Platelet-to-Lymphocyte Ratio as Marker of Platelet Activation in Patients on Potent P2Y<sub>12</sub> Inhibitors

Patricia P. Wadowski, MD, PhD<sup>1</sup>, Joseph Pultar<sup>1</sup>, Constantin Weikert<sup>1</sup>, Beate Eichelberger<sup>2</sup>, Maximilian Tscharre, MD, PhD<sup>1,3</sup>, Renate Koppensteiner, MD<sup>1</sup>, Simon Panzer, MD<sup>2</sup>, and Thomas Gremmel, MD, MBA<sup>1,4,5</sup>

Abstract
A high platelet-to-lymphocyte ratio (PLR) has recently been associated with ischemic outcomes in cardiovascular disease. Increased platelet reactivity and leukocyte-platelet aggregate formation are directly involved in the progress of atherosclerosis and have been linked to ischemic events following percutaneous coronary intervention (PCI). In order to understand the relation of PLR with platelet reactivity, we assessed PLR as well as agonist-inducible platelet aggregation and neutrophil-platelet aggregate (NPA) formation in 182 acute coronary syndrome (ACS) patients on dual antiplatelet therapy with aspirin and prasugrel (n = 96) or ticagrelor (n = 86) 3 days after PCI. PLR was calculated from the blood count. Platelet aggregation was measured by multiple electrode aggregometry and NPA formation was determined by flow cytometry, both in response to ADP and SFLLRN. A PLR ≥ 91 was considered as high PLR based on previous data showing an association of this threshold with adverse ischemic outcomes. In the overall cohort and in prasugrel-treated patients, high PLR was associated with higher SFLLRN-inducible platelet aggregation (67 AU [50-85 AU] vs 59.5 AU [44.3-71.3 AU], P = .01, and 73 AU [50-85 AU] vs 61.5 AU [46-69 AU], P = .02, respectively). Further, prasugrel-treated patients with high PLR exhibited higher ADP- (15% [11%-23%] vs 10.9% [7.6%-15.9%], P = .007) and SFLLRN-inducible NPA formation (64.3% [55.4%-73.8%] vs 53.8% [44.1%-70.1%], P = .01) as compared to patients with low PLR. These differences were not seen in ticagrelor-treated patients. In conclusion, high PLR is associated with increased on-treatment platelet reactivity in prasugrel-treated patients, but not in patients on ticagrelor.

Keywords
platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, protease-activated receptors, prasugrel, ticagrelor

Introduction
Platelets and leukocytes play key roles in the perpetuation and enhancement of inflammation, in particular in atherosclerosis. They provide numerous cytokines and growth factors for their interplay.<sup>1,2</sup> For instance, this mutual influence is apparent as high counts of platelets adhering to leukocytes are seen in acute myocardial infarction, but also in many chronic inflammatory diseases.<sup>3</sup> Neutrophils are the most abundant type of white blood cells in the circulation and a crucial part of the response to acute inflammation. In atherosclerotic processes, they infiltrate plaques and play a major role in plaque destabilization and rupture.<sup>4</sup>

Platelet-to-lymphocyte ratio (PLR) is an easily-obtainable marker comprising the patient’s platelet count and specifics of the white blood cell count, that is, the number of lymphocytes. An elevated PLR has been associated with in-hospital and

1 Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria
2 Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Vienna, Austria
3 Department of Internal Medicine, Cardiology and Nephrology, Landesklinikum Wiener Neustadt, Wiener Neustadt, Austria
4 Department of Internal Medicine I, Cardiology and Intensive Care Medicine, Landesklinikum Mistelbach-Ganserndorf, Mistelbach, Austria
5 Institute of Antithrombotic Therapy in Cardiovascular Disease, Karl Landsteiner Society, St. Polten, Austria

Manuscript submitted: November 20, 2021; accepted: April 07, 2022.

Corresponding Author:
Thomas Gremmel, Department of Internal Medicine II, Medical University of Vienna, Währinger Gurtel 18-20, 1090 Vienna, Austria.
Email: thomas.gremmel@meduniwien.ac.at

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
long-term adverse events in patients with acute coronary syndromes (ACS).\textsuperscript{5-7} Moreover, high PLR has been associated with poor overall survival in patients with acute heart failure\textsuperscript{8} and predicted target vessel restenosis in patients undergoing infrainguinal angioplasty with stent implantation or percutaneous coronary intervention (PCI).\textsuperscript{9,10}

High on-treatment residual platelet reactivity (HRPR) in response to ADP and SFLLRN has been associated with adverse ischemic events following angioplasty and stenting.\textsuperscript{11} In addition, leukocyte-platelet aggregates are increased in patients after myocardial infarction.\textsuperscript{12}

In order to understand the relation of PLR with on-treatment platelet reactivity and leukocyte-platelet aggregate formation, we assessed platelet aggregation and neutrophil-platelet aggregate (NPA) formation in 182 patients undergoing PCI with stent implantation for ACS.

