

Iron-induced platelet aggregation measurement: a novel method to measure platelet function in stenting for ST segment elevation myocardial infarction

J. J. J. Smit^{*}, W. van Oeveren[†], J. P. Ottervanger^{*}, R. J. Slingerland[‡], J. A. Remijn^{**‡}, F. Zijlstra[¶] and A. W. J. van 't Hof^{*}

^{*}Isala Klinieken, Zwolle, [†]Haemoscan, Groningen, [‡]Gelre Ziekenhuizen, Apeldoorn, [§]Universitair Medisch Centrum Groningen, The Netherlands

ABSTRACT

Background Iron and (stainless) steel are potent platelet aggregation activators, and may be involved in stent thrombosis, a serious complication after intracoronary stenting. Current platelet function tests are suboptimal, because of inappropriate agonists and/or lack of reproducibility. We tested the feasibility and reproducibility of a novel platelet function test using stainless steel as an agonist and compared it with other platelet function tests.

Materials and methods In 111 patients with acute ST segment elevation myocardial infarction (STEMI), duplo measurements of iron (Fe)-induced platelet aggregation (FIPA) were performed after clopidogrel, acetylsalicylic acid and/or tirofiban treatment. Within 1 h, citrated blood samples drawn from the femoral sheath before primary percutaneous coronary intervention were added to 100 mg of low carbon steel and after 5 s mixing with vortex, the samples were incubated for 15 min. The ratio between the non-aggregated platelets in the agonist sample and platelets in a reference sample was calculated as the platelet aggregation inhibition.

Results FIPA measurement was highly reproducible (correlation coefficient (R) = 0.942, $P < 0.001$ between duplo samples). FIPA correlated well with adenosine diphosphate-induced platelet aggregation ($R = 0.83$, $P < 0.001$) but weakly with platelet function analyser-100 bleeding time ($R = 0.56$, $P < 0.001$). FIPA could be measured in patients in which platelet aggregation could not be measured by platelet function analyser-100 or after adenosine diphosphate.

Conclusion This study showed good reproducibility of a novel platelet function test using stainless steel as an agonist and showed correlation with validated platelet function tests. We found that the novel platelet function test is a suitable test for measurement of platelet aggregation inhibition in patients undergoing stenting for STEMI, even when they are taking multiple antiplatelet regimens.

Keywords iron-induced platelet aggregation, platelets, platelet function, STEMI

Eur J Clin Invest 2009; 39 (2): 103–109

Introduction

Iron and (stainless) steel are potent platelet aggregation activators, and this has been considered as a limitation of using these materials in endovascular stents [1–7]. This is of particular importance since (subacute) stent thrombosis is a potential life-threatening complication after intracoronary stenting [8]. Current platelet function tests are not optimal, possibly because of inappropriate agonists and lack of reproducibility [9]. We developed a platelet function test using stainless steel as an

agonist. We hypothesize our platelet function test to be reproducible and feasible to detect platelet function in patients with acute coronary syndrome. In this study, we tested the feasibility of iron (Fe)-induced platelet aggregation (FIPA) measurement and compared our FIPA test with other platelet function tests in ST segment elevation myocardial infarction (STEMI) patients before percutaneous coronary intervention (PCI).

Methods

This paper concerns a substudy of 111 patients of the Ongoing Tirofiban in Myocardial Infarction Evaluation (On-TIME) 2 pilot study. The On-TIME 2 study is a randomized, open label, investigator-initiated, multicentre trial to evaluate the value of pre-hospital administration of high dose (bolus $25 \mu\text{g kg}^{-1}$, followed by a maintenance infusion of $0.15 \mu\text{g kg}^{-1} \text{min}^{-1}$) tirofiban vs. placebo (standard treatment) in STEMI patients on improving the extent of myocardial reperfusion (primary endpoint) [10]. The protocol was approved by our institution's Review Board and Ethical Committee, and written informed consent was obtained from all patients. PCI was performed immediately after coronary angiography in all patients. All patients were treated with clopidogrel (600 mg loading dose followed by 75 mg daily for 1 year), acetylsalicylic acid, beta blocker, statin and angiotensin-converting enzyme inhibitor. In our substudy, additional blood samples were collected in citrate (0.109 M) in plastic tubes before PCI, but after the patients received the study medication (tirofiban or placebo) in addition to 600 mg oral clopidogrel, 5000 IU of heparin and 500 mg of acetylsalicylic acid were administered intravenously. The samples were drawn from the femoral sheath at the start of catheterization, before additional heparin infusion, using the Vacutainer[®] system (Becton Dickinson and Company, Franklin Lakes, NJ, USA).

