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ORIGINAL ARTICLE

Is platelet inhibition due to thienopyridines increased in elderly patients, in patients with previous stroke and patients with low body weight as a possible explanation of an increased bleeding risk?

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Abstract

Background The TRITON-TIMI 38 study has identified three subgroups of patients with a higher risk of bleeding during treatment with the thienopyridine prasugrel: patients with a history of stroke or transient ischaemic attack (TIA), patients ≥ 75 years and patients with a body weight < 60 kg. However, the underlying pathobiology leading to this increased bleeding risk remains to be elucidated. The higher bleeding rate may be due to a stronger prasugrel-induced inhibition of platelet aggregation in these subgroups. The aim of the present study was to determine whether on-treatment platelet reactivity is lower in these risk subgroups as compared with other patients in a large cohort on the thienopyridine clopidogrel undergoing elective coronary stenting.

Methods A total of 1069 consecutive patients were enrolled. On-clopidogrel platelet reactivity was measured in

parallel by light transmittance aggregometry, the Verify-Now[®] P2Y12 assay and the PFA-100 collagen/ADP cartridge.

Results Fourteen patients (1.5%) had a prior history of stroke or TIA, 138 patients (14.5%) were older than 75 years and 30 patients (3.2%) had a body weight < 60 kg. Age ≥ 75 years and a history of stroke were independent predictors of a higher on-treatment platelet reactivity. In contrast, a body weight < 60 kg was significantly associated with a lower on-treatment platelet reactivity.

Conclusion In two high-risk subgroups for bleeding, patients ≥ 75 years and patients with previous stroke, on-clopidogrel platelet reactivity is increased. In contrast, in patients with a low body weight, on-clopidogrel platelet reactivity is decreased, suggesting that a stronger response to a thienopyridine might only lead to more bleeds in patients with low body weight

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Introduction

Dual antiplatelet therapy with aspirin and the thienopyridine clopidogrel is the therapy of choice in patients undergoing percutaneous coronary intervention (PCI) with stent implantation [1, 2]. However, despite this treatment ischaemic events still occur, and multiple studies have clearly demonstrated a relationship between the magnitude of on-treatment platelet reactivity and the occurrence of atherothrombotic events [3–8]. Therefore, novel antiplatelet agents with more consistent response rates among patients

have been introduced. One of these is the thienopyridine prasugrel which, similar to clopidogrel, is a specific, irreversible adenosine diphosphate (ADP)-receptor antagonist, but it is faster acting and a more potent platelet inhibitor. The Trial to Assess Improvement in Therapeutic Outcomes by Optimising Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38) demonstrated a significant risk reduction for the occurrence of thrombotic events in patients with an acute coronary syndrome (ACS) undergoing PCI with prasugrel as compared with clopidogrel [9]. This reduction, however, was counterbalanced by a 30% increased risk of bleeding, suggesting a link between platelet reactivity inhibition and bleeding [10, 11].

Three subgroups of patients were identified as having less clinical efficacy from prasugrel and greater absolute risk of bleeding than the overall cohort. These included (1) patients with a prior history of stroke or transient ischaemic attack (TIA), (2) the elderly (≥ 75 years of age), and (3) patients with a body weight < 60 kg [9]. However, the underlying pathobiology leading to this increased bleeding risk remains to be elucidated.

It has been hypothesised that the higher bleeding rate may be due to a stronger prasugrel-induced inhibition of ADP-induced platelet aggregation in these subgroups. Since prasugrel has been introduced only recently, few pharmacodynamic data are available. However, in the POPular study (The Do Platelet Function Assays Predict Clinical Outcomes in Clopidogrel Pretreated Patients Undergoing Elective PCI study), the influence of the other thienopyridine clopidogrel on the inhibition of platelet reactivity was determined in patients undergoing elective coronary stent implantation. The aim of the present sub-analysis study was to establish whether the on-clopidogrel platelet reactivity is lower in the three subpopulations at risk for bleeding as compared with in other patients in a large cohort of patients on clopidogrel undergoing elective coronary stenting.

Methods

Study population

The POPular study was a prospective, observational study that included consecutive patients with established coronary artery disease scheduled for elective PCI with stent implantation. The entry and exclusion criteria were described in the original publication [3]. All patients had been on dual antiplatelet therapy with clopidogrel and low-dose aspirin of 80–100 mg daily for at least 10 days, unless they were on long-term treatment with coumarin derivatives. This study complied with the Declaration of Helsinki and was approved by the local institutional review board. Written

informed consent was obtained from every patient prior to elective PCI.

