Do immature platelet levels in chest pain patients presenting to the emergency department aid in the diagnosis of acute coronary syndrome?

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.1111/ijlh.12250</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:33776183">http://nrs.harvard.edu/urn-3:HUL.InstRepos:33776183</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Do Immature Platelet Levels in Chest Pain Patients Presenting to the Emergency Department Aid in the Diagnosis of Acute Coronary Syndrome?

Michelle A. Berny-Lang, PhD\textsuperscript{a}, Chad E. Darling, MD\textsuperscript{b}, Andrew L. Frelinger III, PhD\textsuperscript{a}, Marc R. Barnard, MS\textsuperscript{a}, Craig S. Smith, MD\textsuperscript{c}, and Alan D. Michelson, MD\textsuperscript{a}

\textsuperscript{a}Center for Platelet Research Studies, Division of Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

\textsuperscript{b}Department of Emergency Medicine, University of Massachusetts Medical School, Worcester, MA

\textsuperscript{c}Department of Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA

Abstract

Introduction—Early and accurate identification of acute coronary syndrome (ACS) versus non-cardiac chest pain in patients presenting to the emergency department (ED) is problematic and new diagnostic markers are needed. Previous studies reported that elevated mean platelet volume (MPV) is associated with ACS and predictive of cardiovascular risk. MPV is closely related to the immature platelet fraction (IPF), and recent studies have suggested that IPF may be a more sensitive marker of ACS than MPV. The objective of the present study was to determine if the measurement of IPF assists in the diagnosis of ACS in patients presenting to the ED with chest pain.

Methods—In this single-center, prospective, cross-sectional study, adult patients presenting to the ED with chest pain and/or suspected ACS were considered for enrollment. Blood samples from 236 ACS-negative and 44 ACS-positive patients were analyzed in a Sysmex XE-2100 for platelet count, MPV, IPF and the absolute count of immature platelets (IPC).

Results—Total platelet counts, MPV, IPF and IPC were not statistically different between ACS-negative and -positive patients. The IPF was $4.6 \pm 2.7$ and $5.0 \pm 2.8\%$ (mean ± SD, p = 0.24) and the IPC was $10.0 \pm 4.6$ and $11.5 \pm 7.5 \times 10^3/\mu$L (p = 0.27) for ACS-negative and ACS-positive patients, respectively.

Conclusion—In 280 patients presenting to the ED with chest pain and/or suspected ACS, no differences in IPF, IPC or MPV were observed in ACS-negative versus ACS-positive patients, suggesting that these parameters do not assist in the diagnosis of ACS.

Corresponding Author: Alan D. Michelson, MD, Boston Children’s Hospital, Karp 08213, 300 Longwood Avenue, Boston, MA 02115-5737. alan.michelson@childrens.harvard.edu. Telephone: (617) 919-2116. Fax: (617) 730-4631.

Authorship
CED, ALF, MRB, and ADM designed the study. MAB, CED, ALF, MRB, CSS, and ADM analyzed and interpreted the data, wrote and/or critically revised the manuscript, and provided final approval of the version to be published.
Keywords
acute coronary syndrome; emergency medicine; immature platelet fraction; myocardial infarction; platelets

INTRODUCTION
Emergency medicine physicians currently utilize a combination of medical history, physical examination, electrocardiographic (ECG) changes, serum markers of myocardial necrosis (troponin I, troponin T, creatine kinase-myocardial band [CK-MB]) and possibly coronary computed tomographic (CT) angiography, to identify acute coronary syndromes (ACS) in patients presenting to the emergency department (ED) with chest pain and/or suspected ACS. However, early and accurate identification of patients with ACS versus non-cardiac chest pain and further classification of ACS into ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI) or unstable angina remains problematic, particularly in patients displaying atypical or inconclusive symptoms. Early diagnosis and the resultant earlier application of appropriate medical intervention for ACS improves patient outcomes and reduces the cost of unnecessary treatment and hospitalization. The development of an additional early marker to aid in ACS diagnosis could therefore improve patient care.