Fe-induced platelet aggregation

Platelet aggregation was measured using AISI 434 low carbon stainless steel as a stimulus. Citrated whole blood was used, collected within 1 h before platelet function testing. Duplicate samples of 2 mL citrated blood were added to tubes containing 100 mg steel wool (Haemoscan, Groningen, The Netherlands), and after a 5-s mixing in a vortex (type MIX TM01, Retsch, Haan, Germany), the citrated blood was incubated for 15 min at room temperature. Subsequently, platelet count was performed on each sample using a routine blood cell counter (Sysmex K4500, Sysmex Corp., Kobe, Japan) and on a reference tube. In the presence of the agonist steel, platelets aggregate and adhere to the steel surface. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio between the non-aggregated platelets in the agonist sample and the platelet count in the reference tube without steel multiplied by 100% was used as the platelet aggregation inhibition.

Adenosine diphosphate-induced platelet aggregation

For measurement of the adenosine diphosphate (ADP) platelet aggregation inhibition, we used the Sysmex K4500 method [11]. Blood samples were collected in plastic tubes containing EDTA and tubes containing PPACK with $20 \mu\text{M L}^{-1}$ ADP (Plateletworks[®], Helena Laboratories, Beaumont, TX, USA). A routine platelet count was performed on each sample. The platelet count in the

EDTA tube was used as a reference. In the presence of the agonist ADP, platelets aggregate and associate. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio between the non-aggregated platelets in the agonist sample and the platelet count in the reference tube multiplied by 100% was calculated as the platelet aggregation inhibition. In our laboratory, we reported a correlation coefficient of 0.90 between the Sysmex K4500 and the ICHOR point-of-care platelet analyser (Helena Laboratories) to validate the Sysmex K4500 platelet aggregation measurement [12,13].

PFA-100[®]

Platelet function was measured using a platelet function analyser (PFA-100, Dade Behring, Marburg, Germany), an instrument that provides a quantitative measurement of platelet adhesion and aggregation in whole blood flowing through a small aperture under high shear conditions [14–17]. The aperture ($147 \mu\text{m}$) is coated with $2 \mu\text{g}$ type I collagen and $50 \mu\text{g}$ epinephrine bitartrate. The closure time of the aperture, referred to as bleeding constant, is an indicator of platelet function.

Statistical analysis

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as mean \pm standard deviation, and categorical data as percentage, unless otherwise denoted. Analysis of variance and the chi-squared test were appropriately used for continuous and categorical variables, respectively. For comparison of the reproducibility of the duplo FIPA measurements, a Wilcoxon test was used, and for comparison with other platelet function tests, a Spearman Rho correlation coefficient was determined using the Pearson product-moment correlation. Furthermore, a Bland-Altman graph was drawn to compare the two different outcome values of the duplo FIPA sample with the mean of these values and to compare the mean of differences between FIPA and ADP aggregation. The limits of agreement, the borderline of 2 standard deviations from the mean, were determined to investigate clinical usefulness. A *P*-value of < 0.05 was considered statistically significant.

Results

From April 2005 to December 2005, platelet aggregation was measured in 111 consecutive patients who were randomized to either pre-hospital high-dose tirofiban ($n = 53$) or placebo ($n = 58$). Baseline characteristics of these patients are described in Table 1.

