

# Impact of immature platelets on platelet response to ticagrelor and prasugrel in patients with acute coronary syndrome

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## Aims

The influence of reticulated platelets (RPs) on platelet inhibition by ticagrelor when compared with prasugrel is unknown. We aimed to determine the influence of RPs on adenosine diphosphate- (ADP-) induced platelet aggregation in patients with acute coronary syndrome who were randomly assigned to receive either ticagrelor or prasugrel for P2Y<sub>12</sub> receptor inhibition.

## Methods and results

One hundred and twenty-four patients were prospectively enrolled. The immature platelet fraction (IPF) was measured by an automated haematoanalyzer and platelet aggregation was assessed by multiple electrode aggregometry. In a subgroup of patients ( $n = 28$ ) ADP-induced P-selectin expression was measured in dependence of the time point of study drug intake in RPs that were discriminated from mature platelets by thiazole-orange (TO) staining. The primary endpoint was the correlation of platelet aggregation and IPF in ticagrelor- vs. prasugrel-treated patients. Platelet aggregation significantly correlated with IPF in patients who received prasugrel ( $r = 0.41$ ,  $P < 0.001$ ). In contrast, no correlation between IPF and platelet aggregation was observed in ticagrelor-treated patients ( $r = 0.08$ ,  $P = 0.51$ ,  $P$  for difference of correlation coefficients  $P = 0.05$ ). Within the TO-positive RP fraction, P-selectin expression was significantly higher in prasugrel when compared with ticagrelor-treated individuals (prasugrel  $18.6 \pm 16.0\%$  vs. ticagrelor  $11.5 \pm 6.0\%$ ,  $P = 0.047$ ). A time-dependent P-selectin expression was observed in prasugrel but not in ticagrelor-treated patients (prasugrel *early*  $11.9 \pm 9.4\%$  vs. *late*  $26.3 \pm 19.0\%$ ,  $P = 0.031$ , ticagrelor *early*  $9.6 \pm 4.9\%$  vs. *late*  $13.5 \pm 6.6\%$ ,  $P = 0.127$ ).

## Conclusion

Reticulated platelets show a greater impact on platelet reactivity in response to prasugrel when compared with ticagrelor.

## Keywords

Reticulated platelets • Platelet aggregation • Ticagrelor • Prasugrel • Acute coronary syndrome

## Clinical Perspective

Reticulated platelets differently influence adenosine diphosphate-induced platelet aggregation in dependence of the selected P2Y<sub>12</sub> receptor inhibitor in patients with acute coronary syndrome. Aiming at a personalized antiplatelet therapy, measurement of RPs may influence the choice of P2Y<sub>12</sub> receptor inhibitors or the timing interval of drug intake especially in patients with a high platelet turnover.

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## Introduction

Current guidelines recommend a dual antiplatelet treatment strategy with aspirin and one of the two novel P2Y<sub>12</sub> receptor antagonists ticagrelor or prasugrel to prevent subsequent thrombotic complications in patients with acute coronary syndrome (ACS) and planned interventional treatment strategy.<sup>1</sup> However, the pharmacodynamic efficacy of P2Y<sub>12</sub> receptor inhibitors differs considerably among individuals and numerous studies have demonstrated that platelet reactivity measured after stimulation with adenosine diphosphate (ADP) predicts adverse cardiovascular events.<sup>2–4</sup> Several factors influence platelet reactivity on therapy with P2Y<sub>12</sub> receptor inhibitors.<sup>5</sup> Recent findings indicate that platelet reactivity might be influenced by RPs, as well. These are young and immature platelets that are newly released from the bone marrow by megakaryocytes.<sup>6</sup> They differ considerably from mature platelets by being larger in size and by having cytoplasmic content of mRNA enabling on-going protein synthesis<sup>7</sup> which might aggravate their prothrombotic potential.<sup>8</sup> About 12% of circulating platelets are replaced every 24 h in order to sustain a sufficient platelet count and their rate in peripheral blood can be increased by a factor of 10 or more in conditions of stress, when demand for thrombocytes rises<sup>9</sup> and upon platelet activation.<sup>10</sup> As a result, production of RPs is increased in ACS patients.<sup>11,12</sup> Of note, platelet response to the thienopyridines clopidogrel and prasugrel is significantly decreased in dependence of platelet turnover and the amount of circulating RPs<sup>13–16</sup> resulting in a worse clinical outcome in these patients.<sup>17–19</sup> These findings indicate that there is a rationale for assessment of RPs for risk stratification in ACS patients receiving dual antiplatelet therapy consisting of aspirin and thienopyridines.<sup>13,19,20</sup>

The pharmacologic properties of ticagrelor differ considerably from thienopyridines like prasugrel mainly due to a reversible binding capacity at the P2Y<sub>12</sub> receptor. As a result, RPs might have significantly less influence on overall platelet reactivity in response to ticagrelor when compared with prasugrel, which has not been investigated before. Thus, the primary objective of this study was to assess the influence of the immature platelet fraction (IPF) on ADP-induced platelet aggregation in ACS patients treated with either ticagrelor or prasugrel. In addition, we aimed at providing mechanistic insights that might account for the interaction between RPs and platelet reactivity by assessment of P-selectin expression as a marker for platelet activation in the reticulated when compared with non-reticulated platelet (RP) fraction.