Methods

Study Population

The study population consisted of 182 ACS patients on daily aspirin (100 mg/day), and either prasugrel (10 mg/d, n = 96), or ticagrelor (180 mg/d, n = 86) therapy (Figure 1). Blood sampling was performed 3 days after successful acute PCI after an overnight fast. Exclusion criteria were a known aspirin, prasugrel or ticagrelor intolerance (allergic reactions, gastrointestinal bleeding), a therapy with vitamin K antagonists (warfarin, phenprocoumon, acenocoumarol) or direct oral anticoagulants (rivaroxaban, apixaban, edoxaban), treatment with ticlopidine, dipyridamol or nonsteroidal anti-inflammatory drugs, a family or personal history of bleeding disorders, malignant myeloproliferative disorders or heparin-induced thrombocytopenia, severe hepatic failure, known qualitative defects in platelet function, a major surgical procedure within 1 week before enrollment, a platelet count <100,000 or >450,000/µL and a hematocrit <30%.

The study protocol was in accordance with the Declaration of Helsinki and its later amendments and approved by the Ethics Committee of the Medical University of Vienna. All study participants gave their written informed consent.

Measurement of Platelet, Neutrophil, and Lymphocyte Count

The hemogram was measured on the Sysmex Xe-5000 System, Sysmex Corporation, Japan, in the central laboratory of the Medical University of Vienna according to standardized protocols.

Multiple Electrode Aggregometry

Whole blood impedance aggregometry was performed with the Multiplate analyzer (Roche Diagnostics, Mannheim, Germany) as previously described.\textsuperscript{13,14} One Multiplate test cell contains 2 independent sensor units and 1 unit consists of 2 silver-coated highly conductive copper wires with a length of 3.2 mm. After dilution (1:2 with 0.9% NaCl solution) of hirudin-anticoagulated whole blood and stirring in the test cuvettes for 3 minutes at 37°C, adenosine diphosphate (ADP, 6.4 µM, Roche Diagnostics, Mannheim, Germany) or SFLLRN (protease activated receptor [PAR]-1 agonist, 32µM, Roche Diagnostics, Mannheim, Germany), was added and aggregation was continuously recorded for 6 minutes. The concentrations of all agonists were chosen according to the manufacturer’s recommendations. The adhesion of activated platelets to the electrodes led to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time. The AU at 6 minutes were used for calculations. One AU corresponds to 10 AU*min (area under the curve of AU).

NPA Formation

NPA were measured as previously published with small modifications.\textsuperscript{15,16}

In brief, HEPES buffer, or ADP (1.5 µM), or SFLLRN (7.1 µM) were added to 5 µL whole blood, which had been diluted with 55 µL HEPES-buffered saline. The concentrations
of all agonists were determined in previous titration experiments with increasing dosages of each agonist in 10 healthy controls. The selected concentrations of agonists induced about 60% to 70% of the maximal achievable increase in NPA formation in healthy controls. After 10 minutes, monoclonal antibodies (anti-CD45-peridinin chlorophyll protein (Becton Dickinson (BD)), anti-CD41-phycocerythrin, (Immunotech, Marseilles, France), and anti-CD14-allophycocyanin, (BD), or isotype-matched controls were added. After 15 min, samples were diluted with FACSlysing solution and at least 10,000 CD45+ events were acquired immediately. Neutrophils were identified based on their side scatter versus CD14 characteristics, and NPIs were determined by recording CD45+CD14+ events (Figures S1-S3).

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 24.0, SPSS, Chicago, Illinois, USA). Median and interquartile range of continuous variables are shown. Categorical variables are given as number (%). We performed the non-parametric Mann Whitney U tests to detect differences in continuous variables. The chi-square test was used to assess differences in categorical variables. Spearman rank correlation was used to assess correlations. Multivariable linear regression analyses using a backward elimination algorithm with a $P$ value $\leq .1$ for removal were used to adjust for patient characteristics. Adjustment was performed for the following variables after thoughtful variable selection: age, sex, body mass index, type of P2Y12 inhibitor, type of ACS, arterial hypertension, diabetes mellitus, hyperlipoproteinemia, smoking, history of myocardial infarction, creatinine, and high-sensitivity C-reactive protein (hsCRP).

Two-sided $P$ values $<.05$ were considered statistically significant. HRPR was defined according to a recent consensus document. The respective cut-off value for HRPR ADP by MEA was AU $\geq 46$. The cut-off value for PAR-1 mediated platelet aggregation was derived from a group of 55 healthy Caucasian volunteers (male/female 21/34; aged 42 $\pm$ 13 years), who served as control population in a previously published study. The corresponding cut-off value were AU $\geq 71$ for normal PAR-1 mediated platelet aggregation (SFLLRN as agonist) by MEA. The cut-off value for high PLR was a PLR $\geq 91$, according to a previous study linking this threshold with target vessel restenosis (TVR) following angioplasty and stenting for peripheral artery disease.