FIPA

The duplo measurement of the FIPA test showed good reproducible results ($R = 0.942$, $P < 0.001$, Fig. 1). In 72% of the patients who received high-dose tirofiban, platelet aggregation

Table 1 Baseline characteristics of patients according to randomization

	Group 1 (n = 53)	Group 2 (n = 58)	P-value
Male gender	77%	71%	0.424
Hypertension	40%	36%	0.711
Diabetes	8%	10%	0.745
Smoking	54%	42%	0.212
Hypercholesterolaemia	23%	17%	0.476
Family history	43%	42%	0.891
Previous angina	43%	36%	0.439
Previous myocardial infarction	13%	12%	0.857
Previous percutaneous coronary intervention	8%	5%	0.707
Previous coronary artery bypass grafting	4%	5%	1.000
Previous cerebrovascular accident	0%	0%	
Age (years, ± SD)	61.9 ± 11.7	63.3 ± 10.8	0.540
Blood pressure systolic (mmHg, ± SD)	142 ± 25	130 ± 26	0.024
Blood pressure diastolic (mmHg, ± SD)	86 ± 15	80 ± 19	0.071
Heart rate (b.p.m.)	75 ± 21	75 ± 20	0.872
Length (cm, ± SD)	176.0 ± 8.7	174.8 ± 9.0	0.589
Weight (kg, ± SD)	82 ± 12	83 ± 16	0.583

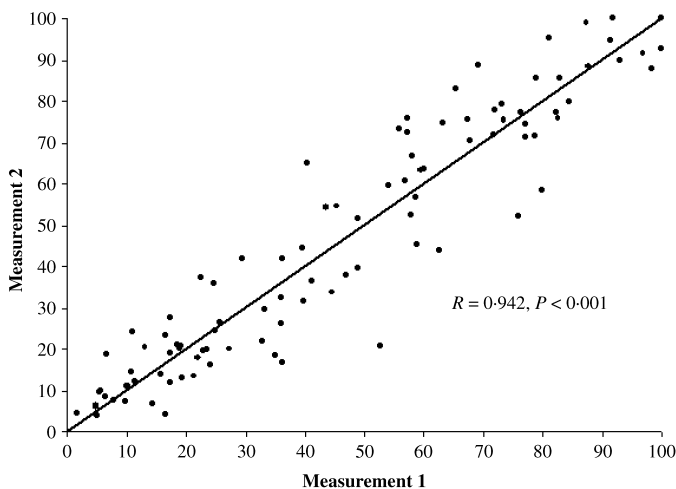


Figure 1 Duplo measurements and correlation of the FIPA inhibition (n = 111, R = 0.942, P < 0.001).

inhibition was below 80%. The Bland–Altman graph shows the mean of both duplo measurements and limits of agreement of plus or minus 20% (Fig. 2). There was no association between an increase in FIPA inhibition and increased difference between the two measurements (Wilcoxon test: P = 0.864).

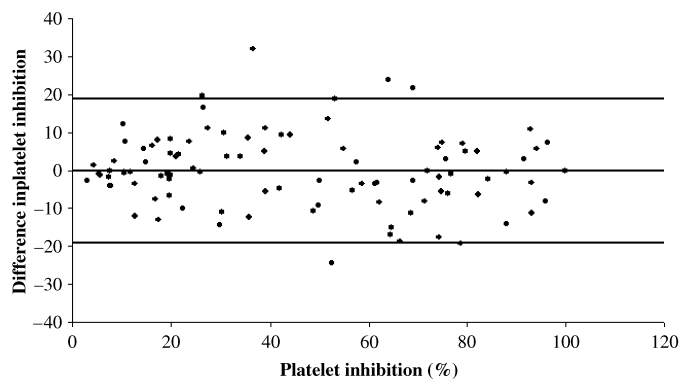


Figure 2 Bland Altman graph of the mean of both duplo measurements of FIPA inhibition (n = 111).