## Material and methods

### Study population

The present study included a cohort of 124 patients with ACS, who were all part of the ongoing randomized multi-centre ISAR-REACT 5 trial.<sup>21</sup> A detailed description of the study protocol as well as inclusion and exclusion criteria for the ISAR-REACT 5 study is listed elsewhere.<sup>21</sup> In brief, all patients received either ticagrelor or prasugrel as a loading dose prior to PCI followed by a maintenance dose of either drug for 12 months together with aspirin as a dual antiplatelet treatment regime.

For this pre-specified RP substudy, patients were prospectively recruited at two participating centres of the ISAR-REACT 5 trial (Deutsches Herzzentrum München or Klinikum rechts der Isar,

Technische Universität, Munich, Germany). All patients who were enrolled in the ISAR-REACT 5 trial in these two centres were prospectively screened for eligibility from the beginning of the ISAR-REACT 5 study until our pre-specified sample size of  $n = 124$  patients was reached. Only patients with available on-prasugrel or on-ticagrelor treatment platelet aggregation values plus corresponding IPF values at the same time point were included. Patients were excluded from the present substudy (i) if they did not receive dual antiplatelet treatment after angiography, (ii) if GPIIb/IIIa antagonists were administered and (iii) if patients were on-clopidogrel maintenance treatment before inclusion into the ISAR-REACT 5 study (Figure 1). In a subgroup of patients ( $n = 28$ ) ADP-induced P-selectin expression was measured in dependence of the time point of drug intake in RPs that were discriminated from mature platelets by thiazole-orange (TO) staining. Due to logistic reasons and the necessity of immediate blood processing, only blood from patients who were enrolled during core times of our laboratory in one study centre, namely the Klinikum rechts der Isar, could be processed for these analyses.

The study was performed in accordance with the provisions of the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee. All patients gave written informed consent before entering the study.

### Blood sampling

Venous whole blood was obtained from an antecubital vein from each patient at baseline before study drug application and 6–48 h after prasugrel or ticagrelor loading dose administration followed by maintenance treatment to assure sufficient pharmacodynamic action of either drug.

### Platelet function testing

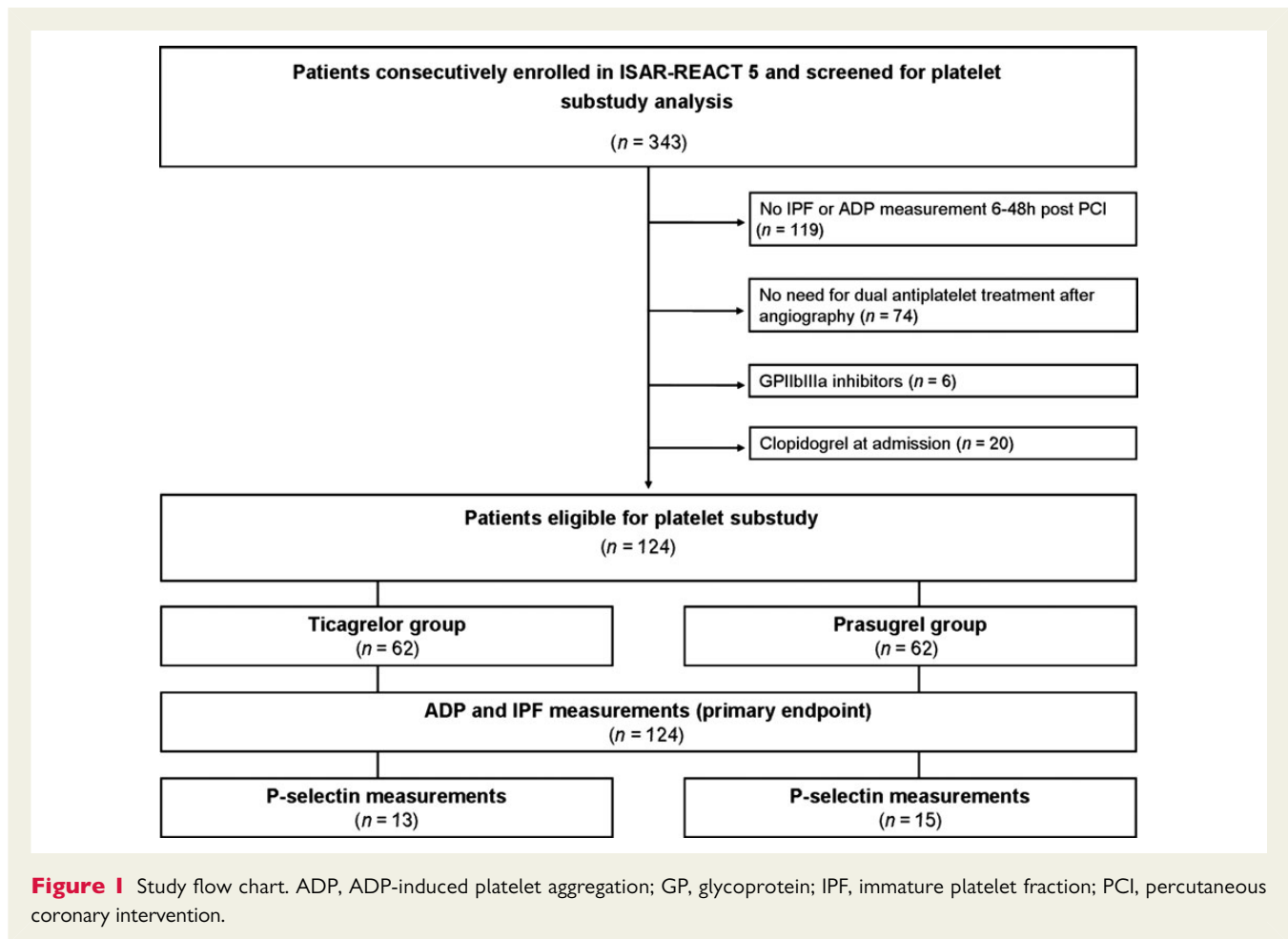
Adenosine diphosphate-induced platelet aggregation was measured in whole blood using the Multiplate analyser (Roche Diagnostics, Switzerland), as previously described.<sup>22</sup> Platelet aggregation was quantified as area under the curve (AUC = AU × min) of aggregation units (AU).