**Results**

Clinical, laboratory and procedural characteristics of the study population are given in Table 1. Patients on ticagrelor treatment were older and had higher creatinine levels than those treated with prasugrel. No other significant differences were observed.

| Table 1. Clinical and Laboratory Characteristics of Prasugrel- and Ticagrelor-Treated Patients. |
|---------------------------------------------|
| Characteristics | Prasugrel ($n = 96$) | Ticagrelor ($n = 86$) | $P$ |
| Demographics | | | |
| Age, years | 56 (48-62) | 59 (52-70) | .01 |
| Male sex, n (%) | 78 (81) | 69 (80) | .9 |
| BMI, kg/m$^2$ | 28 (25-30) | 27 (25-30) | .8 |
| Medical history | | | |
| Previous MI, n (%) | 14 (15) | 14 (16) | .7 |
| Previous TIA/stroke, n (%) | 2 (2) | 2 (2) | .9 |
| Hypertension, n (%) | 59 (61) | 59 (69) | .3 |
| Hyperlipidemia, n (%) | 73 (76) | 58 (67) | .3 |
| Diabetes mellitus, n (%) | 20 (21) | 24 (30) | .2 |
| Smoking, n (%) | 57 (59) | 44 (51) | .3 |
| Stent implantation, n (%) | 100 (100) | 100 (100) | .1 |
| Number of stents/patient | 1 (1-2) | 1 (1-2) | .3 |
| Laboratory data | | | |
| Serum creatinine, μmol/L | 80 (67-88) | 88 (74-104) | .001 |
| Platelet count, G/l | 222 (198-251) | 224 (187-266) | .76 |
| High sensitivity CRP, mg/L | 13 (6-38) | 12 (4-34) | .18 |
| Hemoglobin, mmol/L | 8.7 (8.2-9.3) | 8.6 (7.9-9.1) | .19 |
| WBC, G/L | 8.9 (7.9-10.3) | 8.7 (6.7-10.3) | .43 |
| Medication | | | |
| Statins, n (%) | 95 (99) | 85 (89) | .9 |
| Beta blockers, n (%) | 93 (97) | 84 (98) | .7 |
| ACE inhibitors, n (%) | 83 (86) | 68 (79) | .2 |
| Angiotensin receptor blockers, n (%) | 11 (13) | 17 (20) | .1 |
| Calcium channel blockers, n (%) | 6 (6) | 8 (9) | .4 |

Abbreviations: BMI, body mass index; ACE, angiotensin converting enzyme; CRP, C-reactive protein; MI, myocardial infarction; TIA, transient ischemic attack; WBC, white blood cell count.

$^a$Continuous data are shown as median (interquartile range). Dichotomous data are shown as n (%).

Agonist-inducible platelet aggregation and NPA formation is depicted in Table 2. Platelet aggregation in response to ADP and SFLLRN did not differ between patients on prasugrel and patients on ticagrelor. Of note, HRPR based on the ADP consensus cut-off value of AU $>46$ was seen in only 2 patients, both on prasugrel.

Based on the previously published cut-off value of AU $\geq 71$, high SFLLRN-inducible platelet aggregation was present in 72 patients (40%). Of these patients, 41 and 31 received prasugrel and ticagrelor, respectively (42.7% of prasugrel-treated patients vs 36% of ticagrelor-treated patients; $P = .36$).

NPA formation in response to ADP was similar in prasugrel- and in ticagrelor-treated patients. NPA formation in response to SFLLRN was significantly higher in patients on prasugrel than in those on ticagrelor (Table 2). Unstimulated levels of NPA are given in Table 2.

In the overall study population, ADP-inducible platelet aggregation correlated with ADP-inducible NPA formation ($r = 0.16, P = .03$). This correlation was due to the correlation
in prasugrel-treated patients ($r = 0.37, P < .001$), while no significant correlation was seen in ticagrelor-treated patients ($r = -0.02, P = .83$).

Likewise, there was a correlation between SFLLRN-inducible platelet aggregation and SFLLRN-inducible NPA formation in the overall study population ($r = 0.23, P = .002$), which was again due to the correlation in prasugrel-, but not in ticagrelor-treated patients (prasugrel: $r = 0.25, P = .01$; ticagrelor: $r = 0.19, P = .08$).

In the overall study population ADP- and SFLLRN-inducible platelet aggregation correlated with each other ($r = 0.58, P < .001$), which was similar in prasugrel-($r = 0.57, P < .001$) and ticagrelor-treated patients ($r = 0.61, P < .001$). Likewise, ADP- and SFLLRN-inducible NPA formation correlated in the overall study population ($r = 0.44, P < .001$) as well as in prasugrel- and ticagrelor-treated patients (both $r = 0.42, both P < .001$).

A high PLR (PLR ≥ 91%) was seen in 136 (75%) patients in the overall study population. PLR differed significantly between prasugrel- and ticagrelor-treated patients (104.6 [86.6-137.3] vs 120 [96.7-155.3], $P = .006$; Figure 2). After dichotomization, high PLR was seen in 66 (69%) of prasugrel-treated patients and in 70 patients (81%) on ticagrelor ($P = .05$).

Patients with high PLR were older than those with low PLR (58.6 years [51.6-67 years] vs 54.6 years [47.6-62.2 years], $P = .048$). There was no association of creatinine levels with high or low PLR in the overall study population (data not shown). There were no significant differences in age or serum creatinine between patients on prasugrel ($P = .05$ and $P = .43$) and ticagrelor ($P = .71$ and $P = .52$) with high or low PLR (data not shown).