Comparison with other tests

FIPA correlated well with ADP-induced platelet aggregation (R = 0.834, P-value < 0.001, Fig. 3, Bland–Altman graph in Fig. 4), but there was only a weak association between FIPA and the quantitative platelet adhesion measurement by PFA-100 (R = 0.564, P < 0.001, Fig. 5). FIPA could be measured also in patients in which platelet aggregation could not be measured by PFA-100 or after ADP (Figs 3 and 5). As compared to PFA-100,

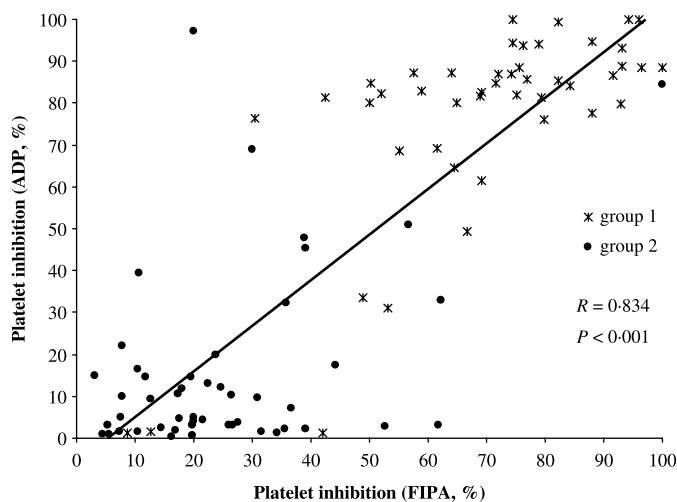


Figure 3 Comparison of FIPA inhibition and ADP induced platelet aggregation inhibition ($n = 111$).

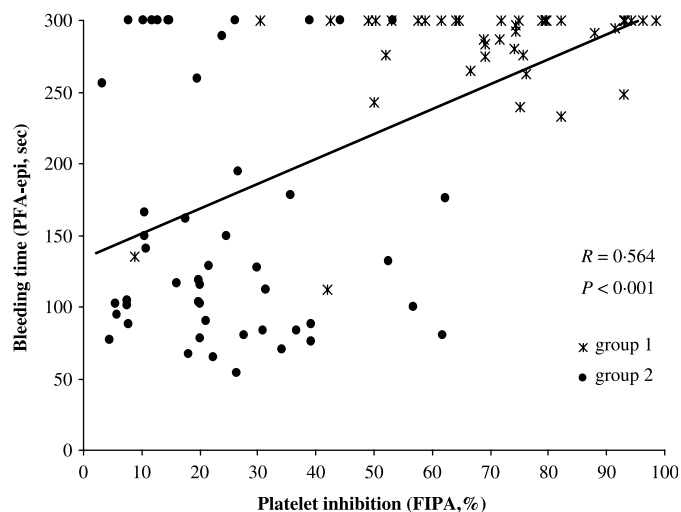


Figure 5 Comparison of FIPA inhibition and PFA-100 bleeding time ($n = 111$).

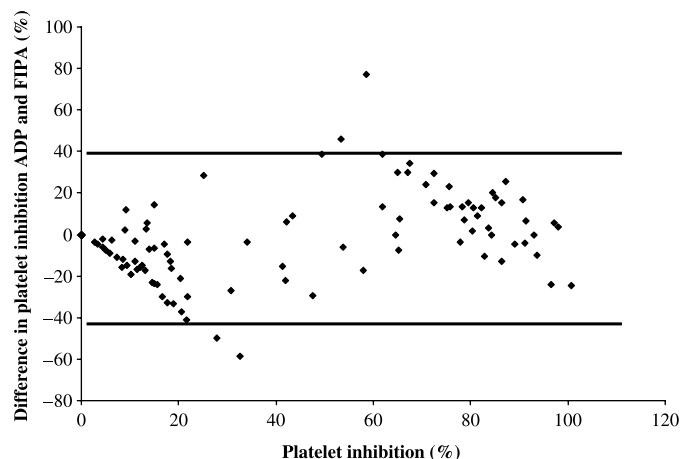


Figure 4 Bland-Altman graph of the comparison between the mean of differences of ADP aggregation inhibition [%] and FIPA inhibition (%), ($n = 111$).

only few patients had maximal platelet inhibition using the FIPA test, indicating that FIPA measurement can be performed in patients with more extensive platelet inhibition (Fig. 5). As compared to the ADP-induced platelet aggregation inhibition, in few patients minimal platelet aggregation inhibition was found using our FIPA method, indicating that platelet function could be measured in patients with maximal platelet activation (Fig. 3).