### Quantification of reticulated platelets by automated haematoanalyser

Reticulated platelets were assessed as percentage of the total optical platelet count (IPF) by flow cytometry using a fully automated analyser (Sysmex XE-5000, Sysmex, Kobe, Japan) in the departments of clinical chemistry in the two participating hospitals. Further assessed parameters included the high immature platelet fraction (H-IPF), mean platelet volume (MPV) and immature platelet count (IPC).

### Assessment of expression of the surface protein P-selectin in reticulated and non-reticulated platelets after stimulation with adenosine diphosphate

For logistic reasons, measurement of the expression of P-selectin on reticulated and non-RPs was only available during the core times of our basic clinical research laboratory. As a result, only blood from  $n = 15$  prasugrel-treated patients and  $n = 13$  ticagrelor-treated patients, who were consecutively enrolled, could be processed for P-selectin measurements. Whole blood was taken in dependence of the time point of the study drug intake at (i) 2 h after the last (*early*) and (ii) 1 h before the next (*late*) intake of prasugrel or ticagrelor maintenance dose. This resulted in a total of 26 measurements (14 *early* and 12 *late*) in the prasugrel group and 22 measurements (11 *early* and 11 *late*) in the ticagrelor group. Platelet rich plasma was manually separated from the tube after centrifugation and stained with anti-CD62-PE antibody after



stimulation with ADP. Reticulated platelets were identified and discriminated from non-RPs through TO labelling of mRNA through incubation of fixed platelets with Reticount<sup>®</sup> (BD Biosciences). The cells were measured using a Gallios flow cytometer (Beckman Coulter) and data analysis were performed with Kaluza<sup>®</sup> Version 1.2.12286.11234 (Beckman Coulter).

## Statistical analysis

The trial was designed to compare the correlation of platelet aggregation and IPF in ticagrelor- vs. prasugrel-treated patients. Sample size calculation was based on the following assumptions: a very weak correlation coefficient of  $r = 0.1$  between platelet aggregation and IPF for ticagrelor-treated patients and a moderate correlation coefficient of  $r = 0.55$  for prasugrel-treated patients, a power of 0.80 and a two-sided alpha level of 0.05. With these assumptions, enrolment of a total of 124 patients (62 patients per group) was required. Variables are presented as mean  $\pm$  SD, numbers (percentages), or median with interquartile range (IQR). Continuous data were compared across groups with non-parametric Mann–Whitney  $U$ -test or the two-sided Student's  $t$ -test depending on the type of distribution of the analysed variable. Categorical variables were compared using  $\chi^2$ -test or Fisher's exact test, as appropriate. Spearman correlation coefficient ( $r$ ) was used to test for the relationship between platelet aggregation values and IPF. Correlation coefficients between independent groups were compared with a  $z$  test after application of Fisher's  $r$ -to- $Z$  transformation using the R library cocor.<sup>23</sup> Analyses were performed using the software package

SPSS Statistics 22 (IBM SPSS, Inc.), the software package R for statistical computing version 2.15.0 (R Foundation, Vienna, Austria) and the software package MedCalc (MedCalc software, Belgium). All statistical tests were performed two sided and a  $P$ -value  $< 0.05$  was considered statistically significant.

