British Journal of Anaesthesia **112** (3): 570–5 (2014)
Advance Access publication 22 October 2013 · doi:10.1093/bja/aet339

BJA

TRANSLATIONAL RESEARCH

Effects of *ex vivo* platelet supplementation on platelet aggregability in blood samples from patients treated with acetylsalicylic acid, clopidogrel, or ticagrelor

E. C. Hansson[1†], C. Shams Hakimi[1†], K. Åström-Olsson[2], C. Hesse[3], H. Wallén[4], M. Dellborg[5,6], P. Albertsson[2] and A. Jeppsson[1,6*]

[1] Department of Cardiothoracic Surgery, [2] Department of Cardiology and [3] Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
[4] Department of Clinical Sciences, Karolinska Institute, Danderyd Hospital, Stockholm, Sweden
[5] Department of Medicine, Sahlgrenska University Hospital/Östra, Gothenburg, Sweden
[6] Department of Molecular and Clinical Medicine, Institute of Medicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

* Corresponding author. E-mail: anders.jeppsson@vgregion.se

Editor's key points

- Platelet transfusion is often used in treating patients on dual antiplatelet therapy (DAPT), with unclear efficacy.
- The effects of *ex vivo* platelet supplementation on platelet aggregation were monitored in whole blood from patients taking platelet inhibitors.
- Platelet aggregation was restored in samples from aspirin-treated patients, but less effectively in samples from patients receiving DAPT, especially with ticagrelor.

Background. Transfusion of platelet concentrate is often used to treat bleeding in patients on platelet inhibitors, but little is known about its efficacy between different inhibitors. We assessed the effect of *ex vivo* platelet supplementation on platelet aggregability in blood samples from patients treated with acetylsalicylic acid (ASA), clopidogrel, or ticagrelor.

Methods. Platelet aggregability was investigated with multiple electrode aggregometry with adenosine diphosphate (ADP), arachidonic acid (to assess ASA-dependent aggregability), and thrombin receptor activating peptide-6 (TRAP) as activators in whole-blood samples from patients treated with ASA (n=10), ASA+clopidogrel (n=15), or ASA+ticagrelor (n=15), and from healthy controls (n=10). Aggregability was measured before and after supplementation of AB0-compatible fresh apheresis platelets (+46, +92, and +138 × 10^9 litre^{-1}).

Results. Both ASA-dependent and ADP-dependent aggregability improved in a dose-dependent fashion after platelet supplementation. ASA-dependent aggregability was completely restored in all patient groups, but there was only a small improvement in ADP-dependent aggregability in patients on dual antiplatelet therapy. There was less effect of platelet supplementation on ADP- and ASA-dependent aggregability in ticagrelor-treated patients than in clopidogrel-treated patients [3.9 (95% confidence interval 1.6–6.3) *vs* 9.0 (5.2–12.8) AU × min (P=0.021) and 48 (36–59) *vs* 69 (60–78) AU × min (P=0.004), respectively, at the highest platelet dose].

Conclusions. Platelet supplementation improved platelet aggregability independently of antiplatelet therapy. The effect on ADP-dependent platelet inhibition was limited however. Reduced effect of platelet transfusion is more likely within 2 h of drug intake in patients treated with ASA+ticagrelor compared with ASA+clopidogrel.

Keywords: haemorrhage; haemostasis, surgical; platelet aggregation inhibitors; platelet transfusion

Accepted for publication: 12 July 2013

Antiplatelet therapy is used to prevent thrombotic episodes, but is associated with an increased risk for spontaneous and perioperative bleeding complications. Dual antiplatelet therapy (DAPT) with acetylsalicylic acid (ASA) and the $P2Y_{12}$-inhibitor clopidogrel further reduces the risk of thrombotic episodes in acute coronary syndrome (ACS) but is also associated with a significantly increased risk of surgical bleeding complications compared with ASA alone.[1] The risk for bleeding complications is even higher with the new more effective $P2Y_{12}$-inhibitors prasugrel and ticagrelor.[2 3]

There are limited treatment options available if excessive bleeding occurs in patients on antiplatelet therapy, since

[†] These two authors contributed equally to the publication.

there are no direct antidotes to oral platelet inhibitors. Current guidelines recommend discontinuation of the $P2Y_{12}$-inhibitor in elective surgical procedures, whereas ASA treatment should be maintained.[4] However, patients on DAPT undergoing acute surgery will have sustained high-grade platelet inhibition during and after the operation. Platelet transfusion is often used to improve haemostasis in patients with ongoing bleeding, but little is known about its efficacy in patients taking platelet inhibitors, or if efficacy varies depending on the type of antiplatelet therapy used.