Discussion

In the present study, we tested the feasibility and reproducibility of a novel platelet function test using stainless steel as an agonist for platelet aggregation. Our test, in contrast to other platelet function tests, had good reproducibility, and platelet aggregation could even be measured in patients with enhanced levels of platelet aggregation inhibition or activation.

Antiplatelet therapy has become increasingly important in patients with coronary artery disease, particularly in those with acute coronary syndrome and in those undergoing PCI [18]. The intended platelet aggregation inhibition should be a balance between prevention of thrombotic complications on one side and bleeding risks on the other side [11,19]. Platelet function measurement may facilitate individual dosing and type of antiplatelet therapy. Platelet function testing is not embedded into routine clinical practice, because no optimal, easy, reproducible and multipathway platelet aggregation test can be accomplished *in vitro*. Furthermore, only few studies have associated platelet aggregation inhibition with clinical outcome in myocardial infarction. Conflicting results have been present in particular in patients with subacute thrombosis after stenting [20–25]. High platelet reactivity was found in patients who experienced stent thrombosis, and patients with clopidogrel resistance were at increased risk for recurrent atherothrombotic events [20,21]. Furthermore, in STEMI, increased levels of platelet aggregation (related to infarct size) were found as compared to unstable angina or control patients [22]. A thrombolysis study reported platelet receptor occupancy to be associated with better angiographic

and electrocardiographic outcome [23]. Finally, in STEMI patients undergoing primary PCI, increased inhibition of platelet aggregation by abciximab was recently found to be associated with better myocardial reperfusion [24]. However, in our recent On-TIME 1 study, no relationship was found between the levels of platelet aggregation inhibition and clinical outcome in STEMI patients undergoing primary PCI [25]. The lack of correlation between platelet aggregation inhibition and clinical outcome might be explained by the small number of major adverse cardiac events by exclusion of moderate heart failure patients, or it could be due to an insufficient method of platelet aggregation measurement at that time of the study. In the On-TIME 1 study, we used ADP as an agonist for platelet aggregation inhibition measurement. It is questionable whether this single pathway platelet aggregation agonist is sufficient to simulate the multipathway platelet aggregation in acute coronary syndrome. We invented a simple platelet aggregation test for practical clinical use, using a multipathway agonist for platelet aggregation, resembling stents in coronary arteries. This platelet function test might identify patients with increased levels of platelet aggregation, who might be at high risk for worse clinical outcome, and be in need of tailored antiplatelet regimens. Our FIPA test will be used in the On-TIME 2 study to identify patients at high risk for thrombotic complications after primary PCI for STEMI.

Stainless steel activates platelet adhesion, aggregation and thromboxane release [1–7]. The potent platelet activation characteristics of stainless steel might be due to coverage of stainless steel with proteins other than albumin, such as fibrinogen, von Willebrand factor, immunoglobulin and fibronectin [26]. As a consequence, the protective cover of albumin, which does not interact with platelets, is less present in stainless steel surfaces, resulting in increased platelet activation by steel surfaces [26].

In the present study, we found an association between FIPA and ADP-induced platelet aggregation. Therefore, our FIPA test might be importantly ADP regulated. However, since FIPA is a multipathway stimulus for platelet aggregation, it might better resemble the clinical platelet aggregation by metal stents in the setting of an acute coronary syndrome. However, no gold standard platelet function test is available. Therefore, we compared our FIPA test to two other platelet function tests instead of one.