The methods and protocols used in the present study are provided in more detail in Supplementary material, Material and Methods.

## Results

### Study population

Between September 2013 and August 2014 all patients who were consecutively enrolled in the ISAR-REACT 5 trial were screened for inclusion in this substudy. *Figure 1* summarizes the study flow. In 119 patients, measurement of RPs and/or ADP-induced platelet aggregation was not available. Seventy-four patients were excluded because they were not in need of a dual antiplatelet treatment regime after coronary angiography. Six patients disqualified for analysis because they received GPIIb/IIIa receptor inhibitors during PCI and 20 patients were excluded because they were on clopidogrel maintenance dose at admission. This resulted in a total of 124 ACS patients who were enrolled in this ISAR-REACT 5 RP study. In a subgroup of these patients ( $n = 13$  in the ticagrelor group and  $n = 15$  in the prasugrel group), who were enrolled during core

times of our laboratory, measurements of P-selectin expression after stimulation with ADP were performed additionally (Figure 1).

Table 1 shows the demographic and laboratory baseline characteristics of the study population, which did not differ significantly between the two treatment arms. The clinical presentation of ACS was similar between the two groups with a relevant number of patients with STEMI (48.4% in the ticagrelor group and 37.1% in the prasugrel group, Table 1).

### Platelet aggregation and immature platelet fraction at hospital admission in dependence of the clinical presentation of acute coronary syndrome

In patients presenting with STEMI, platelet aggregation values were significantly higher when compared with patients presenting with NSTEMI or UA [892 (597–1194) AU × min vs. 716 (389–945) AU × min,  $P = 0.002$ , Figure 2A]. Immature platelet fraction [median (IQR)] did not significantly differ in STEMI compared with NSTEMI

patients [3.9 (2.9–5.5) % vs. 3.5 (2.7–5.1) %,  $P = 0.28$ , Figure 2B] and no significant association between IPF- and ADP-induced platelet aggregation was observed at baseline ( $r = 0.11$ ,  $P = 0.29$ ).

### Association between immature platelet fraction and platelet aggregation in response to prasugrel and ticagrelor

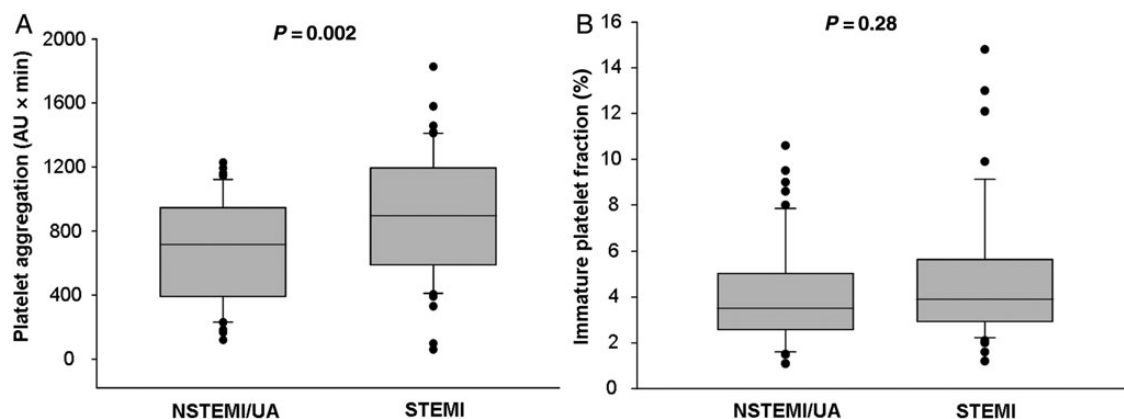
Platelet aggregation and IPF were measured after drug administration at a median (IQR) time interval of 19 h (14–25) in the ticagrelor group and at 18 h (14–22) in the prasugrel group ( $P = 0.52$ ). Individual measurements of ADP-induced platelet aggregation and IPF as a function of time of blood sampling are shown in Supplementary material online, Figure S1. Despite the wide spread of blood sampling times, the majority of measurements (74.2% in the ticagrelor group and 80.6% in the prasugrel group) were performed within the first 24 h after study drug intake and no relevant influence of blood sampling times on ADP-induced platelet aggregation or IPF values was observed. In prasugrel-treated patients, the ADP-induced