Blood sampling and platelet function testing

Prior to heparinisation, whole blood was drawn from the femoral or radial artery sheath. After discarding the first 10 ml of blood, samples were collected into citrated tubes (3.2% for light transmittance aggregometry (LTA) and the VerifyNow[®] system and 3.8% for PFA). The magnitude of platelet reactivity was measured using three platelet function tests in parallel; the platelet function analyser (PFA-100) and ‘classical’ LTA. All methods were performed between 30 min and 2 h after blood collection.

Light transmittance aggregometry

LTA was quantified in non-adjusted platelet-rich plasma on a four-channel APACT 4004 aggregometer (LABiTec, Arensburg, Germany). Platelet-poor plasma was set as 100% aggregation, and maximal (peak) platelet aggregation (%) was measured spontaneously and after stimulation by ADP in final concentrations of 5 and 20 $\mu\text{mol/L}$.

The VerifyNow[®] P2Y12 assay

The VerifyNow[®] P2Y12 assay (Accumetrics, Inc, San Diego, USA) is an automated whole blood, cartridge-based method to determine the magnitude of platelet agglutination as induced by ADP/prostaglandin E_1 [12]. The results are reported in P2Y12 Reaction Units.

PFA-100

The PFA-100 System (Siemens Healthcare Diagnostics Products GmbH, Germany) measures platelet function, in particular adhesion and aggregation, in whole blood under high shear conditions (5000 s^{-1}) [13]. The time needed to form a platelet plug occluding the aperture cut into a membrane coated with collagen/ADP, an agonist, was determined and reported as closure time in seconds, which is inversely related to platelet reactivity. A closure time of > 300 s was referred to as ‘non-closure’.

Statistical analysis

Continuous variables are presented as mean (SD). Categorical data are reported as frequencies (percentages). Categorical variables were compared using the χ^2 test or Fisher’s exact test when frequencies were < 5 . The distribution of variables was determined by the Kolmogorov–Smirnov goodness-of-fit test. Normally distributed continuous variables were compared with a two-sided unpaired t test.

Logistic regression modelling was performed to identify independent correlates of the magnitude of platelet reactivity and to adjust for potential confounders. Being part of a high-risk group was entered as a dichotomous variable. All univariate variables with a *p* value <0.10 were included in multivariable analysis (binary logistic regression).

Results

Patient characteristics

A total of 1069 consecutive patients were enrolled, of whom 951 were on aspirin >10 days. The latter comprised the present study population. Owing to irregularities in platelet assay supply, as well as technical failure in a minority of platelet function tests, not all platelet function assays were performed in every patient.

Baseline characteristics of the total population are depicted in Table 1. Fourteen patients (1.5%) had a history of stroke or TIA, 138 patients (14.5%) were older than 75 years of age and 30 patients (3.2%) had a body weight <60 kg. Patients ≥ 75 years were more often female and smoker and had a lower haemoglobin. They were less often treated with statins and the proportion of hypercholesterolaemia, renal failure and an impaired ejection fraction was higher. Patients with a history of stroke or TIA more frequently received a loading dose of clopidogrel and more often had a previous history of coronary artery bypass grafting. Patient with a body weight <60 kg were significantly older, had lower haemoglobin and were less often treated with statins and β -blockade. The proportion of females was higher in this group, the frequency of diabetes mellitus and hypertension was lower and the minimal stent diameter was smaller.

Old age as risk factor for low platelet reactivity

Elderly patients ≥ 75 years had a significantly higher magnitude of on-treatment platelet reactivity as compared with patients <75 years, regardless of the platelet function test used. (Fig. 1a; Table 2) After adjustment for factors known to influence platelet function (diabetes mellitus, smoking, gender, concomitant use of proton pump inhibitors and the administration of a loading dose of clopidogrel), an age ≥ 75 years remained an independent predictor of a higher magnitude of platelet reactivity, except when 20 $\mu\text{mol/L}$ -induced LTA was used (Table 2).