Bleeding time measurement, in patients with an acute myocardial infarction, using PFA-100, before antiplatelet therapy administration was correlated inversely with the extent of myocardial cell necrosis [22]. In a study of mixed stable and acute coronary syndrome patients, shortened bleeding time with PFA-100 (< 190 s) was associated with a higher risk for the reoccurrence of cardiovascular events or death [27,28]. A characteristic of PFA-100 is that bleeding time is highly dependent on the levels of von Willebrand factor [29,30]. As a result, high von Willebrand factor levels may mask the inhibitory effects of

antiplatelet therapy because the high-shear rates will promote direct binding of von Willebrand factor to glycoprotein IIb/IIIa [31]. The currently available ADP cartridges are not very sensitive to clopidogrel [32,33]. Since patients were randomized to either high-dose tirofiban or placebo on top of clopidogrel and acetylsalicylic acid in the On-TIME 2 study, this might explain the diversity in the results of the PFA-100 bleeding time found in our study.

Limitations

Since thrombosis of coronary arteries is known to be the result of the multifactorial interplay of substance release from the endothelial wall, activation of platelets, shear stress due to roughness of the internal vessel wall and mechanical complications of PCI, inflammation markers released by the immune system, thrombogenicity of stent material, and the form and geometry of stents, it is wrong to suggest that *in vitro* platelet aggregation test using stainless medical steel represents the complete platelet aggregation response in patients with an acute STEMI undergoing primary PCI [34]. Another limitation is the lack of comparison with the Verify-now[®] test.

Conclusion

In this pilot study, FIPA measurement showed to be a novel, feasible and easy method for platelet function measurement, resembling the agonist for platelet aggregation in stents in coronary arteries. Furthermore, FIPA could be measured in acute STEMI patients using potent platelet inhibitors such as glycoprotein IIb/IIIa blockers plus clopidogrel and acetylsalicylic acid in contrast to other methods of platelet function.

Acknowledgement

This study was supported with an educational grant from Merck Sharp & Dohme BV (Haarlem, The Netherlands).

Address

Department of Cardiology, Isala Klinieken, Zwolle, The Netherlands (J. J. J. Smit, J. P. Ottervanger, A. W. J. van 't Hof); Haemoscan, Groningen, The Netherlands (W. van Oeveren); Department of Clinical Chemistry, Isala Klinieken, Zwolle, The Netherlands (R. J. Slingerland, J. A. Remijn); Department of Clinical Chemistry and Hematology, Gelre Ziekenhuizen, Apeldoorn, The Netherlands (J. A. Remijn); and Department of Cardiology, Universitair Medisch Centrum Groningen, The Netherlands (F. Zijlstra).

Correspondence to: A. W. J. van 't Hof, MD, PhD, Department of Cardiology, Isala Klinieken, Groot Wezenland 20 8011 JW Zwolle, The Netherlands. Tel.: +31 38 4242198; fax: +31 38 4243222; e-mail:v.r.c.derks@isala.nl