Current laboratory tests to rule in ACS (troponin I, troponin T, CK-MB) are elevated in response to myocardial damage, but these are relatively late markers that only become elevated 4 – 6 hours after cardiac necrosis. In contrast, platelets play a critical earlier role in ACS by initiating thrombus formation at sites of ruptured atherosclerotic plaque. Multiple studies suggest that platelet markers are altered in ACS. One such platelet marker is the measurement of platelet size by the mean platelet volume (MPV). Dating back to at least the 1960’s, studies have reported an increase in MPV in patients with myocardial infarction (MI). The mechanistic basis for these findings may be that larger platelets have increased reactivity compared to smaller platelets.

Because young, newly generated platelets are larger than older circulating platelets, the increased MPV in ACS may be associated with heightened turnover of platelets. The number of circulating immature platelets (also known as reticulated platelets) can be defined by large platelets that contain messenger RNA (mRNA). Therefore, MPV may be an indirect measure of immature platelets and, indeed, studies suggest that the immature platelet fraction (IPF) is increased in ACS. Elevated IPF has been detected in STEMI patients versus healthy controls, stable coronary artery disease (CAD) patients and NSTEMI patients, and increased immature platelets were found in STEMI and NSTEMI patients versus unstable or stable angina patients.

Based upon the above-suggested role of immature platelets in ACS and the need for new early diagnostic markers for ACS, the current prospective, cross-sectional study was designed to determine if immature platelet levels can aid in the diagnosis of ACS in patients presenting to the ED with chest pain and/or suspected ACS.
METHODS

Study Design and Patient Population

The study was approved by the Committee for Protection of Human Subjects in Research at the University of Massachusetts Medical School. Adult patients (≥18 years of age) presenting with chest pain and/or suspected ACS to the ED of UMass Memorial Medical Center (Worcester, MA) over a period of 22 months (May 2007 to March 2009) were considered for enrollment. Patients were excluded due to trauma, pregnancy, evidence of renal insufficiency (serum creatinine >1.5 mg/dL) or evidence of gastrointestinal or other bleeding. In addition to routine ED admission laboratory tests, an additional blood sample was drawn by venipuncture into a K$_2$ EDTA Becton Dickinson blood collection tube (Franklin Lakes, NJ). IRB-approved written informed consent was obtained for the use of data from patients meeting enrollment criteria. For patients who were clinically unstable at presentation, data were obtained and consent was sought later. In instances in which delayed consent was not granted, all data were discarded.

Patients were interviewed regarding use of antiplatelet agents (aspirin, clopidogrel, non-steroidal anti-inflammatory agents [NSAIDs]). The medical record was reviewed for the following cardiovascular risk factors: diabetes mellitus, hypertension, hypercholesterolemia, smoking and family history of CAD. Based upon the risk factors, the thrombolysis in myocardial infarction (TIMI) risk score was calculated, as described by Antman et al.\textsuperscript{17}

Platelet Analysis

EDTA-anticoagulated blood samples were analyzed in a Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan) for platelet count (by impedance), IPF, absolute immature platelet count (IPC) and MPV. As previously described, the Sysmex XE-2100 identifies immature platelets by: 1) fluorescent mRNA staining and 2) light scatter characteristics.\textsuperscript{18} The instrument was calibrated daily with Sysmex calibration reagents. To establish the normal range of the instrument, 28 EDTA-anticoagulated blood samples from healthy subjects were measured. The platelet count was 242 ± 72 × 10$^3$/µL (range 179–383), the MPV was 10.1 ± 0.9 fL (range 8.7–12.4), the IPF was 3.3 ± 2.2 % (range 0.8–10.1), and the IPC was 7.4 ± 4.2 × 10$^3$/µL (range 2.1–19.6). Blood samples for all analyses were stored at room temperature and measured within 24 hours of collection, which was within the two day post-collection window of IPF stability in EDTA anticoagulated blood.\textsuperscript{19}