Cerebrovascular accident as risk factor for low platelet reactivity

In patients with a history of stroke or TIA, the magnitude of platelet reactivity was significantly higher as compared

Table 1 Baseline characteristics

	Total population
Clinical parameters	
Age (years)	64 \pm 10.6
BMI (kg/m ²)	27.3 \pm 3.9
Gender (male)	717/951 (75.4%)
Hypertension	737/951 (77.5%)
Hypercholesterolaemia	769/951 (80.9%)
Diabetes mellitus	175/951 (18.4%)
Family history	580/951 (61.0%)
Current smoking	107/951 (11.3%)
Impaired ejection fraction	133/951 (14.0%)
Renal insufficiency	72/951 (7.6%)
Prior myocardial infarction	519/951 (54.6%)
Prior PCI	304/951 (32.0%)
Prior CABG	93/951 (9.8%)
Medication	
Loading dose clopidogrel	489/951 (51.4%)
Statin	767/951 (80.7%)
β -blocker	733/951 (77.1%)
ACE inhibitor	345/951 (36.3%)
Proton pump inhibitor	270/951 (28.4%)
Calcium channel blocker	365/951 (38.4%)
Oral anti-diabetic agents	62/951 (6.5%)
Coumarin derivatives	24/951 (2.5%)
Laboratory parameters	
Platelet count ($\times 10^9$)	273.4 \pm 78.8
White blood cell count ($\times 10^9$)	7.7 \pm 2.3
Haemoglobin (mmol/L)	8.6 \pm 2.2
Procedural parameters	
No. of stents implanted	1489/950 1.57
No. of lesions treated	1317/951 1.38
Minimal stent diameter (mm)	3.1 \pm 0.8
Total stent length (mm)	28.3 \pm 17.1
Bifurcation lesion	32/951 (3.4%)
Drug-eluting stent	604/946 (63.8%)
Left anterior descending artery	450/951 (47.3%)
Graft	28/951 (2.9%)

ACE angiotensin-converting enzyme, BMI body mass index, CABG coronary artery bypass grafting

with patients without a previous cerebrovascular accident when platelet reactivity was established using LTA (both 5 and 20 $\mu\text{mol/L}$ ADP-induced aggregation) (Fig. 1b; Table 2). After adjustment for potential confounders, a history of stroke or TIA remained an independent predictor of a higher level of aggregation. In contrast, no significant difference was found between the group with and without a history of stroke or TIA when platelet reactivity was assessed using the VerifyNow[®] P2Y12 assay or the PFA COL/ADP cartridge (Table 2).