Received 29 July 2008; accepted 6 November 2008

References

- 1 Bertrand OF, Sipehia R, Mongrain R, Rodes J, Tardif JC, Bilodeau L *et al.* Biocompatibility aspects of new stent technology. *J Am Coll Cardiol* 1998;**32**:562–71.
- 2 Santin M, Mikhalovska L, Lloyd AW, Mikhalovsky S, Sigfrid L, Denyer SP *et al.* *In vitro* host response assessment of biomaterials for cardiovascular stent manufacture. *J Mater Sci Mater Med* 2004;**15**:473–7.
- 3 Rhodes NP, Shortland AP, Rattray A, Williams DF. Platelet reactions to modified surfaces under dynamic conditions. *J Mater Sci Mater Med* 1998;**9**:767–72.
- 4 Hietala EM, Maasilta P, Juuti H, Nuutinen JP, Harjula AL, Salminen US *et al.* Platelet deposition on stainless steel, spiral, and braided polylactide stents. A comparative study. *Thromb Haemost* 2004;**92**:1394–401.
- 5 Mrowietz C, Franke RP, Seyfert UT, Park JW, Jung F. Haemocompatibility of polymer-coated stainless steel stents as compared to uncoated stents. *Clin Hemorheol Microcirc* 2005;**32**:89–103.
- 6 Kolandaivelu K, Edelman ER. Environmental influences on endovascular stent platelet reactivity: an *in vitro* comparison of stainless steel and gold surfaces. *J Biomed Mater Res A* 2004;**70**:186–93.
- 7 Monnink SH, van Boven AJ, Peels HO, Tigchelaar I, de Kam PJ, Crijns HJ *et al.* Silicon-carbide coated coronary stents have low platelet and leukocyte adhesion during platelet activation. *J Investig Med* 1999;**47**:304–10.
- 8 Smit JJJ, van 't Hof AWJ, De Boer MJ, Hoorntje JCA, Dambrink JHE, Gosselink ATM *et al.* Incidence and predictors of subacute thrombosis in patients undergoing primary angioplasty for an acute myocardial infarction. *Thromb Haemost* 2006;**96**:190–5.
- 9 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.
- 10 van 't Hof AWJ, Hamm C, Rasoul S, Guptha S, Paolini J, ten Berg J. on behalf of the On-TIME 2 investigators. Ongoing tirofiban in myocardial infarction evaluation (On-TIME) 2 trial: rationale and study design. *Eurointerv* 2007;**3**:371–80.
- 11 Ernst NMSKJ, Suryapranata H, Miedema K, Slingerland RJ, Ottervanger JP, Hoorntje JCA *et al.* Achieved platelet aggregation inhibition after different antiplatelet regimens during percutaneous coronary intervention for ST segment elevation myocardial infarction. *J Am Coll Cardiol* 2004;**44**:1187–93.
- 12 Carville DG, Schleckser PA, Guyker KE, Corsello M, Walsh MM. Whole blood platelet function assay on the ICHOR point-of-care haematology analyzer. *J Extra Corpor Technol* 1998;**30**:171–7.
- 13 Lakkis NM, George S, Thomas E, Ali M, Guyer K, Carville D. Use of ICHOR-platelet works to assess platelet function in patients treated with GP IIb/IIIa inhibitors. *Catheter Cardiovasc Interv* 2001;**53**:346–51.
- 14 Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L *et al.* Profile and prevalence of Aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001;**88**:230–5.
- 15 Crescente M, Di Castelnuovo A, Iacoviello L, Vermuyen J, Cerletti C, de Gaetano G. Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100. A systematic review. *Thromb Haemost* 2008;**99**:14–26.
- 16 Marshall PW, Williams AJ, Dixon RM, Growcott HW, Warburton S, Armstrong J *et al.* A comparison of the effects of aspirin on bleeding time measured using the Simplate method and closure time measured using the PFA-100, in healthy volunteers. *Br J Clin Pharmacol* 1997;**44**:151–5.
- 17 Smit JJJ, Hoorntje JCA, Miedema K, Van Oeveren W. Impaired platelet inhibitory effect of a single dose of acetylsalicylic acid in patients with unstable coronary artery syndrome in comparison with healthy volunteers. *Neth Heart J* 2004;**12**:265–70.
- 18 Van 't Hof AWJ, Ernst N, De Boer MJ, de Winter R, Boersma E, Bunt T *et al.* On-TIME study group. Facilitation of primary coronary angioplasty by early start of a glycoprotein 2b/3a inhibitor: results of the ongoing tirofiban in myocardial infarction evaluation (On-TIME) trial. *Eur Heart J* 2004;**25**:837–46.