We investigated the effect of allogeneic platelet supplementation on *ex vivo* platelet aggregation in blood samples from healthy individuals and in coronary artery disease patients treated with different platelet inhibitors, that is, ASA alone or ASA in combination with clopidogrel or ticagrelor.

Methods

Patients

The study included 40 coronary artery disease subjects [mean age 67 (59–78) yr] and 10 healthy age-matched control subjects without antiplatelet therapy [mean age 60 (48–72) yr]. There were three groups on antiplatelet therapy: (i) ASA-treated consecutive patients with stable angina pectoris (n=10); (ii) ACS patients treated with ASA+clopidogrel (n=15); and (iii) ACS patients treated with ASA+ticagrelor (n=15). The time from initiation of DAPT to blood sampling ranged from 20 to 96 h in the ticagrelor+ASA group and from 20 to 192 h in the ASA+clopidogrel group. Concomitant medications are shown in Table 1.

ACS patients were included during a time when ticagrelor was being introduced in western Sweden to replace clopidogrel as first-line treatment in ACS patients. During this period, both ACS patients treated with ASA+ticagrelor and ACS patients treated with ASA+clopidogrel were admitted to the tertiary coronary care unit at Sahlgrenska University Hospital from the hospital's own catchment area and surrounding hospitals in western Sweden. The study was not randomized; instead, consecutive patients with the respective treatment were asked to participate after receiving oral and written information and were included after giving written consent.

Characteristics of the study subjects are given in Table 1. To prevent reactions due to alloimmunization that can occur at transfusion or during pregnancy, only men without previous history of transfusion were included. Further exclusion criteria were a known bleeding disorder, known renal or liver disease, platelet count $<150\times10^9$ litre^{-1}, or treatment with glycoprotein IIb/IIIa blocking agents within 6 h. The study was approved by the regional research ethics committee and was performed in accordance with the 1975 Declaration of Helsinki.

Study design

Venous blood samples for aggregability analysis were collected in hirudin-anticoagulated 3 ml tubes (>0.15 mg litre^{-1}) 2 h after intake of the morning dose of platelet inhibitor(s). Antiplatelet therapy consisted of 75 mg of ASA once daily; or 75 mg of clopidogrel once daily after a loading dose of 300 mg, and 75 mg of ASA; or 90 mg of ticagrelor twice daily after a loading dose of 180 mg, and 75 mg of ASA. Samples for haemoglobin, haematocrit, and platelet count were also collected.

Table 1 Subject characteristics. Mean and 95% confidence interval or number (frequency). ASA, acetylsalicylic acid; ACS, acute coronary syndrome; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction. *Within 24 h of sampling. †Discontinued 6 h before sampling

	Healthy subjects	ASA	ASA+clopidogrel	ASA+ticagrelor
n	10	10	15	15
Age (yr)	60 (48–72)	70 (62–78)	67 (61–73)	66 (59–72)
Platelet count ($\times10^9$ litre^{-1})	215 (179–251)	294 (230–358)	232 (197–266)	225 (204–285)
Haemoglobin (g litre^{-1})	146 (139–152)	136 (130–143)	136 (128–144)	142 (136–147)
Body mass index	25 (23–27)	28 (24–31)	27 (25–29)	27 (25–30)
Diabetes mellitus	0	3 (30%)	1 (6.7%)	2 (13%)
Previous myocardial infarction	0	2 (20%)	11 (73%)	4 (27%)
Type of ACS				
STEMI	—	—	5 (33%)	2 (13%)
NSTEMI	—	—	4 (27%)	4 (27%)
Unstable angina	—	—	6 (40%)	9 (60%)
Medication*				
Fondaparinux	—	—	3 (20%)	3 (20%)
Eptifibatid	—	—	1 (6.7%)†	0
Bivalirudin	—	—	4 (27%)	2 (13%)
Heparin	—	—	5 (33%)	5 (33%)
Simvastatin	—	6 (60%)	11 (73%)	8 (53%)
Atorvastatin	—	0	2 (13%)	3 (20%)
Pravastatin	—	0	0	1 (6.7%)

