Immature platelet fraction and immature platelet count as novel biomarkers of elevated platelet reactivity in NSTE-ACS patients receiving dual antiplatelet therapy

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Abstract

Background. Antiplatelet therapy is the cornerstone of treatment for patients presenting with acute coronary syndrome (ACS) treated with percutaneous coronary intervention (PCI). Some patients may not respond to such therapy adequately, which is associated with a greater risk of ischemic events. Reticulated platelets are the youngest, largest, and most active platelet subtype. They have been initially shown to be associated with an increased risk of cardiovascular (CV) events and increased platelet activity.

Objectives. The aim of the presented study was to evaluate whether the immature platelet fraction (IPF) reflects the response to antiplatelet treatment in invasively managed ACS patients.

Materials and methods. This prospective study enrolled ACS patients treated with PCI and dual antiplatelet therapy (DAPT) comprising acetylsalicylic acid (ASA) and clopidogrel or ticagrelor. In all patients, venous blood was collected within 24 h after the procedure. Platelet parameters were measured, including IPF using the Sysmex hematological analyzer and adenosine diphosphate (ADP)-induced platelet reactivity using the Multiplate® Analyzer.

Results. A total of 108 patients were enrolled, including 62 with ST-segment elevation ACS (STE-ACS) and 46 with non-ST-segment elevation ACS (NSTE-ACS). Of them, 20.4% had diabetes mellitus, 26.9% had a history of MI and 59.2% of smoking. Spearman's correlation analysis demonstrated that higher IPF and immature platelet count (IPC) values are associated with increased ADP-induced platelet reactivity (respectively: rho = 0.387, 95% confidence interval (95% CI): 0.101–0.615, p = 0.008; and rho = 0.458, 95% CI: 0.185–0.666, p = 0.001) in NSTE-ACS but not in STE-ACS patients.

Conclusions. Immature platelet count and IPF may be valuable markers of platelet activity in patients with NSTE-ACS treated invasively and receiving DAPT (ClinicalTrials.gov No. NCT06177587).

Key words: platelet reactivity, acute coronary syndrome, dual anti-platelet therapy, immature platelet fraction

Background

Platelets have a significant role in the pathophysiology of cardiovascular (CV) events, including acute coronary syndrome (ACS), especially concerning patients treated invasively.[1,2] Therefore, therapy aimed at inhibiting platelet activity is an essential part of treatment to prevent, i.a., stent thrombosis (ST) or recurrent myocardial infarction (MI). As standard, such patients receive 2 antiplatelet drugs that act by different mechanisms: acetylsalicylic acid (ASA) and a $P2Y_{12}$ inhibitor, for 1 year, according to guidelines.[3,4] However, the response to treatment varies significantly depending on individual patients' characteristics, which may require adjusting the intensity or duration of treatment.[5,6] To date, there are no simple and accessible methods to effectively predict, and therefore prevent, high on-treatment platelet reactivity (HTPR).

Immature, newly released to the circulation reticulated platelets (RPs) are more reactive than mature ones.[7] Studies have shown that their levels represented as a percentage of RPs among all platelets, named immature platelet fraction (IPF), may have a predictive value for the occurrence of CV events in patients treated with dual antiplatelet therapy (DAPT).[8–11] However, their exact significance in assessing response to treatment is not fully understood.

Objectives

The aim of the presented study was to evaluate whether IPF could be a valuable parameter for determining on-treatment platelet reactivity and predicting response to antiplatelet therapy in ACS patients treated with percutaneous coronary intervention (PCI).

Materials and methods

This was a prospective, single-center study conducted in a tertiary cardiology clinical center. Written informed consent was obtained from each participant. This study was conducted according to the principles outlined in the Declaration of Helsinki and by the Bioethics Committee of Medical University of Warsaw under reference No. KB/242/2015. The clinical trial was registered with ClinicalTrials.gov under the identifier NCT06177587.

Patients

Consecutive patients presenting with ACS between July 2017 and May 2018 were enrolled. The inclusion criteria were: age >18 years, admission due to ACS, the need for immediate (<2 h) or early (<24 h) invasive treatment with stent implantation, treatment with DAPT, and ability to sign informed consent. The patients were excluded if they received any other medication that affects platelet activity or blood coagulation, had any contraindications to take ASA or $P2Y_{12}$ inhibitor, or had coagulation disorders. All patients received a loading dose of ASA (300 mg) and $P2Y_{12}$ inhibitor (300 mg of clopidogrel or 180 mg of ticagrelor) periprocedurally, and were treated thereafter with 75 mg of ASA daily and either clopidogrel (75 mg once a day) or ticagrelor (90 mg twice a day).