Patient Diagnosis

For all patients enrolled in the study, final diagnosis (ACS-positive versus ACS-negative) was established via standardized chart review. All reviews were conducted by study physicians in a blinded manner, without knowledge of platelet data. For all patients who had positive cardiac biomarkers during admission, underwent cardiac catheterization, had a positive provocative cardiovascular test (e.g., exercise stress test) and/or had ischemic ECG changes, the diagnosis was adjudicated by a cardiologist. Patients were considered to be ACS-positive when the final diagnosis was categorized into one of three groups: (i) STEMI, defined as 1 mm ST segment elevation in at least two contiguous ECG leads, a history consistent with ACS and/or cardiac catheterization findings consistent with acute coronary
occlusion; (ii) NSTEMI, defined as any ECG finding other than STEMI, history and inpatient cardiac testing consistent with ACS and positive diagnostic cardiac markers within 24 hours after hospital arrival; or (iii) unstable angina, defined as any ECG findings, a history consistent with ACS, non-diagnostic cardiac markers within 24 hours of arrival and/or cardiac diagnostic testing (e.g., cardiac catheterization) consistent with ACS. Patients who did not meet these criteria (those with a diagnosis of non-cardiac chest pain/symptoms) were classified as ACS-negative. Patients classified as ACS-negative, but diagnosed with a non-cardiac thrombosis were excluded.

**Statistical Methods**

Demographic, clinical and platelet data were stratified into ACS-positive or ACS-negative patient groups. The ACS-positive group was further stratified into STEMI, NSTEMI and unstable angina groups. When data required for clinical diagnosis were missing, the entire data set for the subject was excluded; all other subjects with missing data not required for clinical diagnosis were included. Statistical analyses were performed in GraphPad Prism 5.01 (La Jolla, CA). Continuous data are reported as mean ± standard deviation (SD). Data were evaluated for normality by the D’Agostino and Pearson omnibus normality test. The distribution of all study measurements was non-Gaussian and, therefore, the non-parametric Mann-Whitney test was used to compare continuous data (sum of ranks comparison) from ACS-positive versus ACS-negative patients. Comparisons of categorical data were by Fisher’s exact test. Comparisons of STEMI versus NSTEMI versus unstable angina patients were by Kruskal-Wallis one way analysis of variance (ANOVA) with Dunn’s posttest. A p value of <0.05 was considered statistically significant for all measurements.

The primary end point of this study was the IPF. Secondary end points were IPC and MPV. Based upon the standard deviation of IPF in ACS-positive and -negative patients, the current study had 80% power (with α = 0.05, two-sided) to detect an absolute difference in IPF of 1.3% or greater between ACS-positive and -negative patients. The secondary end points were powered to detect mean differences of 3.3 × 10^3/µL or greater and 0.4 fL or greater between ACS-positive and -negative patients for IPC and MPV, respectively.

**RESULTS**

**Study populations**

A total of 294 separate patients were enrolled in the study. Figure 1 outlines the flow of study patients from initial enrollment to diagnosis. Twelve patients were excluded due to exclusions outlined in the study protocol (elevated serum creatinine, n=6), consent withdrawal (n=1), repeat ED visit (n=1) and inconclusive diagnoses/incomplete clinical data (n=4). Two patients were classified as having a non-cardiac thrombosis (aortic dissection, cerebral aneurysm with aortic and atrial thrombus) and were excluded from further analyses. In total, 44 patients included in the final analyses were classified as ACS-positive, including STEMI, NSTEMI or unstable angina. The remaining 236 patients were classified as ACS-negative. The demographic and clinical characteristics of the study populations are detailed in Table 1. The average patient age was 62 years in the ACS-positive group and 59 years in the ACS-negative group. Male sex, clopidogrel and NSAID use, smoking, hypertension,
hypercholesterolemia, diabetes mellitus and family history of CAD were numerically greater in the ACS-positive group, but not significantly different from the ACS-negative patients. However, as expected, the TIMI risk score, a measurement combining medical history and clinical characteristics to identify risk of death and ischemic events, was significantly elevated in the ACS-positive patients.

### Platelet measurements

As shown in Table 2, platelet counts were similar in ACS-positive and -negative patient populations. The MPV was not significantly different for ACS-positive versus ACS-negative patients (Table 2, Figure 2). Furthermore, no significant differences in the IPF or the IPC were observed between ACS-positive and -negative groups (Table 2, Figure 3).

When the ACS-positive group was further stratified into STEMI (n=6), NSTEMI (n=19) and unstable angina (n=19) populations, and evaluated by the Kruskal-Wallis one way ANOVA, there were no significant differences between STEMI versus NSTEMI versus unstable angina groups for platelet count (p = 0.3029), MPV (p = 0.3405), IPF (p = 0.6425) and IPC.