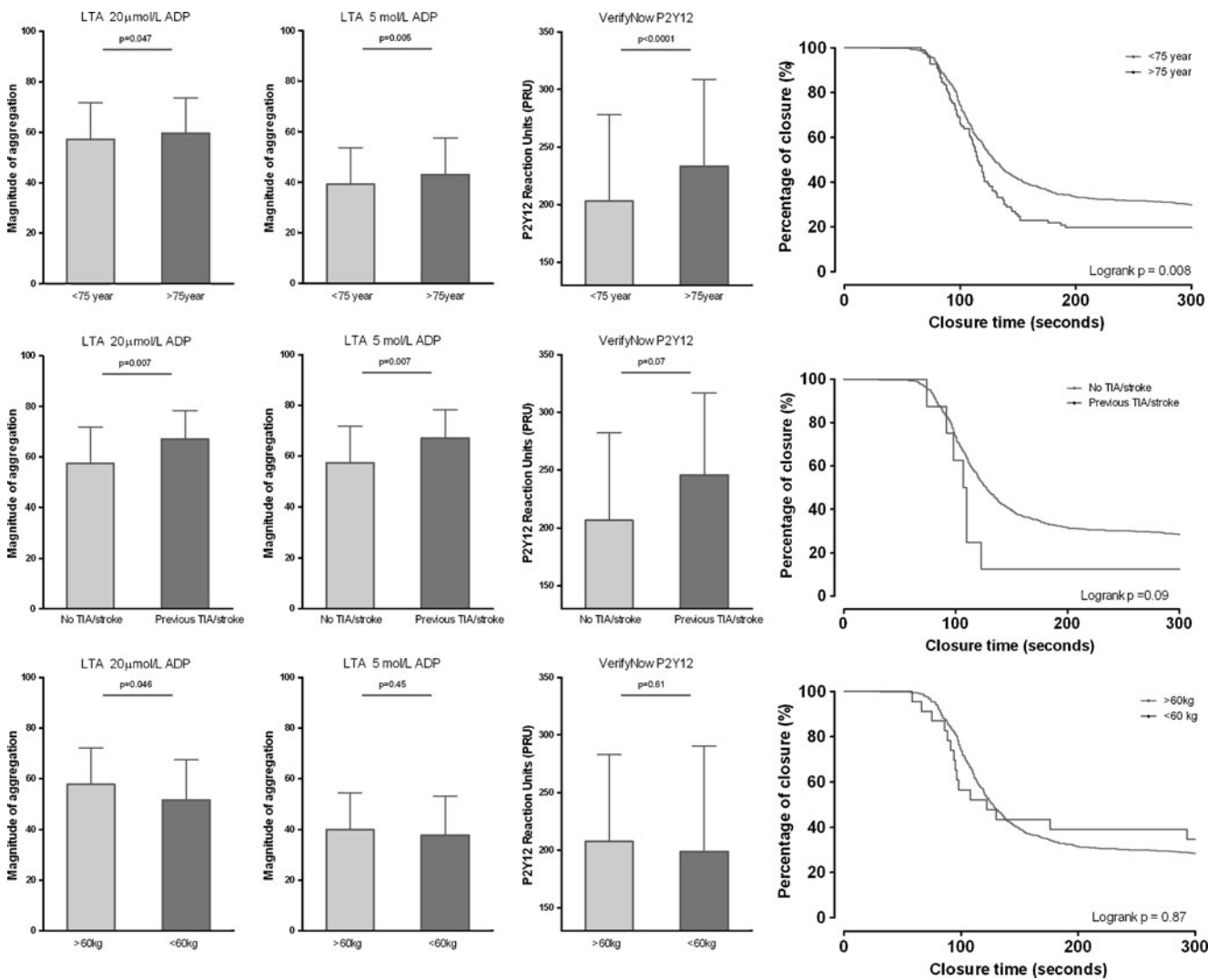


Fig. 1 Magnitude of platelet reactivity. Magnitude of platelet reactivity according to the three tests used. Since the PFA-100® System confines detection of a closure time to a 300-s window, the results of the PFA-100® System are depicted as a cumulative Kaplan–

Meier time-to-aperture-closure plot and a log-rank test was used. **a** In patients <75 years vs. patients ≥75 years of age. **b** In patients with a history of TIA or stroke vs. patients without a history of TIA or stroke. **c** In patients <60 kg vs. patients ≥60 kg

Low body weight as risk factor for low platelet reactivity

Aggregation as measured by 20 μmol/L ADP-induced LTA was significantly lower in patients with a body weight <60 kg as compared with patients with a higher body weight. None of the other tests identified significant differences between patients with a low body weight and patients with a body weight >60 kg (Fig. 1c, Table 2). After adjustment for potential confounding factors, lower body weight remained significantly associated with an intensified platelet response to clopidogrel as established by either 20 μmol/L ADP-induced LTA and became significant when measured with the VerifyNow® P2Y12 assay (Table 2).

Discussion

Whereas TRITON-TIMI 38 demonstrated that prasugrel, a thienopyridine resulting in lower on-treatment platelet reactivity as compared with clopidogrel, was associated with less recurrent atherothrombotic events in ACS patients undergoing PCI, an increased risk of bleeding was observed in patients treated with prasugrel [9]. The presence of a therapeutic window was already acknowledged by Paracelsus, who stated as early as in the fifteenth century that ‘All drugs are poisons, the benefit depends on the dosage’ [14]. There is currently a growing body of evidence supporting the association between bleeding and adverse outcomes, including myocardial infarction, stroke and death