Laboratory tests

Blood sampling for all analyzed parameters was obtained from the peripheral vein in the first 24 h after PCI. Blood collection had taken place while the patients were still in the catheterization laboratory, before they were transported to the ward, so in 88% of cases, it was performed within the first 2 h after the PCI. Platelet count (PLT), hemoglobin, platelet distribution width, mean platelet volume (MPV), and IPF were assessed in whole blood anticoagulated with ethylenediaminetetraacetic (K3EDTA) using an automated hematological analyzer (Sysmex XN 2000; Sysmex, Kope, Japan). In the case of 2 IPF measurements, the average value was used for analyses. Immature platelet count (IPC) was calculated as a product of IPF and PLT. For platelet reactivity measurements, blood samples were drawn from the peripheral vein and collected in hirudin-containing tubes. Impedance aggregometry using Multiplate® Analyzer (Roche Diagnostics, Basel, Switzerland) with adenosine diphosphate (ADP) as agonist was performed 30–120 min after sampling. The test was carried out as instructed by the manufacturer. Maximum platelet aggregation and aggregation velocity are expressed in arbitrary units AUC (area under the curve of aggregation units (AU) over time (min)). Clinical data was collected from an electronic patients' database.

Statistical analyses

The statistical analysis was performed using IBM SPSS Statistics v. 28.0 (IBM Corp., Armonk, USA). The distribution of continuous data was assessed with Shapiro – Wilk test. Data were presented as mean and standard deviation (SD) and compared with Student's t-test, or as median with interquartile range (IQR) and compared with Mann–Whitney U test for parametric and nonparametric variables, respectively. Categorical data were presented as number and percentage. The Spearman's rank correlation coefficient was used to assess the relationship between platelet aggregation and RPs parameters. Two-sided p-values <0.05 were considered statistically significant.

Results

A total of 108 ACS patients were enrolled; 62 of them presented with ST-segment elevation ACS (STE-ACS) and 46 with non-ST-segment elevation ACS (NSTE-ACS). Baseline characteristics (Table 1) did not differ significantly

Table 1. Baseline characteristics (values in bold are statistically significant)

Variable		All (108)	NSTE-ACS (46)	STE-ACS (62)	p-value
Female gender, n (%)		28 (25.9)	12 (26.1)	16 (25.8)	0.974
Age [years], mean (SD)		66.7 (10.7)	69.0 (9.2)	65.8 (11.9)	0.084
HT, n (%)		70 (64.8)	35 (76.1)	35 (56.5)	**0.022**
DM, n (%)		22 (20.4)	11 (23.9)	11 (17.7)	0.364
HL, n (%)		73 (67.6)	31 (67.4)	42 (67.7)	0.903
HF, n (%)		36 (33.3)	15 (32.6)	21 (33.9)	0.954
CKD, n (%)		14 (13.0)	10 (21.7)	4 (6.5)	**0.017**
Current smoker, n (%)		39 (36.1)	16 (34.8)	23 (37.1)	0.908
Past smoker, n (%)		25 (23.1)	8 (17.4)	17 (27.4)	0.166
Previous MI, n (%)		29 (26.9)	15 (32.6)	14 (22.6)	0.245
Previous PCI, n (%)		19 (17.6)	11 (23.9)	8 (12.9)	0.110
MVD, n (%)		53 (49.1)	23 (50.0)	30 (48.4)	0.781
Clopidogrel, n (%)		82 (75.9)	36 (78.3)	46 (74.2)	0.625
Ticagrelor, n (%)		26 (24.1)	10 (21.7)	16 (25.8)	
Creatinine [mg/dL], median (IQR)		1.02 (0.34)	1.03 (0.38)	1.04 (0.34)	0.320
eGFR [mL/min/1.73m^2], median (IQR)		74.0 (28.0)	68.0 (34.5)	74.5 (25.3)	0.100
RBC [10^6/μL], median (IQR)		4.53 (0.69)	4.46 (0.64)	4.63 (0.70)	0.131
HGB [g/dL], median (IQR)		14.1 (2.2)	13.8 (2.1)	14.1 (1.9)	0.32
PLT [10^3/μL], median (IQR)		217 (63)	210 (77)	219 (60)	0.546
Cholesterol [mg/dL], mean (SD)		167 (43)	159 (33)	173 (48)	**0.010**
HDL [mg/dL], median (IQR)		41.5 (21.0)	42.5 (21.0)	41.0 (22.5)	0.540
LDL [mg/dL], mean (SD)		94.3 (37.6)	83.3 (30.4)	103.6 (39.0)	**0.008**
TG [mg/dL], median (IQR)		114 (58)	115 (54)	114 (53)	0.208
EF (%), median (IQR)		49.0 (12.8)	53.5 (9.5)	45.0 (13.5)	**<0.001**
Troponin [ng/mL], median (IQR)		10.3 (29.0)	6.0 (17.0)	17.1 (55.1)	**0.012**
Number of vessels	1	39 (36.1)	14 (30.4)	25 (40.3)	0.228
	2	25 (23.1)	10 (21.7)	15 (24.2)	
	3	24 (22.2)	13 (28.3)	11 (17.7)	
	4	14 (13.0)	5 (10.9)	9 (14.5)	
	5	6 (5.6)	4 (8.7)	2 (3.2)	
Final TIMI flow, mean (SD)		2.9 (0.5)	3.0 (0.0)	2.8 (0.6)	0.111
ASA prior to hospitalization, n (%)		23 (21.3)	11 (23.9)	12 (19.4)	0.567
Satin, n (%)		106 (98.1)	45 (97.8)	61 (98.4)	0.831
β-blocker, n (%)		97 (89.8)	45 (97.8)	52 (83.9)	**0.018**
ACEI/ARB, n (%)		103 (95.4)	44 (95.7)	59 (95.2)	0.904
CCB, n (%)		12 (11.1)	9 (19.6)	3 (4.8)	**0.016**
PPI, n (%)		97 (89.8)	40 (87.0)	57 (91.9)	0.398