- 19 Yusuf S, Mehta SR, Chrolavicius S, Afzal R, Pogue J, Granger CB *et al.* Fifth Organization to Assess Strategies in Acute Ischemic Syndromes Investigators. Comparison of fondaparinux and enoxaparin in acute coronary syndromes. *N Engl J Med* 2006;**354**:1464–76.
- 20 Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I *et al.* Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004;**109**:3171–5.
- 21 Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissaha MZ *et al.* Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol* 2005;**46**:1827–32.
- 22 Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H *et al.* Platelet function predicts myocardial damage in patients with acute myocardial infarction. *Circulation* 2004;**110**:1392–7.
- 23 Gibson CM, Jennings LK, Murphy SA, Lorenz DP, Giugliano RP, Harrington RA *et al.* INTEGRITI Study Group. Association between platelet receptor occupancy after eptifibatid (integrilin) therapy and patency, myocardial perfusion, and ST-segment resolution among patients with ST-segment-elevation myocardial infarction: an INTEGRITI (Integrilin and Tenecteplase in Acute Myocardial Infarction) substudy. *Circulation* 2004;**110**:679–84.
- 24 de Prado AP, Fernandez-Vazquez F, Cuellas JC, Alonso-Orcajo N, Carbonell R, Pascual C *et al.* Association between level of platelet inhibition after early use of abciximab and myocardial reperfusion in ST-elevation acute myocardial infarction treated by primary percutaneous coronary intervention. *Am J Cardiol* 2006;**97**:798–803.
- 25 Smit JJJ, Ernst NM, Slingerland RJ, Kolkman JJ, Suryapranata H, Hoorntje JCA *et al.* on behalf of the On-TIME study group. Platelet microaggregation inhibition in patients with acute myocardial infarction pretreated with tirofiban and relationship with angiographic and clinical outcome. *Am Heart J* 2006;**151**:1102–7.
- 26 Welle A, Grunze M, Tur D. Plasma protein adsorption and platelet adhesion on poly[bis(trifluoroethoxy)phosphazene] and reference material surfaces. *J Colloid Interface Sci* 1998;**197**:263–74.
- 27 Gianetti J, Parri MS, Sbrana S, Paoli F, Maffei S, Paradossi U *et al.* Platelet activation predicts recurrent ischemic events after percutaneous coronary angioplasty: a 6 months prospective study. *Thromb Res* 2006;**118**:487–93.
- 28 Jacopo G, Elisabetta V, Silverio S, Massimiliano M, Sergio B, Grazia AM *et al.* Identification of platelet hyper-reactivity measured with a portable device immediately after percutaneous coronary intervention predicts in stent thrombosis. *Thromb Res* 2007;**121**:407–12.
- 29 Chakroun T, Gerotziapas G, Robert F, Lecrubier C, Samama MM, Hatmi M *et al.* *In vitro* aspirin resistance detected by PFA-100 closure time: pivotal role of plasma von Willebrand factor. *Br J Haematol* 2004;**124**:80–5.
- 30 Haubelt H, Anders C, Vogt A, Hoerdt P, Seyfert UT, Hellstern P. Variables influencing Platelet Function Analyzer-100 closure times in healthy individuals. *Br J Haematol* 2005;**130**:759–67.
- 31 Reiter RA, Mayr F, Blazicek H, Galehr E, Jilma-Stohlawetz P,

- Domanovits H *et al.* Desmopressin antagonizes the *in vitro* platelet dysfunction induced by GPIIb/IIIa inhibitors and aspirin. *Blood* 2003;**102**:4594–9.
- 32 Golanski J, Pluta J, Baraniak J, Watala C. Limited usefulness of the PFA-100 for the monitoring of ADP receptor antagonists – *in vitro* experience. *Clin Chem Lab Med* 2004;**42**:25–9.
- 33 Hézard N, Metz D, Nazeyrollas P, Droulle C, Potron G, Nguyen P. PFA-100 and flow cytometry: can they challenge aggregometry to assess antiplatelet agents, other than GpIIbIIIa blockers, in coronary angioplasty? *Thromb Res* 2002;**108**:43–7.
- 34 Sheth S, Litvack F, Dev V, Fishbein MC, Forrester JS, Eigler N. Subacute thrombosis and vascular injury resulting from slotted-tube nitinol and stainless steel stents in a rabbit carotid artery model. *Circulation* 1996;**94**:1733–40.