In univariate analyses, PLR did not correlate with ADP- or SFLLRN-inducible platelet aggregation, but with ADP-inducible NPA formation, due to the significant correlation in prasugrel-treated patients (Table 3). PLR did not correlate with SFLLRN-inducible NPA formation, even if patients on prasugrel and ticagrelor were evaluated separately (Table 3).

In multivariable linear regression analyses adjusting for confounders PLR was associated with ADP-inducible NPA formation ($β = 0.214, P = .009$) and with SFLLRN-inducible platelet aggregation ($β = 0.208, P = .013$), whereas no association of PLR with ADP-inducible platelet aggregation and SFLLRN-inducible NPA formation was detectable (Table 4).

Next, we were interested to evaluate if either high PLR or low PLR values were linked to on-treatment platelet aggregation or NPA formation.

Patients with high and low PLR had similar levels of ADP-inducible platelet aggregation (20 AU [15-24 AU] vs 18.5 AU [14.8-23 AU]; $P = .27$). Also, ADP-inducible platelet aggregation was similar in prasugrel-treated patients with high vs low PLR (20 AU [15-23 AU] vs 18.5 AU [15-22.3 AU]; $P = .9$) and in those on ticagrelor with high vs low PLR (20 AU [15-25 AU] vs 17.5 AU [12.5-23.8 AU]; $P = .23$, respectively).

In contrast, high PLR was linked to higher SFLLRN-inducible platelet aggregation compared to low PLR (67 AU [50-85 AU] vs 59.5 AU [44.3-71.3 AU]; $P = .01$). This observation in the entire cohort was attributable to the results obtained in prasugrel-treated patients, who had higher SFLLRN-inducible platelet aggregation in case of high PLR as compared to low PLR (73 AU [50-85 AU] vs 61.5 AU [46-69 AU]; $P = .02$; Figure 3). No such difference was discernible in patients on ticagrelor (high PLR: 65 AU [48.8-82.3 AU] vs low PLR 53 AU [37.5-74.8 AU]; $P = .12$; Figure 3).

NPA formation in response to ADP did not differ significantly between patients with high and low PLR in the overall study population (13.3% [10.3%-17.9%] vs 10.9% [7.9%-18.7%], $P = .06$). However, in prasugrel-treated patients, we observed a significant difference between those with high vs those with low PLR regarding the formation of NPA in...
Table 3. Correlations of PLR With MEA and NPA Formation in Response to ADP and SFLLRN in the Overall Study Population as well as in Prasugrel- and Ticagrelor-Treated Patients.

| Correlations of PLR with | All patients (n = 182) | Prasugrel (n = 96) | Ticagrelor (n = 86) |
|--------------------------|------------------------|-------------------|-------------------|
| MEA-ADP (AU)             | r = 0.03, P = .74      | r = -0.03, P = .8  | r = 0.06, P = .61  |
| MEA-SFLLRN (AU)          | r = 0.12, P = .11      | r = 0.13, P = .2   | r = 0.16, P = .14  |
| NPA-ADP (%)              | r = 0.2, P = .009      | r = 0.28, P = .007 | r = 0.08, P = .46  |
| NPA-SFLLRN (%)           | r = 0.12, P = .11      | r = 0.12, P = .25  | r = 0.17, P = .12  |

Abbreviations: ADP, adenosine diphosphate; AU, aggregation units; MEA, multiple electrode aggregometry; NPA, neutrophil-platelet aggregates; PLR, platelet-to-lymphocyte ratio.

Table 4. Association of PLR With MEA and NPA Formation in Response to ADP and SFLLRN in the Overall Study Population Adjusting for Confounders.

| Parameters of the final model | β coefficient | P  |
|-------------------------------|---------------|----|
| ADP-inducible NPA formation (%) |              |    |
| Platelet-to-lymphocyte ratio  | 0.214         | .009 |
| Age, years                    | 0.201         | .015 |
| NSTEMI at presentation        | 0.202         | .056 |
| Prasugrel                     | 0.261         | .016 |
| MEA SFLLRN (AU)               |              |    |
| Platelet-to-lymphocyte ratio  | 0.208         | .012 |
| Body mass index, kg/m²        | 0.204         | .011 |
| Smoking                       | -0.223        | .008 |
| Hyperlipoproteinemia          | -0.144        | .070 |
| Arterial hypertension         | 0.136         | .094 |

Abbreviations: ADP, adenosine diphosphate; AU, aggregation units; MEA, multiple electrode aggregometry; NPA, neutrophil-platelet aggregate; PLR, platelet-to-lymphocyte ratio.

High PLR has repeatedly been identified as predictor of adverse outcomes in patients receiving dual antiplatelet therapy after cardiac and peripheral stenting. Moreover, ST-elevation myocardial infarction (STEMI) patients with high PLR exhibited larger infarct sizes and a higher rate of no-reflow phenomenon compared to patients with low PLR. Also, PLR has been associated with the extent of coronary and carotid artery disease, and repeatedly linked to adverse outcomes in ACS patients. Further, high PLR was associated with disease severity and longer hospitalizations during the current pandemic in patients infected with SARS-CoV-2.