ASA – acetylsalicylic acid; ACEI – angiotensin-converting enzyme inhibitor; ARB – angiotensin receptor blockers; CCB – calcium channel blocker; CKD – chronic kidney disease; DM – diabetes mellitus; EF – ejection fraction; eGFR – estimated glomerular filtration rate; HDL – high-density lipoprotein; HF – heart failure; HGB – hemoglobin; HL – hyperlipidemia; HT – hypertension; PPI – proton pump inhibitor; IQR – interquartile range; LDL – low-density lipoprotein; MI – myocardial infarction; MVD – multi-vessel disease; n – number; NSTE-ACS – non-ST-elevation acute coronary syndrome; PCI – percutaneous coronary intervention; PLT – platelets; RBC – red blood cells; SD – standard deviation; STE-ACS – ST-elevation acute coronary syndrome; TIMI – thrombolysis in myocardial infarction; TG – triglycerides.

between the groups, except for a higher prevalence of hypertension in STE-ACS patients and a greater incidence of chronic kidney disease in the NSTE-ACS group. Additionally, the NSTE-ACS group exhibited lower troponin and cholesterol levels, including LDL, as well as a higher ejection fraction compared to the STE-ACS group. Ticagrelor was received by 26 (24.1%) and clopidogrel by 82 (75.9%) patients.

The analysis revealed that the level of IPF correlates with ADP-induced platelet reactivity in NSTE-ACS patients (rho = 0.387, 95% confidence interval (95% CI): 0.101–0.615,