### DISCUSSION

The main finding of this prospective, cross-sectional study is that the IPF, IPC and MPV were not different in patients classified as ACS-positive or -negative after presenting to the ED with chest pain and/or suspected ACS. Furthermore, the IPF, IPC and MPV did not differ between patients with STEMI, NSTEMI and unstable angina. Therefore, this study suggests that the measurement of these platelet indices does not assist in the diagnosis or classification of ACS and that ACS does not increase the rate of platelet production.

The strength of the current study of patients presenting to the ED with chest pain and/or suspected ACS is its cross-sectional design. The majority of previous studies on immature platelets in ACS were cohort or case-control studies that measured immature platelets in defined populations, including healthy subjects and patients with CAD, ACS, STEMI and NSTEMI. For example, a case-control study of 202 patients with ACS and 202 healthy subjects without a history of vascular or thromboembolic disease, reported significant elevation of the IPF in STEMI or NSTEMI patients compared with healthy controls. The different result as compared with the current study is likely due to the study design and patient groups studied (healthy controls versus ACS as compared to, in the current study, all patients presenting to the ED). In contrast to two studies that found differences in IPF among ACS patients, the present study did not detect differences in IPF between STEMI, NSTEMI or unstable angina patients. The present study was powered to compare ACS-positive and -negative patient groups irrespective of ACS type, and included only 6 patients with a STEMI diagnosis.

Similar to immature platelets, although elevated MPV has been reported to be strongly associated with ACS, the present study did not find the MPV to be elevated in the ACS-positive patient group versus the ACS-negative patient group. This is in agreement with another cross-sectional study of patients admitted to a coronary care unit, wherein
MPV was no different in patients with MI, angina or acute cardiac ischemia, leading the authors to conclude that MPV has no clinical relevance in the coronary care unit.\textsuperscript{21}

Although we did not find a role for immature platelet or MPV measurements in the diagnosis of ACS, the level of immature platelets may nevertheless play a role in the prevention and management of ACS. In agreement with the present study, a study of hospitalized ACS patients found no difference in IPF between patients with and without ST-segment elevation. However, this study reported a significant increase in mortality in patients with high IPF values.\textsuperscript{27} This finding is similar to the results of the AMI-Florence 2 Study in which higher IPF was detected in patients that had died at 1 year of follow-up.\textsuperscript{28} In addition to an association with mortality, studies in healthy subjects and patients with CAD or previous stent thrombosis have reported that individuals with increased levels of immature platelets are hyporesponsive to antiplatelet agents, including aspirin and clopidogrel, as measured by increased reactivity in platelet function assays.\textsuperscript{29–34}

**Study limitations and strengths**

The present study was powered to detect a percent change of 4, 28 and 33\% or greater in MPV, IPF and IPC, respectively, between ACS-positive and -negative patients. Thus, if the true differences were less, they would not have been detected by our study. Strengths of the present study include its cross-sectional design and the use of a well-standardized method to measure the immature platelet fraction. Some previous studies have measured immature platelets by flow cytometry,\textsuperscript{16,29,31} which is difficult to standardize resulting in variable measurements from laboratory to laboratory. The full automation of the presently used Sysmex XE-2100 method provides faster results with better precision and reproducibility.

**Conclusions**

In this prospective, cross-sectional study of 280 patients presenting to the ED with chest pain and/or suspected ACS, no differences in IPF, IPC or MPV were detected in ACS-positive versus ACS-negative patients. This study suggests that measurements of IPF, IPC and MPV do not aid in the diagnosis of ACS.

**Acknowledgements**

The authors thank Michelle Maynard for excellent technical assistance. MAB was supported by an NIH T32 Training Grant (5T32HL007574-31). CED was supported in part by a Research Training Grant from the Society for Academic Emergency Medicine (SAEM), Des Plaines, IL. This study was funded in part by a grant from Sysmex Corporation; however, Sysmex Corporation had no role in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

**Abbreviations and Acronyms**

| ACS   | acute coronary syndrome |
| CAD   | coronary artery disease |
| CK-MB | creatine kinase-myocardial band |
| CT    | computed tomographic |
ED  emergency department
ECG  electrocardiogram
IPC  absolute immature platelet count
IPF  immature platelet fraction
MPV  mean platelet volume
MI  myocardial infarction
NSAID  non-steroidal anti-inflammatory drug
NSTEMI  non-ST-segment elevation myocardial infarction
STEMI  ST-segment elevation myocardial infarction
TIMI  thrombolysis in myocardial infarction

References


Figure 1. Study flow diagram
The chart outlines the flow of patients from study enrollment to diagnosis.
Figure 2. The mean platelet volume (MPV) in ACS-positive and -negative patients presenting to the ED with chest pain and/or suspected ACS.