Potent platelet inhibition is a mainstay in the pharmacological treatment of patients with atherosclerotic disease, particularly when undergoing stent implantation, and on-treatment residual platelet reactivity has been numerous linked to adverse outcomes. MEA is an easily-obtainable and highly standardized platelet function test, which has been related to outcome data in clopidogrel-treated patients. We measured NPA formation because neutrophil-platelet interaction and neutrophil density have previously been associated with impaired coronary microcirculation, myocardial damage and left ventricular dysfunction in patients with STEMI. Neutrophils can augment coagulation and atherosclerosis by inducing thrombin generation via neutrophil extracellular traps (NETs), leading to platelet activation and the development of ischemic events. The important interplay between platelets and leukocytes in the development of atherosclerosis is possibly reflected in PLR and may therefore make it a new valuable prognostic marker in atherosclerosis. Indeed, we found a significant association of PLR with ADP-inducible NPA in our cohort.

Current antiplatelet therapy with the novel P2Y12 inhibitors has been shown to be superior to clopidogrel in ACS patients by exerting a more potent inhibition of ADP-inducible platelet aggregation. This fact might explain the lack of association of PLR with ADP-inducible platelet aggregation in our cohort, contrary to a previous report demonstrating a significant association of PLR with high residual platelet reactivity (defined as change in maximal aggregation ≤20% from baseline aggregation in response to ADP) in patients treated with clopidogrel. However, platelet activation via other surface receptors

Discussion

To the best of our knowledge, this is the first study associating on-treatment platelet aggregation and NPA formation with PLR in ACS patients treated with prasugrel or ticagrelor. We demonstrate that PLR was independently associated with ADP-inducible NPA formation and SFLLRN-inducible platelet aggregation. Moreover, patients with high PLR had higher SFLLRN-inducible platelet aggregation than patients with low PLR. Finally, prasugrel-treated patients with high PLR exhibited higher ADP- and SFLLRN-inducible NPA formation as compared to patients with low PLR.
remains largely unaffected. Thrombin acts as a strong endo-
genous platelet agonist activating platelets predominantly via
PAR-1.41-44 PAR-1 activation can occur despite potent P2Y12
inhibition,21,22,45 and we have previously shown that PAR-1
mediated platelet activation predicts long-term ischemic events
in patients with lower extremity artery disease.11,21,22 This is of
particular interest, as PLR was associated with SFLLRN-
inducible platelet aggregation in our cohort and, hence, high
PLR might reflect a prothrombotic milieu and explain the asso-
ciation of PLR with adverse outcomes in previous reports.9,23
Also, we have identified a number of patients with high
SFLLRN-inducible platelet aggregation despite adequate P2Y12
inhibition, which may explain the stronger associations of PLR
with PAR-1 mediated platelet activation as compared to ADP-
inducible platelet activation. Of note, the addition of the PAR-1
inhibitor vorapaxar to antiplatelet therapy decreased ischemic
events in patients with CAD, but significantly increased bleed-
ing risk and has therefore never been included in the standard
antiplatelet regimen.56,47

The differences in the associations of platelet aggregation and
NPA formation with PLR observed between prasugrel-
and ticagrelor-treated patients may be due to different pharmacoki-
netics and –dynamics of the thienopyridine prasugrel and the
cyclopentyl-triazolopyrimidine ticagrelor.48 Ticagrelor is
proposed to bind to a second pocket consisting of transmem-
brane segments and the extracellular loop 2 as well as the
N-terminal domain of P2Y12.49 This binding site is suggested
as a mechanism for platelet inhibition independent of ADP.49
Further biological effects have been attributed to ticagrelor, which
are different from prasugrel and possibly not due to P2Y12
inhibition, like the inhibition of cellular adenosine uptake and of
toll-like receptor 1/2 mediated platelet activation.50-52 The missing
association of PLR with NPA formation in ticagrelor-treated
patients suggests that ticagrelor may at least in part overrule
platelet activation via pathways that are independent of P2Y12
inhibition.52 There is a dispute, however, whether ticagrelor or
prasugrel are more beneficial50,53-55 with a non-significantly more
favorable 1-year outcome in ticagrelor-treated patients as
described by Motovska et al.55 and a significantly more favorable
1-year outcome for patients treated with prasugrel in the Intracor-
onary Stenting and Antithrombotic Regimen: Rapid Early Action
for Coronary Treatment (ISAR-REACT) 5 study.56

**Limitations**

A study limitation is the lack of clinical outcome data. Further,
a potential selection bias exists due to the choice of the P2Y12
antagonist by the treating physician.
Whole blood methods to determine platelet function may differ from results obtained by assays that use separated platelets. However, whole blood assays are considered advantageous to assays using separated platelets, as the process of separation may affect platelet response to agonists. Moreover, whole blood assays better reflect the in vivo conditions of each individual, as the interplay of all blood components is preserved. For the reasons mentioned above, we did not use light transmission aggregometry (LTA), even though it can be considered the historical gold standard of platelet function testing. In initial studies we have seen that results are affected at platelet counts <100,000 and >450,000/μL and a hematocrit <30%. We therefore did not include individuals with these abnormal laboratory values. In detail, 7 patients were not enrolled due to an abnormal platelet count at screening (Figure 1).