**Table 1** Baseline characteristics of the study population

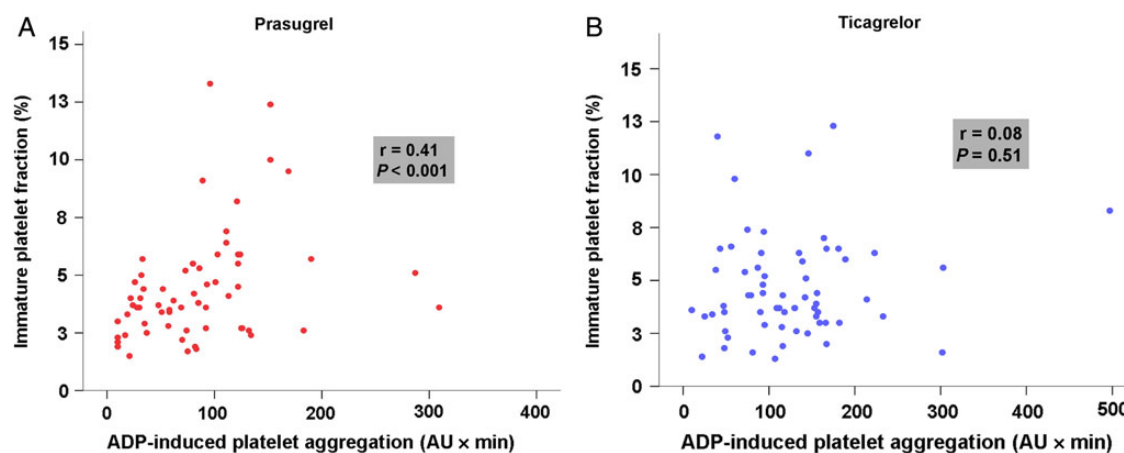
Variable	Ticagrelor group (n = 62)	Prasugrel group (n = 62)	P-value
Demographics			
Age (years)	63.5 ± 11.3	66.3 ± 12.3	0.16
Woman, n (%)	8 (12.9)	15 (24.2)	0.12
Body mass index (kg/m <sup>2</sup> )	26.7 ± 3.5	28.2 ± 5.0	0.06
Diabetes mellitus, n (%)	14 (22.6)	19 (30.6)	0.31
Active smokers, n (%)	21 (33.9)	19 (30.6)	0.70
Hypertension, n (%)	47 (75.8)	51 (82.3)	0.38
Hypercholesterolaemia, n (%)	35 (56.5)	44 (72.1)	0.07
Renal insufficiency, n (%)	12 (19.4)	16 (25.8)	0.39
NSTEMI/UA, n (%)	32 (51.6)	39 (62.9)	0.20
STEMI, n (%)	30 (48.4)	23 (37.1)	0.20
No. of diseased coronary vessels, n (%)			
1	20 (32.3)	12 (19.4)	0.18
2	10 (16.1)	16 (25.8)	
3	32 (51.6)	34 (54.8)	
Previous bypass surgery, n (%)	4 (6.5)	8 (12.9)	0.22
History of MI, n (%)	13 (21.0)	12 (19.4)	0.82
History of PCI, n (%)	15 (24.2)	20 (32.3)	0.32
Laboratory parameters			
ADP-induced platelet aggregation (AU × min)	734 [418–1165]	825 [543–998]	0.95
IPF (%)	3.8 [3.0–4.9]	3.7 [2.5–5.9]	0.69
H-IPF (%)	1.2 [0.9–1.7]	1.1 [0.7–1.7]	0.42
IPC (10 <sup>9</sup> /L)	8.6 [6.1–11.0]	9.2 [6.1–11.7]	0.63
MPV (fL)	10.3 [9.9–11.3]	10.5 [9.7–11.1]	0.74
Platelets (× 10 <sup>3</sup> /μL)	209 [173–243]	227 [188–259]	0.07
Haemoglobin (g/dL)	14.4 [13.4–15.5]	14.4 [13.1–15.5]	0.60
WBC (× 10 <sup>3</sup> /μL)	9.2 [7.2–12.6]	8.0 [6.6–10.7]	0.13
CRP (mg/dL)	0.3 [0.1–0.9]	0.3 [0.1–0.7]	0.80

Data presented are means ± SD, median (IQR) or numbers of patients (percentages). Renal insufficiency was defined as creatinine values ≥ 1.3 mg/dL.

ADP, adenosine diphosphate; CRP, C-reactive protein; H-IPF, high immature platelet fraction; IPC, immature platelet count; IPF, immature platelet function; MI, myocardial infarction; MPV, mean platelet volume; NSTEMI, non-ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction; UA, unstable angina; WBC, white blood cells.



**Figure 2** Box plots of (A) adenosine diphosphate-induced platelet aggregation values and (B) Immature platelet fraction at baseline before study drug administration in patients with NSTEMI/UA compared with patients with STEMI. Boxes indicate 25th and 75th percentiles, whiskers denote 10th and 90th percentile. ACS, acute coronary syndrome; ADP, adenosine diphosphate; AU, aggregation units; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; UA, unstable angina.



