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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Published online: 15 April 2011
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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 VerifyNow® 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.

Keywords Clopidogrel · Platelet reactivity

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 antago-
nist, 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
due to a stronger prasugrel-induced inhibition of ADP-
induced platelet aggregation in these subgroups. Since
prasugrel has been introduced only recently, few pharmaco-
dynamic 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 undergo-
ing 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 de-
scribed 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 derivate. 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 per-
formed 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 μmol/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 E1 [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 χ² 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 μmol/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 with patients without a previous cerebrovascular accident when platelet reactivity was established using LTA (both 5 and 20 μmol/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).
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.

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.
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 high-risk subgroups as compared with other patients, demonstrated that in the two high-risk subgroups 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

| Magnitude of platelet reactivity in the elderly |
|-----------------------------------------------|
|                  | <75 year (n=815) | ≥75 year (n=136) | p value | After adjustment |
|                  |                  |                  |        |                |
| **LTA 20 ADP**   | 57.2±14.5        | 59.7±13.8        | 0.047  | 2.10           |
| **LTA 5 ADP**    | 39.3±14.5        | 43.1±14.4        | 0.0046 | 3.3            |
| **PRU**          | 202.9±74.9       | 233.5±75.2       | <0.0001| 25.9           |
| **PFA COL/ADP**  | NA               | NA               | 0.008  | NA             |

Magnitude of platelet reactivity in patients with a history of a cerebrovascular event

|                  | No TIA/stroke (n=937) | Previous TIA/stroke (n=14) | p value | After adjustment |
|                  |                     |                       |        |                |
| **LTA 20 ADP**   | 57.4±14.4          | 67.0±11.3             | 0.007  | 8.46           |
| **LTA 5 ADP**    | 39.7±14.6          | 48.8±10.6             | 0.007  | 8.46           |
| **PRU**          | 206.8±75.6         | 245.5±71.5            | 0.07   | 37.4           |
| **PFA COL/ADP**  | NA                  | NA                    | 0.09   | NA             |

Magnitude of platelet reactivity in patients with a low body weight

|                  | ≥60 kg (n=921)     | <60 kg (n=30)         | p value | After adjustment |
|                  |                   |                      |        |                |
| **LTA 20 ADP**   | 57.7±14.3         | 51.6±15.8            | 0.046  | −7.2           |
| **LTA 5 ADP**    | 39.9±14.5         | 37.7±15.4            | 0.45   | −3.9           |
| **PRU**          | 207.7±75.1        | 198.7±91.4           | 0.61   | −28.1          |
| **PFA COL/ADP**  | NA                 | NA                   | 0.87   | NA             |
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 confirmed previous studies and identified body weight as increased bleeding risk in this population. excess in platelet inhibition, thereby accounting for the therapy [24], we do not consider prasugrel to result in an with younger individuals, even when not on thienopyridine therapy [24], we do not consider prasugrel to result in an

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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