We cannot completely exclude potential laser coincidence events related to platelet counts. However, by flow cytometry, we assessed each sample first without agonists and then after agonist-induced activation for comparison, staining specifically with a platelet marker.

Conclusion

In conclusion, our study demonstrates the association of on-treatment platelet aggregation and NPA formation with PLR in prasugrel-treated ACS patients. We therefore propose further evaluation of PLR as simple, readily-available and inexpensive marker of a prothrombotic state.

Author Contributions

Patricia P. Wadowski: Patient recruitment, data analysis, and writing the initial draft; Joseph Pultar: Patient recruitment, critical revision, and final approval; Constantin Weikert: Patient recruitment, critical revision, and final approval; Beate Eichelberger: Laboratory measurements, critical revision, and final approval; Maximilian Tscharré: Data analysis, critical revision, and final approval; Renate Koppensteiner: Critical revision and final approval; Simon Panzer: Study design, writing the initial draft, critical revision, and final approval; Thomas Gremmel: Study design, data analysis, writing the initial draft, critical revision, and final approval.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The research was funded by the “Medical Scientific Fund of the Mayor of the City of Vienna,” grant number 14016, and by the “Anniversary Fund of the Austrian National Bank,” grant number 16155, to Thomas Gremmel.

ORCID iDs

Patricia P. Wadowski https://orcid.org/0000-0003-2462-4515
Thomas Gremmel https://orcid.org/0000-0001-9554-7292

Supplemental Material

Supplemental material for this article is available online.

References

1. Chen Y, Zhong H, Zhao Y, Luo X, Gao W. Role of platelet biomarkers in inflammatory response. Biomark Res. 2020;8(1):28. doi:10.1186/s40364-020-00207-2
2. Gremmel T, Koppensteiner R, Kaide A, Eichelberger B, Manhalter C, Panzer S. Impact of variables of the P-selectin—P-selectin glycoprotein ligand-1 axis on leukocyte-platelet interactions in cardiovascular disease. Thromb Haemost. 2015;113(4):806-812. doi:10.1160/TH14-08-0690
3. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation. 2001;104(13):1533-1537.
4. Meng L-B, Yu Z-M, Guo P, et al. Neutrophils and neutrophil-lymphocyte ratio: inflammatory markers associated with intimal-media thickness of atherosclerosis. Thromb Res. 2018;170:45-52. doi:10.1016/j.thromres.2018.08.002
5. Li W, Liu Q, Tang Y. Platelet to lymphocyte ratio in the prediction of adverse outcomes after acute coronary syndrome: a meta-analysis. Sci Rep. 2017;7:40426. doi:10.1038/srep40426
6. Azab B, Shah N, Akerman M, McGinn JT. Value of platelet/lymphocyte ratio as a predictor of all-cause mortality after non-ST-elevation myocardial infarction. J Thromb Thrombolysis. 2012;34(3):326-334. doi:10.1007/s11239-012-0178-6
7. Ugur M, Gul M, Bozbay M, et al. The relationship between platelet to lymphocyte ratio and the clinical outcomes in ST elevation myocardial infarction underwent primary coronary intervention. Blood Coagul Fibrinolysis. 2014;25(8).
8. Ye G-L, Chen Q, Chen X, et al. The prognostic role of platelet-to-lymphocyte ratio in patients with acute heart failure: a cohort study. Sci Rep. 2019;9(1):10639. doi:10.1038/s41598-019-47143-2
9. Lee S, Hoberstorfer T, Wadowski PP, Kopp CW, Panzer S, Gremmel T. Platelet-to-lymphocyte and neutrophil-to-lymphocyte ratios predict target vessel restenosis after infrainguinal angioplasty with stent implantation. J Clin Med. 2020;9(6):1729. doi:10.3390/jcm9061729
10. Li C, Shen Y, Xu R, et al. Evaluation of preprocedural laboratory parameters as predictors of drug-eluting stent restenosis in coronary chronic total occlusion lesions. Angiology. 2018;70(3):272-278. doi:10.1177/0003319717752245
11. Gremmel T, Steinr A, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. In vivo and protease-activated receptor-1-mediated platelet activation but not response to antiplatelet therapy predict two-year outcomes after peripheral angioplasty with stent implantation. Thromb Haemost. 2014;111(3):474-482. doi:10.1160/TH13-07-0558
12. Linden MD, Furman MI, Frelinger AL III, et al. Indices of platelet activation and the stability of coronary artery disease.
13. Gremmel T, Kopp CW, Seidinger D, et al. Differential impact of cytochrome 2C9 allelic variants on clopidogrel-mediated platelet inhibition determined by five different platelet function tests. *Int J Cardiol*. 2013;166(1):126-131. doi:10.1016/j.ijcard.2011.10.010

14. Gremmel T, Kopp CW, Seidinger D, et al. Response to antiplatelet therapy and platelet reactivity to thrombin receptor activating peptide-6 in cardiovascular interventions: differences between peripheral and coronary angioplasty. *Atherosclerosis*. 2014;232(1):119-124. doi:10.1016/j.atherosclerosis.2013.10.027