**Figure 3** Scatterplot of immature platelet fraction and adenosine diphosphate-induced platelet aggregation (A) in prasugrel-treated patients ( $n = 62$ ) and (B) in ticagrelor-treated patients ( $n = 62$ ). Spearman's correlation coefficients ( $r$ ) and corresponding  $P$ -values are provided. ADP, adenosine diphosphate; AU, aggregation units.

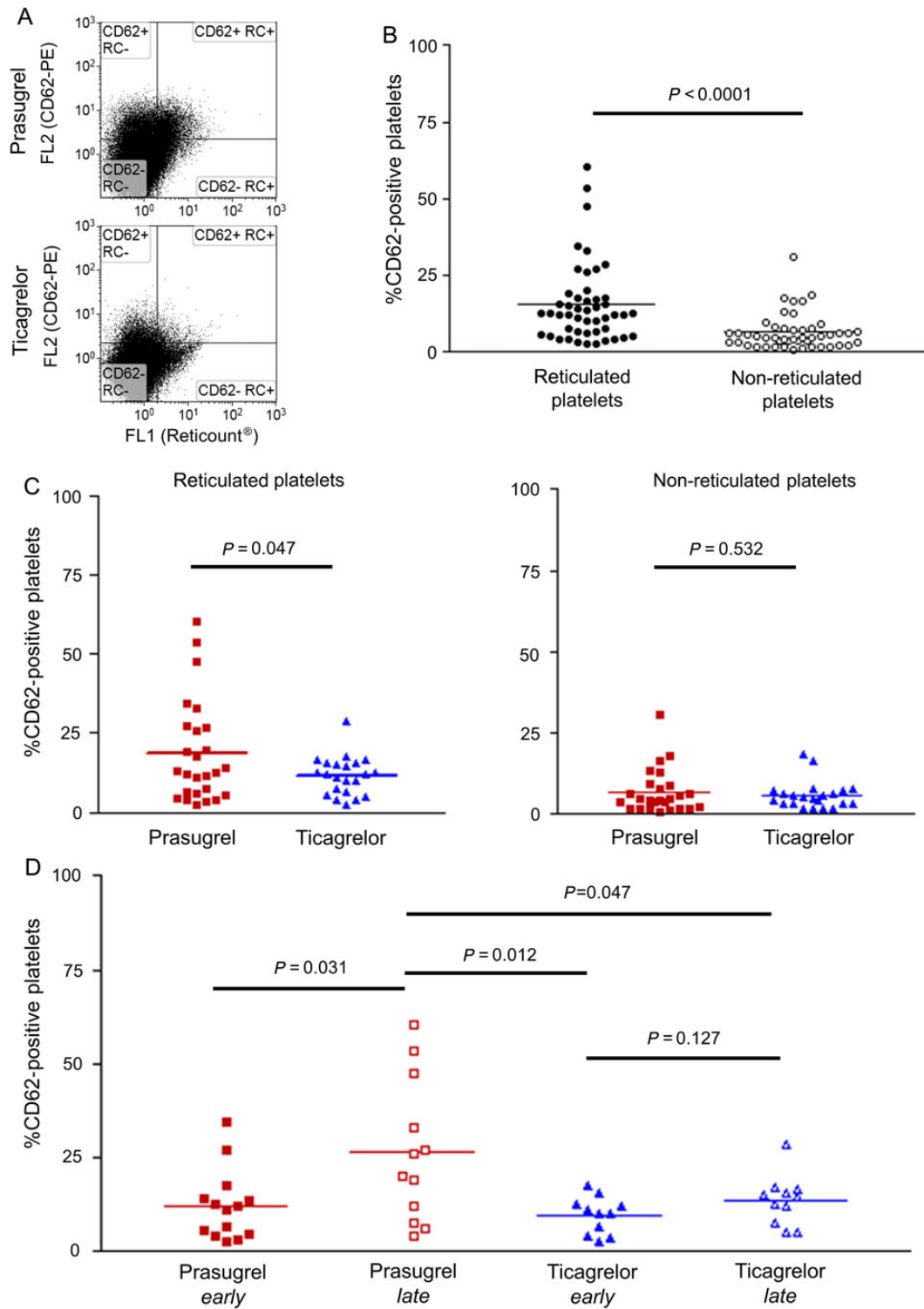
platelet aggregation was significantly lower when compared with ticagrelor-treated patients (81 (34–121) vs. 116 (74–158) AU  $\times$  min,  $P = 0.001$ ). The phenotype of HPR was defined as platelet aggregation values  $\geq 468$  AU  $\times$  min<sup>24</sup> and occurred only in one patient in the ticagrelor group (ADP-induced platelet aggregation 497 AU  $\times$  min), whereas no HPR was observed in the prasugrel group.

