 [1.46–2.21]	0.02
HbA1c (%)	7.5 [6.8–8.1]	7.7 [7–8.7]	0.24
HbA1c (mmol/mol)	58 [51–65]	61 [53–72]	
<i>Insulin resistance and metabolic syndrome:</i>			
Waist circumference (cm)	102 ± 13	110 ± 12	0.007
Waist index	1.26 [1.15–1.36]	1.38 [1.23–1.5]	0.006
HDL cholesterol (g/L)	0.43 [0.35–0.51]	0.46 [0.36–0.53]	0.34
Low HDL* (%)	53 (60%)	16 (59%)	1
Triglycerides (TG; g/L)	1.09 [0.71–1.56]	1.29 [0.79–1.79]	0.55
TG > 1.5 g/L	24 (27%)	9 (33%)	0.63
<i>Insulin resistance (biological data):</i>			
C-peptide (µg/L)	1.6 [1.2–2.3]	1.8 [1–3.4]	0.81
Insulin (mIU/L)	8 [5.5–10.8]	11 [6.4–11.8]	0.09
HOMA-IR	2.7 [1.9–3.9]	5.9 [3.6–9.6]	0.002
QUICKI	0.14 [0.14–0.15]	0.13 [0.12–0.14]	0.002
Leptin (ng/mL)	13 [5.9–25.3]	22.9 [13.6–30.4]	0.01

Continuous data are presented as medians [interquartile range] or means ± standard deviation; categorical data are presented as numbers (n) and proportions (%);

^a by Mann–Whitney or Student's *t* test, as appropriate, for continuous data; by Fisher's exact test for categorical data;

* < 0.5 g/L in women, < 0.4 g/L in men;

HPR: high platelet reactivity; HbA1c: glycated haemoglobin; HDL: high-density lipoprotein; HOMA-IR: homoeostasis model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index

Table IV
 Coronary artery disease (CAD) status in patients with vs without high platelet reactivity (HPR)

Variable	No HPR n = 89 (77%)	HPR n = 27 (23%)	<i>P</i>^a
Mean Agatston score	77 [1–268]	200 [60–802]	0.03
Patients with Agatston scores < 10	19 (37%)	0 (0%)	0.006
Patients with previous CAD	37 (42%)	13 (48%)	0.66

Continuous data are presented as medians [interquartile range] or means \pm standard deviation; categorical data are presented as numbers (n) and proportions (%);

^a by Mann–Whitney or Student’s *t* test, as appropriate, for continuous data; by Fisher’s exact test for categorical data

Table V

Thrombopoietin (TPO) and inflammatory markers in patients with vs without high platelet reactivity (HPR)

Variable	No HPR n = 89 (77%)	HPR n = 27 (23%)	<i>P</i>^a
TPO (pg/mL)	112 [80–154]	131 [90–147]	0.38
White blood cell count (/mm ³)	6900 [5600–8350]	6500 [5400–8400]	0.72
Neutrophil count (/mm ³)	3965 [3135–5315]	3950 [3160–4830]	0.45
Fibrinogen (g/L)	3.21 ± 0.62	3.18 ± 0.55	0.95
C-reactive protein (CRP; mg/L)	1.28 [0.15–2.80]	1.56 [0.76–5.46]	0.08
CRP < 2 mg/L	56 (63%)	13 (52%)	0.35
CRP > 3 mg/L	25 (28%)	10 (40%)	0.33
Interleukin (IL)-10 (pg/mL)	17.2 [14.7–20]	17.4 [14.7–21.8]	0.68
IL-6 (pg/mL)	2.7 [2–4.1]	2.9 [2–3.9]	0.86
Tumour necrosis factor- α (pg/mL)	46.1 [36.3–60.1]	46.4 [29–53.2]	0.31
IDO activity (%)	7.4 [5.3–8.9]	6.7 [4.9–9.1]	0.98

Values are presented as medians [interquartile range] or means \pm standard deviation;

^a by Mann–Whitney or Student's *t* test, as appropriate, for continuous data; by Fisher's exact test for categorical data;

IDO: indoleamine 2,3-dioxygenase

Table VI

Correlations (R) between plasma thromboxane B2 (TXB2) and other major parameters of diabetes, platelet aggregation and inflammation

	R^a	P
Diabetes duration	0.15	0.10
Long-acting insulin dose (/kg)	0.43	0.0007
Body mass index	0.22	0.02
Waist circumference (cm)	0.20	0.03
Waist index	0.20	0.03
Leptin	0.25	0.01
Fasting glucose	0.19	0.05
HbA1c	0.22	0.02
C-peptide	-0.004	0.97
Insulin	0.19	0.32
HOMA-IR	0.42	0.002
QUICKI	-0.44	0.0006
Thrombopoietin	0.03	0.78
Fibrinogen	0.08	0.39
C-reactive protein	0.23	0.02
Interleukin-6	0.07	0.49
Interleukin-10	-0.06	0.57
Tumour necrosis factor- α	0.09	0.33
IDO activity	-0.12	0.25

^a Spearman's rank correlation coefficient;

HbA1c: glycated haemoglobin; HOMA-IR: homoeostasis model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index; IDO: indoleamine 2,3-dioxygenase