Table 2 Magnitude of platelet reactivity

Magnitude of platelet reactivity in the elderly					
	<75 year (n=815)	≥75 year (n=136)	<i>p</i> value	After adjustment	
				Difference	<i>p</i> value
LTA 20 ADP	57.2±14.5	59.7±13.8	0.047	2.10	0.11
LTA 5 ADP	39.3±14.5	43.1±14.4	0.0046	3.3	0.01
PRU	202.9±74.9	233.5±75.2	<0.0001	25.9	0.0001
PFA COL/ADP	NA	NA	0.008	NA	0.008
Magnitude of platelet reactivity in patients with a history of a cerebrovascular event					
	No TIA/stroke (n=937)	Previous TIA/stroke (n=14)	<i>p</i> value	After adjustment	
				Difference	<i>p</i> value
LTA 20 ADP	57.4±14.4	67.0±11.3	0.007	8.46	0.03
LTA 5 ADP	39.7±14.6	48.8±10.6	0.007	8.46	0.03
PRU	206.8±75.6	245.5±71.5	0.07	37.4	0.06
PFA COL/ADP	NA	NA	0.09	NA	0.11
Magnitude of platelet reactivity in patients with a low body weight					
	≥60 kg (n=921)	<60 kg (n=30)	<i>p</i> value	After adjustment	
				Difference	<i>p</i> value
LTA 20 ADP	57.7±14.3	51.6±15.8	0.046	-7.2	0.007
LTA 5 ADP	39.9±14.5	37.7±15.4	0.45	-3.9	0.16
PRU	207.7±75.1	198.7±91.4	0.61	-28.1	0.04
PFA COL/ADP	NA	NA	0.87	NA	0.76

LTA light transmittance aggregometry, NA not applicable, PRU P2Y12 reaction units, PFA COL/ADP platelet function analyser using the collagen/ADP cartridge

[15–17], and several studies have suggested a link between the inhibition of platelet reactivity and the occurrence of bleeding [10, 18–20]. Thus, the identification of a window of platelet inhibition that on the one hand prevents atherothrombotic events and on the other hand does not lead to an increase in bleeding events, is of utmost importance [21].

The TRITON-TIMI 38 study has identified three subgroups of patients with a higher risk of bleeding during treatment with prasugrel: (1) patients with a prior history of stroke or TIA, (2) the elderly (>75 years of age), and (3) patients with a body weight less <60 kg [9]. However, the underlying pathobiology leading to this increased bleeding risk remains to be elucidated. It has been hypothesised that the higher bleeding rate might be the consequence of a stronger prasugrel-induced inhibition of ADP-induced platelet aggregation in these subgroups. Since prasugrel was introduced only recently, few pharmacodynamic data are available. The present study, with the aim to determine whether on-clopidogrel platelet reactivity is lower in these risk subgroups as compared with other patients, demonstrated that in the two high-risk subgroups for bleeding, patients >75 years of age and patients with previous stroke, on-treatment platelet reactivity is contrarily increased. However, in the third high-risk subgroup for bleeding,

patients with a low body weight, on-treatment platelet reactivity is indeed decreased. When these data are applied to the hypothesis that prasugrel leads to a stronger platelet inhibition, it seems that in TRITON-TIMI 38, a stronger response to prasugrel might have only led to more bleeds in patients with low body weight.

These observations are in line with results from a recent analysis of 16 phase-I clinical pharmacological studies performed in healthy patients. In this analysis, no effect of advanced age on the availability of the active metabolite of prasugrel was perceived [22]. On the contrary, in the TRITON-TIMI 38, patients ≥75 years had 19% higher exposure to the active metabolite as compared with those <75 years and even 25% higher exposure as compared with patients <60 years of age [23]. However, in the latter the concentration of the active metabolite was not measured, but estimated from its inactive metabolite. In contrast, body weight had the greatest influence on exposure to the active metabolite of prasugrel in both clinical pharmacology studies and the TRITON-TIMI 38, with an increase in exposure as body weight decreased. Exposure was 40% higher in individuals <60 kg as compared with those ≥60 kg [22, 23]. Modelling data suggest that decreasing the maintenance dose of prasugrel to 5 mg in these subjects would reduce exposure to the active metabolite to levels

consistent with those <75 years and ≥ 60 kg [22]. Both European and American regulatory agents therefore recommend a daily dose of 5 mg in patients <60 kg. For patients ≥ 75 years, the US Food and Drug Administration advises that prasugrel is generally not recommended, but might be considered in patients at high risk of recurrent atherothrombotic events at a maintenance dose of 10 mg in those ≥ 60 kg [23]. On the contrary, the European Medicines Agency recommends that prasugrel is avoided in the elderly, but if used, the dose should be halved to 5 mg [23]. Taking the findings from the present study into account and the fact that elderly patients have a higher risk of bleeding as compared with younger individuals, even when not on thienopyridine therapy [24], we do not consider prasugrel to result in an excess in platelet inhibition, thereby accounting for the increased bleeding risk in this population.

In conclusion, the results from the present analysis confirmed previous studies and identified body weight as the most influential covariate on the magnitude of ADP-induced platelet reactivity, which might have implications for prasugrel maintenance dose in daily clinical practice.

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