Samples from each subject were prepared by addition of increasing amounts of fresh (<6 h old) allogeneic ABO-compatible apheresis platelet concentrate from the hospital blood bank. Platelets were collected with the Trima Accel system version 6.0 (Terumo BCT, Lakewood, CO, USA) according to the manufacturer's instructions and following national and European guidelines.[5] Before donation, the donor platelet count was $>230\times10^9$ litre^{-1}. Platelets were collected in autologous plasma. The target concentration was 1600×10^9 litre^{-1} for each donation. Whole blood to citrate ratio was 10:1. Platelet count in each batch of concentrate was measured and the amount of platelet concentrate needed to achieve consistent doses of added platelets was calculated. Phosphate-buffered saline solution (PBS, 10 mM, pH 7.4) was used to maintain a constant volume of additive. The baseline sample consisted of 1 ml of blood and 300 μl of PBS; the low platelet dose 1 ml of blood, 50 μl of platelets, and 250 μl of PBS; the medium dose 1 ml of blood, 100 μl of platelets, and 200 μl of PBS; and the high platelet dose 1 ml of blood, 150 μl of platelets, and 150 μl of PBS. The numbers of platelets added were 60×10^6, 120×10^6, and 180×10^6, which increased the platelet concentration in the samples by +46, +92, and $+138\times10^9$ litre^{-1}, respectively. The increase in platelet count was calculated to correspond to the increase in platelet count achieved by *in vivo* transfusion of ~2–5 units of single-donor apheresis platelets to a 70 kg patient.

Analyses

Platelet aggregability was analysed with whole-blood impedance aggregometry (Multiplate Roche Diagnostics, Basel, Switzerland) as described.[6] In the test cell of the aggregometer, 300 μl of whole blood, PBS, and platelet mixture were added to 300 μl of saline solution pre-heated to 37°C. Test kits used were ADP high sensitivity kit (ADP final concentration 6.5 μM in combination with prostaglandin E1 final concentration 9.4 nM) for high-sensitivity detection of $P2Y_{12}$-dependent aggregation, ASPI test kit [arachidonic acid (AA) final concentration 0.5 mM] to assess cyclooxygenase-dependent (i.e. ASA-sensitive) platelet aggregation, and TRAP test kit (final concentration of thrombin receptor-activating peptide-6 32 μM) which detects PAR-1 receptor-dependent platelet aggregation and is commonly used to evaluate the effect of glycoprotein IIb/IIIa-inhibiting drugs. The change in impedance when platelets aggregate at the electrodes in the test cell is expressed as a graph where the area under the curve is a quantification of platelet aggregability, and reported in arbitrary aggregation units (AU×min). Single analyses with one test cell (two electrodes) were performed. The maximum tolerated difference between the two electrodes is 20%. The investigator was not blinded to the patient group. The manufacturer's normal range for ADP-high sensitivity test is 43–100 AU×min, for ASPI test 71–115 AU×min, and for TRAP test 84–128 AU×min.

Statistics

The numbers of included subjects were arbitrarily chosen, since no data were available for a sample size calculation when the study started. All data are presented as mean with 95% confidence intervals. A *P*-value of <0.05 was considered statistically significant. Distribution of data was tested for normality with the Kolmogorov–Smirnov test. Aggregation data were normally distributed. Changes from baseline within a group were analysed with the paired *t*-test. Group comparisons of aggregability at baseline were made with one-way analysis of variance (ANOVA) (more than two groups) or with Student's *t*-test (two-group comparisons). Differences in response to the different doses of platelet transfusion between the groups were analysed by ANOVA for repeated measurements followed by the unpaired *t*-test as *post hoc* test. For statistical analysis, STATISTICA 10 software was used (StatSoft, Tulsa, OK, USA).