Box represents 25th to 75th percentiles and whiskers 5th to 95th percentiles, with outliers indicated by black circles. The median is indicated by the middle horizontal line and the mean by ‘+.’ Sum of ranks comparison was by the Mann-Whitney test.
Figure 3. The A) immature platelet fraction (IPF) and B) absolute immature platelet count (IPC) in ACS-positive and -negative patients presenting to the ED with chest pain and/or suspected ACS.

Box represents 25th to 75th percentiles and whiskers 5th to 95th percentile, with outliers indicated by black circles. The median is indicated by the middle horizontal line and the mean by ‘+.’ Sum of ranks comparisons were by the Mann-Whitney test.
Table 1
Demographics and clinical characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>ACS-positive (total n=44)</th>
<th>ACS-negative (total n=236)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>62 ± 16 (n=44)</td>
<td>59 ± 14 (n=236)</td>
<td>0.4265 (ns)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>77 (n=44)</td>
<td>64 (n=236)</td>
<td>0.1177 (ns)</td>
</tr>
<tr>
<td>TIMI risk score (mean ± SD)</td>
<td>3.2 ± 1.6 (n=43)</td>
<td>2.1 ± 1.4 (n=234)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>74 (n=43)</td>
<td>80 (n=234)</td>
<td>0.4185 (ns)</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>25 (n=44)</td>
<td>15 (n=235)</td>
<td>0.1198 (ns)</td>
</tr>
<tr>
<td>NSAIDs (%)</td>
<td>33 (n=40)</td>
<td>27 (n=219)</td>
<td>0.5670 (ns)</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>25 (n=44)</td>
<td>23 (n=234)</td>
<td>0.7007 (ns)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>66 (n=44)</td>
<td>62 (n=234)</td>
<td>0.7351 (ns)</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>75 (n=44)</td>
<td>59 (n=234)</td>
<td>0.0623 (ns)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>36 (n=44)</td>
<td>26 (n=234)</td>
<td>0.2018 (ns)</td>
</tr>
<tr>
<td>Family history of CAD (%)</td>
<td>50 (n=44)</td>
<td>41 (n=234)</td>
<td>0.3215 (ns)</td>
</tr>
</tbody>
</table>

Comparisons of continuous data (sum of ranks comparisons) were by the Mann-Whitney test. Comparisons of categorical data were by Fisher’s exact test. ACS-positive = STEMI, NSTEMI, unstable angina; CAD = coronary artery disease; ns = nonsignificant; TIMI = thrombolysis in myocardial infarction.
Table 2
Platelet parameters of ACS-positive and -negative patients

<table>
<thead>
<tr>
<th></th>
<th>ACS-positive mean ± SD (total n=44)</th>
<th>ACS-negative mean ± SD (total n=236)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (× 10^3/µL)</td>
<td>242 ± 70 (n=43)</td>
<td>233 ± 67 (n=236)</td>
<td>0.4675 (ns)</td>
</tr>
<tr>
<td>Mean platelet volume, MPV (fL)</td>
<td>11.5 ± 0.7 (n=43)</td>
<td>11.3 ± 1.0 (n=234)</td>
<td>0.0918 (ns)</td>
</tr>
<tr>
<td>Immature platelet fraction, IPF (% of total platelets)</td>
<td>5.0 ± 2.8 (n=44)</td>
<td>4.6 ± 2.7 (n=236)</td>
<td>0.2390 (ns)</td>
</tr>
<tr>
<td>Immature platelet count, IPC (× 10^3/µL)</td>
<td>11.5 ± 7.5 (n=44)</td>
<td>10.0 ± 4.6 (n=236)</td>
<td>0.2695 (ns)</td>
</tr>
</tbody>
</table>

Measurements were performed in a Sysmex XE-2100 analyzer. Platelet count was by impedance. Comparisons were by the Mann-Whitney test. ns = nonsignificant.