15. Gremmel T, Kopp CW, Seidinger D, et al. The formation of monocyte–platelet aggregates is independent of on-treatment residual agonists'-inducible platelet reactivity. *Atherosclerosis*. 2009;202(2):608-613. doi:10.1016/j.atherosclerosis.2009.05.037

16. Gremmel T, Michelson AD, Wadowski PP, et al. Sex-specific platelet activation through protease-activated receptor-1 in patients undergoing cardiac catheterization. *Atherosclerosis*. 2021;339:12-19. doi:10.1016/j.atherosclerosis.2021.11.011

17. Gremmel T, Kopp CW, Eichelberger B, Koppensteiner R, Panzer S. Sex differences of leukocyte-platelet interactions and on-treatment platelet reactivity in patients with atherosclerosis. *Atherosclerosis*. 2014;237(2):692-695. doi:10.1016/j.atherosclerosis.2014.10.095

18. Lee S, Eichelberger B, Kopp CW, Panzer S, Gremmel T. Residual platelet reactivity in low-dose aspirin-treated patients with class I obesity. *Vasc Pharmacol*. 2021;136:106819. doi:10.1016/j.vph.2020.106819

19. Sibbing D, Aradi D, Alexopoulos D, et al. Updated consensus statement on platelet function and genetic testing for guidance of antiplatelet therapy in cardioprotection. *JACC Cardiovasc Interv*. 2019;12(16):1521. doi:10.1016/j.jcin.2019.03.034

20. Badr Eslam R, Lang IM, Koppensteiner R, Calatzis A, Panzer S, Gremmel T. Residual platelet activation through protease-activated receptors (PAR)-1 and -4 in patients on P2Y12 inhibitors. *Int J Cardiol*. 2013;168(1):403-406. doi:10.1016/j.ijcard.2012.09.103

21. Wadowski PP, Pultar J, Weikert C, et al. Resistant to aspirin treatment: platelet function and risk of in-hospital mortality in patients with ST-elevated myocardial infarction. *Med Sci Monit*. 2014;20:660-665. doi:10.12659/MSM.890152

22. Qiu Z, Jiang Y, Jiang X, et al. Relationship between platelet to lymphocyte ratio and stable coronary artery disease: meta-analysis of observational studies. *Angiology*. 2020;71(10):909-915. doi:10.1117/0003319720943810

23. Wen H, Yu H. Correlation analysis of carotid plaque in young patients with newly diagnosed type 2 diabetes and platelet-to-lymphocyte ratio and neutrophil–lymphocyte ratio [published online January 27, 2022]. *Vascular*. 2022. doi:10.1177/17085381211052362

24. Dong G, Huang A, Liu L. Platelet-to-lymphocyte ratio and prognosis in STEMI: a meta-analysis. *Eur J Clin Invest*. 2021;51(3):e13386. doi:10.1111/ejc.13386

25. Meng Z, Yang J, Wu J, Zheng X, Zhao Y, He Y. Association between the platelet-lymphocyte ratio and short-term mortality in patients with non-ST-segment elevation myocardial infarction. *Clin Cardiol*. 2021;44(7):994-1001. doi:10.1002/clc.23648

26. Qu R, Ling Y, Zhang Y, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19. *J Med Virol*. 2020;92(9):1533-1541. doi:10.1002/jmv.25767 (Electronic).

27. Simadibatra DM, Pandhita BAW, Ananta ME, Tando T. Platelet-to-lymphocyte ratio, a novel biomarker to predict the severity of COVID-19 patients: a systematic review and meta-analysis. *J Intensive Care Soc*. 2022;23(1). doi:10.11171/1751143720969587

28. Gremmel T, Michelson AD, Frelinger AL, Bhatt DL. Novel aspects of antiplatelet therapy in cardiovascular disease. *Res Pract Thromb Haemost*. 2018;2(3):439-449. doi:10.1002/rth2.12115

29. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377(12):1119-1131. doi:10.1056/NEJMoai1079714

30. Arakawa K, Yasuda S, Hao H, et al. Significant association between neutrophil aggregation in aspirated thrombus and myocardial damage in patients with ST-segment elevation acute myocardial infarction. *Circ J*. 2009;73(1):139-144. doi:10.1253/circj.CJ-08-0609

31. Gould Travis J, Vu Trang T, Swystun Laura L, et al. Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. *Arterioscler Thromb Vasc Biol*. 2014;34(9):1977-1984. doi:10.1161/ATVBAHA.114.304114

32. Horn M, Bertling A, Brodde MF, et al. Human neutrophil alpha-defensins induce formation of fibrinogen and thrombospordin-1 amyloid-like structures and activate platelets via glycoprotein Ib/IIa. *J Thromb Haemost*. 2012;10(4):647-661. doi:10.1111/j.1538-7836.2012.04640.x

33. Lim H-H, Jeong I-H, An G-D, et al. Evaluation of neutrophil extracellular traps as the circulating marker for patients with acute coronary syndrome and acute ischemic stroke. *J Clin Lab Anal*. 2020;34(5):e23190. doi:10.1002/jcla.23190