Results

ADP-induced aggregability

There was a significant difference in baseline ADP-induced aggregability between the groups (ANOVA $P<0.001$) (Table 2). ADP-induced aggregability at baseline was significantly reduced in the ASA+clopidogrel and ASA+ticagrelor groups at baseline compared with healthy and ASA-treated subjects ($P<0.001$ for all comparisons), while the ASA+clopidogrel and ASA+ticagrelor groups were not significantly different in this respect ($P=0.90$). Platelet supplementation improved ADP-induced aggregability significantly in the ASA+clopidogrel group and in the ASA+ticagrelor group, but not in the ASA group or in healthy subjects (Table 2). When ASA+clopidogrel and ASA+ticagrelor-treated samples were compared, the response to platelet supplementation was significantly inferior in the ticagrelor-treated samples at the highest dose of platelets ($P=0.021$) (Fig. 1).

AA-induced aggregability

There was a significant difference in AA-induced aggregability in the different groups at baseline (ANOVA, $P<0.001$) (Table 2). Baseline AA-induced aggregability was significantly reduced in all three groups compared with healthy subjects ($P<0.001$ in all cases). The AA-induced aggregability at baseline was significantly lower in the ASA+clopidogrel and ASA+ticagrelor groups than in the ASA group ($P=0.001$ and 0.006, respectively), while the ASA+clopidogrel and ASA+ticagrelor groups were not significantly different in this respect ($P=0.19$). Platelet supplementation increased AA-induced aggregability in all four groups, with the highest response in the ASA group and the lowest response in the ASA+ticagrelor group (Table 2). In a direct comparison between ASA+clopidogrel and ASA+ticagrelor, the response to platelet supplementation was significantly inferior in ticagrelor-treated patients at all doses of platelets ($P=0.025$, 0.040, and 0.004, respectively) (Fig. 1).

TRAP-induced aggregability

There was a significant difference in baseline TRAP-induced aggregability between the different groups (ANOVA, $P=0.002$) (Table 2). Baseline TRAP-induced platelet aggregability was significantly reduced in the ASA+clopidogrel and ASA+ticagrelor groups compared with the ASA group ($P=0.004$ and 0.006, respectively) or with healthy subjects ($P=0.012$ and 0.017, respectively). The ASA group and healthy subjects

Table 2 Platelet aggregability in AU × min. Mean and 95% confidence interval. AA, arachidonic acid; ADP, adenosine diphosphate; ASA, acetylsalicylic acid; TRAP, thrombin receptor-activating peptide. *$P<0.05$, ***$P<0.001$ compared with the healthy controls at baseline. ‡‡$P<0.01$, ‡‡‡$P<0.001$ compared with the ASA group at baseline. †<0.05, ††$P<0.01$, †††$P<0.001$ compared with baseline within the same group

Group	Baseline	Low platelet dose	Medium platelet dose	High platelet dose
ADP-induced				
Healthy controls	45 (38–51)	40 (30–50)	44 (36–53)	43 (33–53)
ASA	50 (37–63)***	54 (41–67)	60 (50–70)	59 (48–70)
Clopidogrel+ASA	12 (8–16)***,‡‡‡	14 (10–17)	15 (12–18)††	21 (16–26)†††
Ticagrelor+ASA	12 (8–15)***,‡‡‡	14 (11–16)†	15 (12–18)††	16 (13–18)††
AA-induced				
Healthy controls	54 (39–68)	61 (50–74)	70 (56–84)†	78 (62–94)††
ASA	15 (12–18)***	59 (49–70)†††	82 (69–94)†††	95 (88–102)†††
Clopidogrel+ASA	7 (4–10)***,‡‡	39 (28–51)†††	60 (50–69)†††	76 (68–85)†††
Ticagrelor+ASA	10 (7–12)***,‡‡	25 (18–33)††	46 (34–58)†††	57 (46–68)†††
TRAP-induced				
Healthy controls	82 (74–90)	84 (77–91)	90 (84–97)†††	91 (84–97)††
ASA	87 (76–99)	97 (86–108)†	98 (88–108)†	103 (95–110)†††
Clopidogrel+ASA	61 (48–74)*,‡‡	73 (62–84)†††	83 (75–92)†††	89 (82–97)†††
Ticagrelor+ASA	62 (49–74)*,‡‡	76 (63–89)†††	85 (77–93)†††	93 (85–101)†††