In prasugrel-treated patients, the ADP-induced platelet aggregation significantly correlated with IPF ( $r = 0.41$ ,  $P < 0.001$ , Figure 3A). In contrast, there was no correlation between ADP-induced platelet aggregation and the IPF in ticagrelor-treated patients ( $r = 0.08$ ,  $P = 0.51$ , Figure 3B,  $P$  for difference of correlation coefficients  $P = 0.05$ ). Similar findings were observed for correlation coefficients between ADP-induced platelet aggregation and further assessed parameters such as H-IPF, MPV, and IPC (Supplementary material online, Table S1).

### P-selectin expression in reticulated and non-reticulated platelets in response to ticagrelor and prasugrel

To further assess platelet activation in reticulated vs. non-RPs, we measured platelet P-selectin (CD62) expression after stimulation with ADP in a subset of prasugrel- and ticagrelor-treated patients who were consecutively enrolled into the study during core times of our laboratory (Figure 4A). Baseline characteristics of these patients did not differ relevantly between the two treatment arms and are provided in Supplementary material online, Table S2. Measurements were either taken at an early and/or at a late time point after study drug intake resulting in a total of  $n = 48$  measurements (Figure 4B). In RPs, P-selectin expression upon ADP stimulation was significantly higher when compared with





**Figure 4** (A) Flow cytometry dot plots of peripheral blood platelets stained with anti-CD62-PE (FL2; y-axis) and Reticount® (FL1; x-axis) after stimulation with adenosine diphosphate. Representative plots for each sample. For gating hierarchy see Supplementary material online, Figure S2. (B) Percentage of CD62+ platelets after stimulation with adenosine diphosphate. (C) Percentage of CD62+ platelets after stimulation with adenosine diphosphate within the reticulated (left panel) and the non-reticulated platelet fraction (right panel) shown separately for prasugrel- and ticagrelor-treated patients. (D) Percentage of CD62+ platelets after stimulation with adenosine diphosphate within the reticulated platelet fraction 2 h after medication intake (*early*) or 1 h before next dose (*late*) in prasugrel- and ticagrelor-treated patients.

non-RPs in both, prasugrel- and ticagrelor-treated patients (non-reticulated  $6.2 \pm 5.8$  vs. reticulated  $15.3 \pm 12.9\%$ ,  $P < 0.0001$ , Figure 4B) suggesting a higher prothrombotic potential of immature when compared with mature platelets. Within the RP fraction, P-selectin expression after ADP stimulation was significantly higher in patients treated with prasugrel when compared with those on ticagrelor treatment (prasugrel  $18.6 \pm 16.0\%$  vs. ticagrelor  $11.5 \pm 6.0\%$ ,  $P = 0.047$ , Figure 4C, left panel). This difference was not observed in the non-RP fraction (prasugrel  $6.7 \pm 6.8\%$  vs. ticagrelor  $5.7 \pm 4.3\%$ ,  $P = 0.532$ , Figure 4C, right panel).

To assess whether different pharmacokinetic properties might contribute to the distinct effects of ticagrelor and prasugrel on RPs, P-selectin expression was analysed in dependence of the time point of study drug intake at exactly (i) 2 h after the last (*early*) and (ii) 1 h before the next intake of prasugrel or ticagrelor maintenance dose (*late*) in the RP fraction. Significant differences between *early* and *late* platelet P-selectin expression upon ADP stimulation were observed only in prasugrel-treated patients but not in ticagrelor-treated patients (prasugrel *early*  $11.9 \pm 9.4\%$  vs. prasugrel *late*  $26.3 \pm 19.0\%$ ,  $P = 0.031$ , ticagrelor *early*  $9.6 \pm 4.9\%$  vs. ticagrelor *late*  $13.5 \pm 6.6\%$ ,  $P = 0.127$ , Figure 4D).

## Discussion

This is the first study that prospectively assessed the influence of immature platelets on ADP-induced platelet aggregation in ACS patients being randomly assigned to either ticagrelor or prasugrel treatment. The major finding of this study is that platelet reactivity significantly correlated with the IPF in patients receiving prasugrel but not in those receiving ticagrelor for P2Y<sub>12</sub> receptor inhibition. Additionally, we provide evidence that P-selectin expression upon ADP stimulation on the surface of RPs is significantly lower in ticagrelor-treated patients when compared with prasugrel-treated patients which might be explained by differences in the pharmacokinetic profiles and dosing intervals of both drugs.