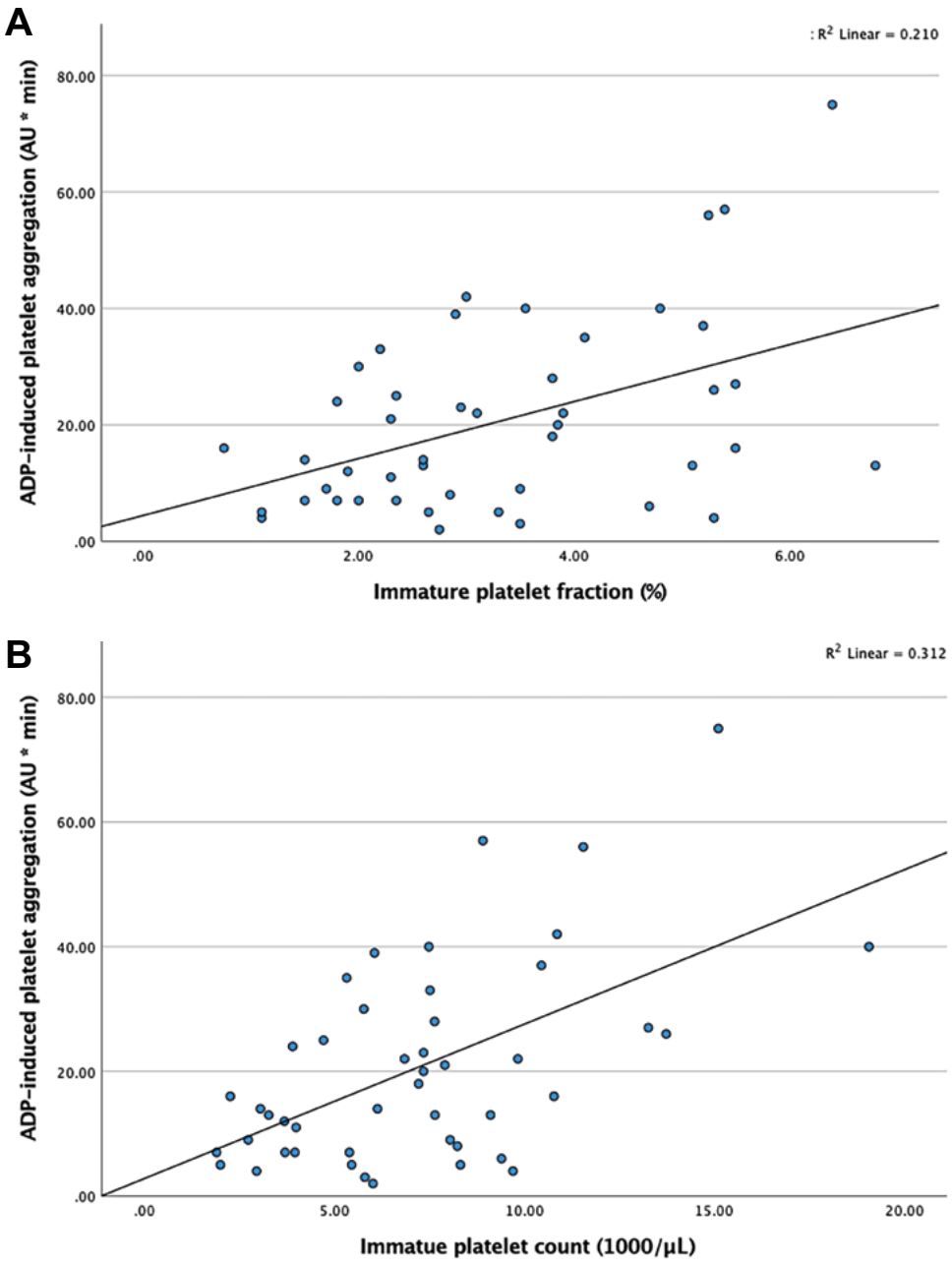

Fig. 1. Correlation between adenosine diphosphate (ADP)-induced platelet reactivity and (A) immature platelet fraction and (B) immature platelet count

p = 0.008); Fig. 1). However, this relationship was not observed in the STE-ACS group.

In the NSTE-ACS group, 36 patients were treated with clopidogrel and 10 with ticagrelor. We analyzed the relationship between ADP-induced platelet reactivity and IPF in both groups separately. For clopidogrel, the positive correlation was also present (rho = 0.346, 95% CI: 0.010–0.612, p = 0.039), whereas in the ticagrelor group, the observed correlation did not reach a statistically significant level (rho = 0.610, 95% CI: −0.054–0.900, p = 0.061).

Analysis concerning IPC revealed an even stronger correlation with ADP-induced platelet reactivity in NSTE-ACS patients (rho = 0.458, 95% CI: 0.185–0.666, p = 0.001). Moreover, this relationship was maintained in both clopidogrel and ticagrelor treated cohorts analyzed separately (rho = 0.378, 95% CI: 0.047–0.635, p = 0.023; and rho = 0.854, 95% CI: 0.467–0.966, p = 0.002, respectively). Again, the relationship was absent in STE-ACS patients.

Partial Spearman's correlation for potentially confounding variables including age, gender, diabetes mellitus, smoking status, and antiplatelet agent was also performed in the NSTE-ACS cohort. It revealed that the relationship between ADP and IPF as well as between ADP and IPC remained while controlling for all the variables mentioned above. The detailed results of the analysis are presented in Supplementary Table 1. Moreover, we showed that clinical presentation did not significantly impact the level of platelet reactivity, also after adjustment for potentially confounding variables (Supplementary Table 2), and that there were no differences in platelet parameters according to diabetes status, insulin treatment and the $P2Y_{12}$ inhibitor received (Supplementary Table 3).

Discussion

We demonstrated that the levels of both studied RP parameters, i.e., IPF and IPC, correlate with ADP-induced platelet aggregometry among patients with NSTE-ACS treated with PCI and DAPT. This relationship was not observed in STE-ACS patients.