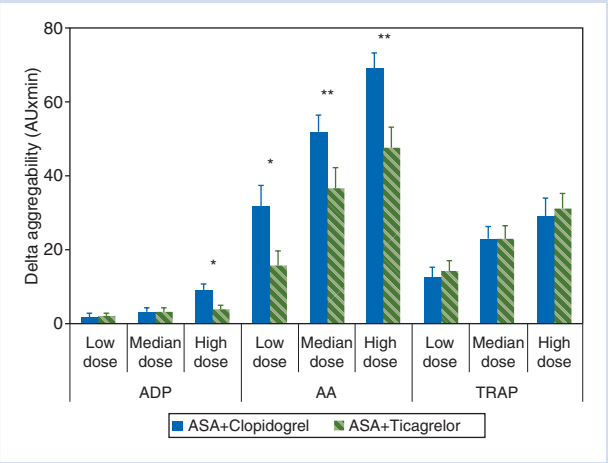

Fig 1 Changes (mean and standard error of the mean) from baseline in aggregability after addition of increasing doses of platelet concentrate in the ASA+clopidogrel group and the ASA+ticagrelor group. AA, arachidonic acid; ADP, adenosine diphosphate; ASA, acetylsalicylic acid; TRAP, thrombin receptor-activating peptide. *$P<0.05$, **$P<0.01$ between the groups.

were not significantly different in this respect ($P=0.89$). Platelet supplementation enhanced TRAP-induced aggregability in all four groups, with the largest increase in the ASA+clopidogrel and ASA+ticagrelor groups. There was no significant difference in response between the ASA+clopidogrel and ASA+ticagrelor groups ($P=0.67$) (Fig. 1).

Discussion

The main findings in the present study were: (i) platelet supplementation improved platelet aggregability independently of the type of antiplatelet drug treatment; and (ii) platelet supplementation was less effective in samples from patients treated with ASA+ticagrelor than those treated with ASA+clopidogrel within 2 h of last drug intake.

Even though platelet transfusion has been used extensively over the years to improve platelet function in bleeding patients on antiplatelet therapy, few investigations have assessed efficacy.[7–9] Furthermore, there have been no studies in coronary artery disease patients, the main consumers of platelet inhibitors, and possible differences in efficacy depending on target pathway and type of inhibitor have not been addressed. In healthy subjects, three previous studies have investigated the effect of platelet transfusion on the reversibility of platelet inhibition by ASA or ASA in combination with clopidogrel. Di Minno and colleagues[7] found that the platelet inhibition afforded by ASA could be restored *ex vivo* with moderate doses of platelet transfusion. Vilahur and colleagues[8] showed that platelet doses corresponding to 2–3 units restored *ex vivo* platelet aggregation in clopidogrel- and ASA-treated healthy subjects. Pruller and colleagues[9] found a partial restitution of platelet function *in vivo* after supplementation of ASA+clopidogrel-treated healthy subjects with autologous platelets. The present study confirms and extends the results from these three studies. AA-induced aggregation was restored and increased above baseline levels after supplementation with platelet doses corresponding to 2–5 units of platelet transfusion. This effect was observed in healthy subjects without ASA, in ASA-treated patients, and in patients treated with ASA in combination with clopidogrel or ticagrelor. However, the final aggregability with the highest platelet dose was markedly higher in ASA-treated patients than in patients treated with ASA+clopidogrel or ASA+ticagrelor. TRAP-induced aggregability was also enhanced with platelet supplementation to levels above baseline in healthy volunteers, and independently of antiplatelet regimen.

Interestingly, patients on DAPT with clopidogrel or ticagrelor had ~25% lower baseline TRAP-induced aggregability than healthy volunteers and ASA-treated patients. This is most likely a reflection of the fact that the platelet-activating effect of TRAP is partly dependent on ADP released from activated platelets, and that $P2Y_{12}$-inhibitors inhibit this amplifying process. A similar mechanism might also explain the significantly lower AA-induced aggregability observed at baseline in patients on DAPT compared with those on monotherapy with ASA, despite receiving the same dose of ASA (75 mg daily).

