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Frakcja niedojrzałych płytek krwi oraz płytkowe mikroRNA w ocenie rokowania oraz odpowiedzi na leczenie przeciwpłytkowe w ostrych zespołach wieńcowych: prospektywne badanie kohortowe

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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STRESZCZENIE W JĘZYKU ANGIELSKIM (SUMMARY)

Title: Immature platelet fraction and platelet microRNAs in the assessment of prognosis and response to antiplatelet therapy in acute coronary syndromes: a prospective cohort study

Background: Acute coronary syndromes (ACS) are a major public health concern worldwide. Platelets play a central role in the pathophysiology of ACS, and antiplatelet therapy is a cornerstone of its management. Despite advances in the understanding of platelet biology and the development of antiplatelet agents, a notable gap in evidence regarding the optimal approach to antiplatelet therapy in ACS patients exists. The need for individualization of treatment strategies is underscored by the heterogeneity of patient responses and different risks of adverse outcomes. While current guidelines acknowledge this challenge, they predominantly rely on general risk scores that assess bleeding and ischemic risks, lacking factors for predicting platelet reactivity due to the absence of suitable biomarkers. The presented work aims to bridge this gap by investigating the role of two promising factors – immature platelet fraction (IPF) and selected platelet microRNAs (miRs) – in the assessment of the prognosis and response to antiplatelet therapy in ACS. IPF reflects the number of newly released platelets into circulation. Due to the greater activity of young platelets, it is suspected that IPF may be useful in assessing cardiovascular risk and monitoring antiplatelet therapy. Insights into the interplay between IPF and antiplatelet therapy offer promise for refining treatment strategies and improving outcomes in ACS patients. MiRs, small RNA molecules, play crucial roles in post-transcriptional gene regulation and contribute to cardiovascular diseases' development, including ACS. Specific platelet-derived miRs can regulate platelets' activation processes and thus affect the response to antiplatelet therapy. Exploring the role of selected platelet miRNAs holds promise for improvement of our understanding of ACS mechanisms and their utility as novel, minimally invasive biomarkers for risk stratification and monitoring dual antiplatelet therapy (DAPT).

Aim: The main objective of the work was to evaluate the roles of IPF and platelet miRs in assessing patients' prognosis and response to antiplatelet therapy in ACS.

Methods: The study was registered with ClinicalTrials.gov (NCT06177587). In the prospective phase of the study, adult patients with ACS upon admission were enrolled in the First Department of Cardiology, Medical University of Warsaw (Publications 1-3). Participants underwent coronary angiography, with percutaneous coronary intervention (PCI) performed as

per current guidelines. Periprocedurally, patients received loading doses of antiplatelet agents including clopidogrel or ticagrelor as $P2Y_{12}$ receptor antagonist. Peripheral vein blood samples were obtained within the first 24 hours post-procedure. IPF analysis was conducted on whole blood anticoagulated with ethylenediaminetetraacetic acid (K3EDTA) using an automated hematological analyzer (Sysmex XN 2000, Kobe, Japan). Impedance aggregometry with adenosine diphosphate (ADP) as an agonist, was performed 30–120 minutes after blood sampling with the Multiplate® Analyzer (Roche Diagnostics, Basel, Switzerland). The aggregation test was conducted following the manufacturer's instructions. Patients were subsequently followed for a period of 60 months to assess long-term outcomes and evaluate the prognostic value of the studied parameters (Publications 1 and 3). The primary endpoint was defined as major adverse cardiovascular events (MACE) comprising all-cause mortality, myocardial infarction (MI), stroke, and unplanned revascularization. The extension regarding miR assessment was performed in the subset of enrolled ACS population, which was expanded to include 18 prasugrel-treated patients and 18 healthy volunteers (Publication 2). Blood sampling for miR expression measurements was performed in the first 24 hours after the procedure. Patients received DAPT including clopidogrel, ticagrelor, or prasugrel as a $P2Y_{12}$ receptor antagonist. The manuscript provides detailed methods regarding the measurement of the expression of selected miRs: miR-126-3p, miR-21-5p, miR-223-3p, miR-197-3p, and miR-24-3p. The final part includes a comprehensive review of the pertinent literature (Publication 4).

Results: 140 patients were included in the first part of the study (Publications 1 and 3). Complete clinical follow-up was available for 130 of them. Overall MACE occurred in 27 patients (20.8%) in the median follow-up time of 57 months. After dividing patients into tertiles based on IPF level, Cox proportional hazard model for MACE revealed a significant difference in the occurrence of MACE in the highest compared to the lowest tertile (HR = 5.341 95% CI: 1.546-18.454, p = 0.008). Multivariable Cox regression analyses showed that baseline IPF level was independently associated with MACE (HR = 1.279, 95% CI: 1.056-1.549, p = 0.012). Time-dependent receiver operating characteristic (ROC) curve analysis revealed area under curve (AUC) of 0.656 for 5-year outcome with an IPF cut point of 3.45% being 63.0% sensitive and 65.0% specific for MACE. The analysis also revealed that the level of IPF correlates with ADP-induced platelet reactivity in non-ST elevation acute coronary syndrome (NSTE-ACS) patients (rho = 0.387, 95% 95% CI: 0.101–0.615, p = 0.008), independently of the $P2Y_{12}$ receptor antagonist received, but was not observed in the ST

elevation acute coronary syndrome (STE-ACS) group (Publication 1). A similar, even stronger relationship was observed for immature platelet count (IPC). The second part of the study (Publication 2), regarding miR measurements recruited 97 patients, including 79 ACS individuals and 18 healthy volunteers. It revealed significantly lower expression of miR-126-3p, miR-223-3p, miR-21-5p, and miR-197-3p in patients treated with ticagrelor compared to clopidogrel (fold changes from -1.43 to -1.27, p-values from 0.028 to 0.048). Positive correlations were observed between platelet reactivity and the expression of miR-223-3p (rho = 0.400, p = 0.019) and miR-21-5p (rho = 0.423, p = 0.013) in patients treated with potent drugs.

Conclusions:

- The heterogenic response to antiplatelet therapy highlights the necessity for personalized treatment strategies, with the incorporation of new biomarkers offering the potential to enhance personalized management.
- The value of IPF in NSTE-ACS patients correlates with ADP-induced platelet reactivity assessed with impedance aggregometry.
- The expression of miR-126-3p, miR-223-3p, miR-21-5p, miR-197-3p significantly differs between patients receiving potent (ticagrelor) and less potent (clopidogrel) $P2Y_{12}$ receptor antagonists as part of DAPT; in addition, the expression of miR-223-3p and miR-21-5p correlates with the impedance aggregometry results.
- IPF measurements in ACS patients have predictive value for long-term MACE, however their sensitivity and specificity at this point appear insufficient as an independent tool for therapeutic decision-making.
- Further research on the utility of IPF and selected platelet miRs as prognostic tools of MACE and response to DAPT in ACS is warranted.