Optimal platelet inhibition stands as a crucial factor influencing the prognosis of post-PCI patients.[12] Inadequate response to antiplatelet treatment remains an open problem related to serious consequences such as ST, MI, or CV death.[13] Despite numerous attempts and tests evaluated so far, routine identification of HTPR on a large scale was not found cost-effective and is currently not recommended in the society guidelines.[3,14]

Several studies have indicated the relationship between the level of RPs and antiplatelet therapy response, particularly notable in patients receiving thienopyridine therapy. However, it was not apparent in the ticagrelor-treated group.[15–18] Most of the patients in our study were treated with clopidogrel. Therefore, the issue of the relationship between IPF and platelet activity in ticagrelor-treated patients remains to be further elucidated. Despite the limited sample size, it is noteworthy that among ticagrelor-treated patients, there was a rising trend in IPF as ADP-induced platelet aggregation levels rose. Moreover, a statistically significant correlation was identified with regard to IPC. Based on the existing literature, the influence of clopidogrel treatment compared to ticagrelor appears to elicit varying effects on IPC levels in a long-term observation.[19] However, our findings, as presented, reveal that baseline platelet parameters and their correlation with platelet reactivity persist irrespective of the administered medication at a saturating dose.

Immature platelets, known for their heightened prothrombotic potential, can be reflected by IPF level – a reliable marker of platelet turnover. Elevated IPF is characteristic for specific patient groups including smokers, diabetics or the ones with ongoing inflammation,[20–23] as well as ACS patients.[24] Baseline IPF serves as a predictor of major adverse CV events (MACE) in patients with coronary artery disease (CAD) treated invasively and with DAPT.[8,9,11] Similar findings extend to IPC, which was also more strongly associated with antiplatelet response.[10,25] Patients with higher baseline levels of both parameters face a higher risk of ischemic events, indicating increased platelet turnover and reactivity despite adequate therapy. Regarding patients treated percutaneously with stent implantation, there is an additional risk of ST.

Interestingly, the correlation in our study did not exist for STE-ACS patients. Prior studies suggested that patients with STE-ACS have a higher IPF level than NSTE-ACS patients.[22] This was not observed in our population, where the distribution of IPF and IPC was similar in both groups. It can be due to the fact that blood parameters were obtained after an initial treatment including PCI and the loading doses of antiplatelet drugs. Perl et al. described the correlation between RPs level and platelet reactivity in STE-ACS patients, yet the measurements in that study were performed 2–4 days after the start of the treatment and later after 30 days.[26] The short interval between the onset of STE-ACS and the measurements in our study could be a factor contributing to this observation. Subsequent studies should focus on selecting the most optimal measurement time when IPF or IPC values reliably reflect platelet activity.

Immature platelet fraction can be easily, inexpensively measured using automatic hematology analyzers during a complete blood count test, providing the results quickly.[27,28] The same applies to IPC, which can be calculated from IPF and PLT. As such, RPs parameters may become useful markers for guiding antiplatelet therapy once the above findings are confirmed in further studies with larger cohorts.

Limitations

Our study predominantly included clopidogrel-treated NSTE-ACS patients, warranting further research specific to ticagrelor. The pharmacokinetics and pharmacodynamics differ among $P2Y_{12}$ inhibitors. It cannot be excluded that the relationship between platelet reactivity and the level of RPs depends on the drug received. Moreover, our focus on parameters shortly after the procedure prevents us from confirming whether this relationship persists in longer-term follow-up.

Conclusions

Immature platelet count and IPF may hold promise as potential markers of platelet reactivity in patients with NSTE-ACS undergoing invasive treatment and receiving DAPT. Given their accessibility, these markers could prove valuable for assessing an individual's responsiveness to antiplatelet therapy or aid in identifying individuals who are at higher risk of thrombotic events. Further research is needed to establish their effectiveness in this regard.

Supplementary data

Supplementary materials are available at https://zenodo.org/doi/10.5281/zenodo.10219636. The package contains the following files:

Supplementary Table 1. Partial Spearman's correlation analysis between ADP and IPF/IPC for potentially confounding variables.

Supplementary Table 2. Multivariate analysis showing the relation between ADP-induced platelet aggregation and the clinical presentation of ACS after adjustment for potential confounding variables.

Supplementary Table 3. The differences between platelet parameters (IPF, IPC and ADP-induced PA) in groups divided by diabetic status, insulin intake or P2Y12 inhibitor used.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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