34. Yüksel M, Yıldız A, Oylumlu M, et al. The association between platelet/lymphocyte ratio and coronary artery disease severity. *Anatol J Cardiol*. 2015;15(8):640-647. doi:10.5152/akd.2014.5565

35. Gary T, Pichler M, Belaj K, et al. Neutrophil-to-lymphocyte ratio and its association with critical limb ischemia in PAOD patients. *PloS One*. 2013;8(2):e56745. doi:10.1371/journal.pone.0056745

36. Viviotis SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357(20):2001-2015. doi:10.1056/NEJMoa0706482

37. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361(11):1045-1057. doi:10.1056/NEJMoa0904327
40. Efe E, Kocayiğıt I, Türker PM, et al. Platelet-to-lymphocyte ratio but not neutrophil-to-lymphocyte ratio predicts high on-treatment platelet reactivity in clopidogrel-treated patients with acute coronary syndrome. *Indian J Pharmacol*. 2016;48(4):355-359. doi:10.4103/0253-7613.186205

41. Ofosu FA, Dewar L, Craven SJ, et al. Coordinate activation of human platelet protease-activated receptor-1 and -4 in response to subnanomolar alpha-thrombin. *J Biol Chem*. 2008;283(40):26886-26893. doi:10.1074/jbc.M802237200

42. Martorell L, Martínez-Gonzalez J, Rodriguez C, et al. Thrombin and protease-activated receptors (PARs) in atherothrombosis. *Thromb Haemost*. 2008;99(2):305-315. doi:10.1160/TH07-08-0481

43. Wu CC, Wu SY, Liao CY, Teng CM, Wu YC, Kuo SC. The roles and mechanisms of PAR4 and P2Y<sub>12</sub>/phosphatidylinositol 3-kinase pathway in maintaining thrombin-induced platelet aggregation. *Br J Pharmacol*. 2010;161(3):643-658. doi:10.1111/j.1476-5381.2010.00921.x

44. Andersen H, Greenberg DL, Fujikawa K, Xu W, Chung DW, Davie EW. Protease-activated receptor 1 is the primary mediator of thrombin-stimulated platelet procoagulant activity. *Proc Natl Acad Sci U S A*. 1999;96(20):11189-11193.

45. Gremmel T, Koppensteiner R, Steiner S, Panzer S. Preserved thrombin-inducible platelet activation in acute coronary syndromes. *Cardiovasc Drugs Ther*. 2020;34(1):53-63. doi:10.1007/s10557-019-06932-7

46. Nylander S, Femia EA, Scavone M, et al. Ticagrelor inhibits human platelet aggregation via adenosine in addition to P2Y<sub>12</sub> antagonism. *J Thromb Haemost*. 2013;11(10):1867-1876. doi:10.1111/jth.12360

47. Pareek M, Bhatt DL. Ticagrelor for patients with acute coronary syndromes: PLATOnic affair or lasting SWEDEHEART? *Eur Heart J*. 2016;37(44):3343-3346. doi:10.1093/eurheartj/ehw356

48. Pultar J, Wadowski PP, Panzer S, Gremmel T. Oral antiplatelet agents in cardiovascular disease. *Vasa*. 2019;48(4):291-302. doi:101024/0301-1526/a000753

49. VAN Giezen JJ, Nilsson L, Berntsson P, et al. Ticagrelor binds to human P2Y<sub>12</sub> independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. *J Thromb Haemost*. 2009;7(9):1555-1565. doi:10.1111/j.1538-7836.2009.03527.x

50. Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. *J Am Coll Cardiol*. 2014;63(23):2503-2509. doi:10.1016/j.jacc.2014.03.031

51. Bonello L, Laine M, Kipson N, et al. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. *J Am Coll Cardiol*. 2014;63(9):872-877. doi:10.1016/j.jacc.2013.09.067

52. Wadowski PP, Weikert C, Pultar J, et al. Ticagrelor inhibits toll-like and protease-activated receptor mediated platelet activation in acute coronary syndromes. *Cardiovasc Drugs Ther*. 2020;34(1):53-63. doi:10.1007/s10557-019-06932-7

53. Nylander S, Femia EA, Scavone M, et al. Ticagrelor inhibits human platelet aggregation via adenosine in addition to P2Y<sub>12</sub> antagonism. *J Thromb Haemost*. 2013;11(10):1867-1876. doi:10.1111/jth.12360

54. Pareek M, Bhatt DL. Ticagrelor for patients with acute coronary syndromes: PLATOnic affair or lasting SWEDEHEART? *Eur Heart J*. 2016;37(44):3343-3346. doi:10.1093/eurheartj/ehw356

55. Motovska Z, Hlinomaz O, Kala P, et al. 1-year outcomes of patients undergoing primary angioplasty for myocardial infarction treated with prasugrel versus ticagrelor. *J Am Coll Cardiol*. 2018;71(4):371-381. doi:10.1016/j.jacc.2017.11.008

56. Schüpke S, Neumann F-J, Menichelli M, et al. Ticagrelor or prasugrel in patients with acute coronary syndrome. *N Engl J Med*. 2019;381(16):1524-1534. doi:10.1056/NEJMoa1908973