In contrast to AA- and TRAP-induced aggregation, the effect of platelet supplementation on ADP-induced aggregation was less pronounced. Although platelet transfusion improved ADP-dependent aggregability significantly in samples from patients treated with ASA and clopidogrel or ASA and ticagrelor, it did not reach more than 50% of the baseline levels in healthy subjects—even with the highest dose of platelets. This indicates that it is more difficult to restore ADP-dependent aggregability than AA- or TRAP-dependent aggregability, which could indicate a potential problem if bleeding occurs in patients treated with DAPT including a $P2Y_{12}$-inhibitor. The levels of aggregation obtained with the highest platelet dose in clopidogrel- and ticagrelor-treated patients (21 and 16 AU×min, respectively) were also markedly lower than a recently suggested cut-off level for an increased bleeding risk in cardiac surgery patients (31 AU×min).[10]

We also observed that platelet supplementation was less effective in samples from ticagrelor-treated patients than from clopidogrel-treated patients, both for ADP-induced and AA-induced aggregability. This might be explained by their different pharmacological properties. Clopidogrel is a thienopyridine that covalently binds to the $P2Y_{12}$-receptor, causing irreversible platelet inhibition, while ticagrelor is a high-affinity ADP analogue that causes reversible inhibition of the receptor.[11] Ticagrelor has a faster onset and offset than clopidogrel in patients with stable angina;[12] it causes more effective platelet inhibition than clopidogrel and there are no non-responders.[11] However, the enhanced inhibition with ticagrelor was not confirmed in the present study, where baseline ADP-dependent aggregability was comparable in clopidogrel- and ticagrelor-treated patients ($P=0.90$). The lack of difference might be explained by the limited number of study subjects, inter-individual variability in platelet aggregation, or both.

The present study was performed on blood samples collected <2 h after the last dose of platelet inhibitor, when the anti-aggregating effects of clopidogrel and ticagrelor would be expected to be at their peak.[11] Ticagrelor should be present in peak plasma concentrations,[12] blocking the $P2Y_{12}$-receptors of the 'fresh' platelets added to the samples (or transfused to the patient). In contrast, the concentration of the active metabolite of clopidogrel is likely low 2 h after ingestion of the drug.[13]

Zafar and colleagues[14] recently presented data on the effect of *ex vivo* platelet supplementation in blood samples from healthy subjects who had received prasugrel. Platelet aggregability increased up to 6 h after drug intake but then was stable. The differences in study design make direct comparisons to the present study impossible, but both studies suggest that it is possible to counteract the antiplatelet effect of $P2Y_{12}$-inhibitors early after intake with platelet transfusion. Future studies should more thoroughly investigate the ability of platelet concentrates to restore aggregability at various time points after discontinuation of the specific $P2Y_{12}$-inhibitors.

Limitations of this study include those inherent in an *ex vivo* study of platelet function, and the extent to which the results can be translated into the clinical setting. We used impedance aggregometry, which utilizes whole blood, although contributions of blood vessels and vascular endothelium are absent. Platelet function was only assessed with multielectrode aggregometry and it is possible that other methods might have yielded different results. Nevertheless, there is satisfactory correlation between multielectrode aggregometry and light transmission aggregometry, the gold standard in the assessment of platelet function.[15 16] Donor platelets were not tested for aggregability before addition to the whole blood samples. It is therefore possible that preexisting platelet dysfunction in the concentrate might have influenced the results. The ADP high sensitivity test kit contains prostaglandin E1, which can reduce platelet aggregability. Platelet dysfunction after addition of platelet concentrate might thus be overestimated. Furthermore, it cannot be assumed that platelet inhibition was at steady state in all ACS patients since treatment times differed. On the other hand, we measured platelet aggregation in all patients and related the effects of platelet supplementation to individual baseline levels. Finally, baseline aggregation cannot be directly compared with other studies since samples were diluted with PBS to maintain the same degree of haemodilution (before and after platelet supplementation) in all samples.

