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Association of Immature Platelets With Adverse Cardiovascular Outcomes

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ABSTRACT

BACKGROUND Immature platelets are less responsive to the effects of antiplatelet drugs and contain messenger ribonucleic acid that is translationally active. They can be measured easily using an automated hematoanalyzer and reported as part of the complete blood count.

OBJECTIVES The purpose of this study was to determine the prognostic significance of elevated immature platelet count (IPC) in patients with coronary artery disease (CAD).

METHODS In this prospective cohort study in patients with CAD, patients underwent IPC measurement and were then followed up for the composite endpoint of major adverse cardiovascular events (MACE), defined as a composite of all-cause mortality, myocardial infarction, unplanned revascularization, or hospitalization for angina. For the purposes of analysis, patients were stratified into tertiles of IPC.

RESULTS Eighty-nine patients were followed up for a median of 31 months. Stratification to the high IPC tertile was associated with higher rates of MACE compared with the intermediate and low tertiles (60% vs. 24% vs. 16%, respectively; $p < 0.001$). Time-dependent receiver-operating characteristic analysis revealed that an IPC level ≥7,632 platelets/μl was 70.7% sensitive and 82.1% specific for MACE. After adjustment for age, admission diagnosis, index revascularization, heart failure, smoking, hematocrit, and baseline platelet count, patients with an IPC level ≥7,632 platelets/μl were more likely to experience a MACE (hazard ratio: 4.65; 95% confidence interval: 1.78 to 12.16; $p < 0.002$).

CONCLUSIONS IPC is a novel biomarker for MACE risk stratification in patients with CAD. Future studies should focus on the utilization of this marker for individualized antiplatelet therapy. (J Am Coll Cardiol 2014;64:2122–9)

A cornerstone event in the pathophysiology of intravascular thrombosis, platelet activation can lead to myocardial infarction (MI) and stroke (1,2). The population of circulating platelets is not homogeneous; different subpopulations of platelets can be categorized according to their activation patterns in response to different agonists (3). Immature platelets, also termed reticulated platelets (RPs) as they are analogous to erythroid reticulocytes, comprise the youngest component of the circulating platelet pool, and appear to participate most actively in thrombosis (4). They contain measurable amounts of cytosolic messenger ribonucleic acid (mRNA) that is translationally active (5–7). RPs also tend to be larger in size, contain more dense granules, and display a greater ex vivo reactivity profile in response to agonists compared with mature non-mRNA-containing platelets (8–10). There is concordance between the level of circulating RPs and the proportion of subjects who are hyporesponsive to aspirin or clopidogrel, indicating that RPs may play a significant role in attenuating the effects of these agents (8–10).

RPs were first described using light microscopy, which revealed a specific staining pattern of their nucleic acid content that is predominantly due to the presence of mRNA (11,12). The next significant

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advance was identifying RPs with flow cytometry after staining blood specimens with thiazole orange (13,14). However, flow cytometry is a time-consuming and costly procedure without a standardized protocol and not well suited for rapid screening in patients in clinical practice. More recently, newer assay techniques have been developed to allow for automated immature platelet determination as part of the standard complete blood count (15). The aim of the current study was to evaluate the ability of the automated method for immature platelet detection to further stratify patients with coronary artery disease (CAD) according to their risk for clinical outcomes.

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METHODS

STUDY DESIGN. In this prospective cohort study, the inclusion criteria were age ≥18 years, the ability to provide informed consent, diagnosis of CAD, and current treatment with dual antiplatelet therapy (unless coronary artery bypass graft [CABG] surgery was performed).

The study protocol was approved by the Houston Methodist Hospital institutional review board, and all patients provided written informed consent. Patients were excluded if they had received any blood products within the 30 days before immature platelet evaluation.

The primary study outcome was the occurrence of major adverse cardiovascular events (MACE), defined as the composite endpoint of all-cause mortality, MI (either ST-segment elevation myocardial infarction [STEMI] or non-ST-segment elevation myocardial infarction [NSTEMI]), unplanned revascularization, or recurrent angina requiring hospitalization. Myocardial infarction was defined as symptoms suggestive of myocardial ischemia (i.e., chest pain or discomfort) associated with biochemical evidence of myocardial necrosis, defined as the level of cardiac troponin >5 times the upper limit of normal (16). Unplanned revascularization was defined as the need for coronary revascularization that was not planned or staged at the index hospitalization. Angina was reported by patients and was defined as discomfort, pain, and/or tightness in the chest, jaw, shoulder, back, and/or arm that was aggravated by exertion and relieved by nitroglycerin or rest (17). To ensure the accuracy of the diagnosis of angina, categorization of an angina episode as an event required the patient to have had an overnight stay in the hospital for the evaluation of anginal symptoms.

Patients were contacted between August and October of 2013 for the evaluation of the occurrence of the primary outcome. Follow-up was conducted by 2 investigators who were blinded to the immature platelet count (IPC) results and who made telephone calls to all of the patients or to their families if a patient was deceased. Follow-up ended at the time of the first contact with the patient or family.

IPC MEASUREMENT. Immature platelets were measured with an automated hematoanalyzer (Sysmex 2100 XE, Sysmex America Inc., Mundelein, Illinois) that uses fluorescent dyes containing polymethine and oxazine. Both dyes penetrate the cell membrane and stain the RNA in erythrocytes and leukocytes as well as platelets. Stained cells are then sorted as they pass through a beam of light created by a semiconductor laser diode. After measurements of cell volume (using forward scatter light) and RNA content (using fluorescence intensity), the device provides scattergrams using a proprietary nonadjustable software algorithm with a preset gate (Sysmex IPF Master). This system discriminates between mature and immature platelets and reports the immature platelet fraction (IPF). The IPC can then be determined by multiplying IPF by the platelet count from the complete blood count.

Immature platelet measurements were conducted on enrollment. In patients who underwent revascularization (either CABG or percutaneous coronary intervention), IPC was evaluated within 72 h of the index revascularization. In all other patients, IPC was evaluated within 72 h of admission to the hospital.

TURBIDIMETRIC PLATELET AGGREGATION. Light transmission aggregometry was used to measure agonist-induced platelet aggregation. We collected 20 ml of whole blood in 0.32% sodium citrate (final concentration). The tubes were centrifuged immediately at 1,700 *g* for 6 min to prepare platelet-rich plasma. The remaining whole blood fraction was further centrifuged at 3,200 *g* for 15 min to separate platelet-poor plasma. The platelet count in platelet-rich plasma was standardized between 200 and 250 × 10^3 cells/μl. Platelet aggregation was induced using 5 and 20 μM adenosine diphosphate (ADP) (final concentrations). The maximum aggregation achieved during a 6-min period was used for analysis.

Aggregation was assessed after an adequate period to ensure full antiplatelet effect (i.e., at least 6 h after 600 mg clopidogrel loading dose, at least 12 h after 300 mg clopidogrel loading dose, or long-term use of clopidogrel 75 mg daily for at least 5 days).

STATISTICAL ANALYSIS. Categorical variables were analyzed using the Fisher exact test. To evaluate the differences between normally distributed continuous

ABBREVIATIONS AND ACRONYMS

AUC = area under the curve
CAD = coronary artery disease
IPC = immature platelet count
IPF = immature platelet fraction
MACE = major adverse cardiovascular event(s)
NSTEMI = non-ST-segment elevation myocardial infarction
ROC = receiver-operating characteristic
RP = reticulated platelets
STEMI = ST-segment elevation myocardial infarction