Although prasugrel and ticagrelor both provided superior efficacy compared with clopidogrel in the pivotal randomized trials,<sup>25,26</sup> ACS patients continue to have a comparatively high risk of ischaemic events which can be related to an insufficient P2Y<sub>12</sub> receptor inhibition.<sup>2,4</sup> Besides genetic and clinical factors influencing platelet reactivity, there is emerging evidence that circulating immature and hyper-reactive RPs contribute to atherothrombosis and should be targeted by a more potent antiplatelet treatment strategy especially in ACS patients.<sup>27</sup>

Diverse mechanisms how RPs might modulate the pharmacodynamic platelet response to P2Y<sub>12</sub> receptor inhibitors are conceivable. We can confirm previous studies showing that RPs have a moderate but significant impact on platelet reactivity in patients who receive thienopyridines such as prasugrel or clopidogrel for P2Y<sub>12</sub> receptor inhibition.<sup>13–15</sup> These findings might be explained by an accelerated platelet turnover during ACS resulting in a greater amount of RPs circulating in the blood stream with non-inhibited P2Y<sub>12</sub> receptors on their surface. Prasugrel is a prodrug which is taken every 24 h, although its active metabolite that irreversibly binds to platelets has an elimination half-life of only 7.5 h at the maximum after drug intake.<sup>28</sup> Keeping in mind that about 100 billion new platelets must be produced daily from megakaryocytes to sustain a sufficient platelet count<sup>9</sup> and that this platelet turnover is even increased in ACS patients,<sup>11,12</sup> it

becomes obvious that P2Y<sub>12</sub> receptors on the surface of RPs are unlikely to be inhibited if they are produced during a time gap between the degradation of prasugrel's active metabolite until the next maintenance dose will be taken. Indeed, our findings indicate that RPs are less inhibited by prasugrel when compared with ticagrelor especially during the last hours of the dosing interval concluding that once daily dosing of prasugrel might be insufficient in patients with a high platelet turnover and a high amount of RPs. Likewise, it has been shown that thienopyridines are ineffective to inhibit donor platelets received from platelet transfusions.<sup>29</sup> Similarly to thienopyridines, the antiplatelet efficacy of low-dose aspirin, which is another agent with a short half live and an irreversible platelet inhibition, has also shown to be influenced by RPs especially in patients with a high platelet turnover suggesting that once daily dosing of aspirin might not provide adequate 24 h blockade of platelet inhibition in these patients as well.<sup>30–32</sup> These cumulative findings could be of relevant clinical significance even if overall platelet inhibition, as assessed by multiple electrode aggregometry, is below the threshold for HPR. Especially, if a few hyper-reactive platelets might be enough to cause severe thrombotic complications. With the results presented here we are the first to show that overall platelet reactivity in response to ticagrelor is not influenced by RPs in patients with ACS undergoing PCI. In this context, we also showed that in RPs, P-selectin expression after ADP stimulation was significantly less inhibited by prasugrel when compared with ticagrelor indicating that ticagrelor might be superior for inhibition of RPs. In contrast to prasugrel, ticagrelor is an active drug that does not require metabolic activation and, of importance, it antagonizes the platelet P2Y<sub>12</sub> receptor through a reversible non-competitive binding manner.<sup>33</sup> These pharmacokinetic properties including a ticagrelor plasma half-life of 11 h<sup>34</sup> together with a dosing interval of every 12 h result in a continuous circulation of active reversibly binding ticagrelor within peripheral blood which—in contrast to thienopyridines—enables a continuous inhibition of P2Y<sub>12</sub> receptors on RPs that are newly released from the bone marrow. In line with these findings, it has been shown that donor platelet transfusions in humans are ineffective to reverse the antiplatelet efficacy of ticagrelor.<sup>35</sup> Additionally, a prior study in rats indicated that immature platelets that were produced after clopidogrel treatment exposed active P2Y<sub>12</sub> receptors that were fully sensitive to reversible P2Y<sub>12</sub> antagonism with ticagrelor.<sup>36</sup>

Interestingly, we found that P-selectin expression was significantly higher in RPs as compared with non-RPs in both, ticagrelor- and prasugrel-treated patients despite a high overall inhibition of ADP-induced platelet aggregation as assessed by the multiplate analyser which could point to an enhanced prothrombotic potential of RPs<sup>8</sup> and might indicate that measurement of ADP-induced platelet aggregation with multiple electrode aggregometry alone might not be sufficient for assessment of platelet inhibition in patients with a high platelet turnover.

## Conclusion

The impact of RPs on platelet reactivity is greater in response to prasugrel when compared with ticagrelor. Whether ticagrelor could be beneficial for the clinical outcome in patients with a high platelet turnover warrants further investigation.

## Study limitations

Since TO staining of RPs and measurement of platelet P-selectin expression was only available during core times of our laboratory, we were not able to provide these data for all of the patients included in this study, which limits generalizability of these findings. In addition, we used only one method for platelet function testing. Finally, the clinical relevance of our findings is unclear and will be clarified by our ongoing investigation on the relative merits of ticagrelor and prasugrel in patients with ACS<sup>21</sup> (ClinicalTrials.gov, NCT01944800).

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

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