In summary, *ex vivo* platelet supplementation improves platelet aggregability in blood samples from coronary artery disease patients treated with ASA, ASA+clopidogrel, and ASA+ticagrelor. AA-induced aggregation, which is used to monitor antiplatelet effects of ASA, was completely restored, while recovery of ADP-dependent aggregation, used to monitor the antiplatelet effects of $P2Y_{12}$-antagonists, was limited *ex vivo*. AA- and ADP-dependent recovery was reduced in ticagrelor-treated patients 2 h after last drug intake compared with clopidogrel-treated patients.

Authors' contributions

E.C.H., C.S.H.: study design, recruitment of patients, data analysis, and drafting of the paper. K.Å.-O.: recruitment of patients, analysis of data, and revision of the manuscript. C.H., H.W., M.D., P.A.: analysis and interpretation of data and revision of the manuscript. A.J.: study conception and design, interpretation of data, and revision of the paper. All authors have approved the final version of the paper.

Declaration of interest

None declared.

Funding

The study was supported by the Swedish Heart and Lung Foundation (grant numbers 20090488 and 20120372 to A.J.); Västra

Götaland region (grant number ALFGBG-146281 to A.J.), and Gothenburg Medical Society grant GLS-329771 to E.C.H.). The study sponsors had no influence on the analysis and interpretation of data, in writing the report, or in the decision to submit the paper for publication.

References

1 Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001; **345**: 494–502

2 Wiviott SD, Braunwald E, McCabe CH, *et al.* Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; **357**: 2001–15

3 Becker RC, Bassand JP, Budaj A, *et al.* Bleeding complications with the P2Y12 receptor antagonists clopidogrel and ticagrelor in the PLATelet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J* 2011; **32**: 2933–44

4 Hamm CW, Bassand JP, Agewall S, *et al.* ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: the Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011; **32**: 2999–3054

5 *Guide to the Preparation, Use and Quality Assurance of Blood Components*, 16th Edn. Strasbourg: Council of Europe Press, 2010

6 Toth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. *Thromb Haemost* 2006; **96**: 781–8

7 Di Minno G, Silver MJ, Murphy S. Monitoring the entry of new platelets into the circulation after ingestion of aspirin. *Blood* 1983; **61**: 1081–5

8 Vilahur G, Choi BG, Zafar MU, *et al.* Normalization of platelet reactivity in clopidogrel-treated subjects. *J Thromb Haemost* 2007; **5**: 82–90

9 Pruller F, Drexler C, Archan S, Macher S, Raggam RB, Mahla E. Low platelet reactivity is recovered by transfusion of stored platelets: a healthy volunteer in vivo study. *J Thromb Haemost* 2011; **9**: 1670–3

10 Ranucci M, Baryshnikova E, Soro G, Ballotta A, De Benedetti D, Conti D. Multiple electrode whole-blood aggregometry and bleeding in cardiac surgery patients receiving thienopyridines. *Ann Thorac Surg* 2011; **91**: 123–9

11 Wallentin L. P2Y(12) inhibitors: differences in properties and mechanisms of action and potential consequences for clinical use. *Eur Heart J* 2009; **30**: 1964–77

12 Gurbel PA, Bliden KP, Butler K, *et al.* Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation* 2009; **120**: 2577–85

13 Tuffal G, Roy S, Lavisse M, *et al.* An improved method for specific and quantitative determination of the clopidogrel active metabolite isomers in human plasma. *Thromb Haemost* 2011; **105**: 696–705

14 Zafar MU, Santos-Gallego C, Vorchheimer DA, *et al.* Platelet function normalization after a prasugrel loading-dose: time-dependent effect of platelet supplementation. *J Thromb Haemost* 2013; **11**: 100–6

15 Sibbing D, Braun S, Jawansky S, *et al.* Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. *Thromb Haemost* 2008; **99**: 121–6

16 Paniccia R, Antonucci E, Maggini N, *et al.* Comparison of methods for monitoring residual platelet reactivity after clopidogrel by point-of-care tests on whole blood in high-risk patients. *Thromb Haemost* 2010; **104**: 287–92

Handling editor: H. C. Hemmings