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# Age modulates the relationship between platelet-to-lymphocyte ratio and coronary artery disease

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ABSTRACT

*Background:* Thrombocytosis and inflammation are vital elements in the pathogenesis of atherosclerosis. The platelet-to-lymphocyte ratio (PLR) is a novel biomarker that combines these parameters and has been shown to be associated with cardiovascular disease (CVD). This study aimed to determine whether PLR correlates with coronary artery disease (CAD) in high-risk patients, and if the relationship is affected by age.

*Methods:* Consecutive patients referred for coronary angiogram were evaluated (n = 822). with 608 patients demonstrating CAD, compared to 214 patients with normal coronary arteries. Patients were stratified into premature CAD (age < 55) and non-premature CAD (age ≥ 55). High and low PLR groups were defined as admission PLR in the highest (≥146.7) and lower two tertiles (<146.7) respectively. Multivariate logistic regression was undertaken to adjust for traditional cardiovascular risk factors.

*Results:* In univariate analysis, high PLR negatively correlated with premature CAD (OR 0.45, 95%CI 0.23–0.87, $P = 0.017$), while its association with CAD in older patients did not reach statistical significance (OR 1.32, 95%CI 0.89–1.97, $P = 0.170$). After adjustment for traditional risk factors, high PLR was significantly associated with increased CAD in older patients (OR 2.22, 95%CI 1.28–3.82, $P = 0.004$) but decreased premature CAD (OR 0.31, 95%CI 0.11–0.87, $P = 0.026$).

*Conclusions:* There is an age-related effect on the correlation between PLR and CAD. While high PLR was an independent marker of CAD in older high-risk patients, it was negatively correlated with premature CAD in younger patients. PLR is widely available and inexpensive, and could be used in highlighting patients at high risk for CAD.



## 1. Introduction

Morbidity and mortality from cardiovascular disease (CVD) are increasing worldwide due to population growth and ageing [1]. Coronary artery disease (CAD) is the most common form of CVD and a major global health problem which imposes great economic burden. Appropriate CVD risk stratification is important for clinical decision-making to balance costs and risks and benefits of therapeutic approaches. Different biomarkers coordinate the progression of atherosclerosis to complex plaque formation and rupture, and may reflect early physiological and morphological transformation prior to disease manifestation. Biomarkers of CAD offer a window of opportunity to prevent disease progression and complications with effective treatments available.

Thrombocytosis and inflammation are two vital elements in the pathophysiology of atherosclerosis that lies beneath CAD [2]. Several biomarkers of inflammation and thrombocytosis have been associated with CAD. High white cell count has been associated with poor outcomes in ischemic heart disease [3], with increased neutrophil and decreased lymphocyte count holding greatest prognostic value [4]. Lymphopenia is suggested to be a result of lymphocyte apoptosis following chronic inflammation in CAD [5]. Low lymphocyte counts were also associated with higher mortality in patients with stable CAD [6] as well as following acute coronary events [7].

The critical role of platelets activation in the pathogenesis of atherosclerosis and its clinical consequences, CAD and acute myocardial infarction (AMI), have been well documented [8]. In cases of long

*Abbreviations:* CVD, cardiovascular disease; CAD, coronary artery disease; ACS, acute coronary syndrome; PLR, platelet-to-lymphocyte ratio; eGFR MDRD, estimated glomerular filtration rate by the Modification of Diet in Renal Disease formula; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; BP, blood pressure; BMI, body mass index; IQR, interquartile range; STEMI, ST-elevated myocardial infarction; NSTEMI, non ST-elevated myocardial infarction; AMI, acute myocardial infarction; CRP, C-reactive protein; Treg, FOXP3 + T regulatory cells; PVD, peripheral vascular disease.

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standing inflammation as in atherosclerosis, elevated platelet count occurs due to increased proliferation in megakaryocytic series [9]. High baseline platelet count has been associated with poor prognosis including thrombotic complications [10] and increased cardiovascular mortality [11,12]. Despite the reported associations of high platelet counts with CAD and cardiovascular mortality, platelet counts typically decrease with age [13].

With the growing recognition that low lymphocyte and high platelet counts reflect progression of atherosclerosis and its sequelae, the platelet-to-lymphocyte ratio (PLR) has emerged as a promising biomarker and prognosticator of CAD. PLR is a widely available biomarker that may be useful in cardiovascular risk stratification, and is inexpensive. This novel marker combines thrombosis and inflammatory parameters and may be more valuable than either the platelet or lymphocyte counts alone in predicting atherosclerotic plaque burden and cardiovascular outcomes.

Despite promising evidence on PLR as a novel biomarker of CVD [5,9,14–22], current data are inadequate for its use clinically. Few studies have examined PLR and its relationship to demonstrated CAD on coronary angiography. Additionally, despite premature CAD accounting for a growing proportion of sudden death in young people, there are limited studies investigating variations in the inflammatory characteristics of CAD in different age groups. Younger patients have been underrepresented in CAD biomarker studies, and the role of PLR has not previously been characterized in premature CAD. This study assessed the relationship between PLR and CAD and hypothesized that PLR correlates with angiographically demonstrated CAD in high-risk patients, in an age-dependent manner.

## 2. Methods

### 2.1. Participants

In this single-center cross-sectional study, we collected clinical, angiographic and hematological data for consecutive patients undergoing coronary angiography at St. Vincent's Hospital, Melbourne, a university-affiliated tertiary hospital, between October 2009 and June 2013. These patients were prospectively enrolled in the BRAVEHEART and MINACS databases. Exclusion criteria were history of malignancy excluding non-melanoma skin cancer (n = 26), active hematological diseases (n = 1), current hepatitis or liver function test parameters >5 times the upper limit of normal (n = 7), end stage renal failure (n = 4), active infection (n = 19), chronic inflammatory or autoimmune diseases (n = 43), systemic anti-inflammatory drug usage (n = 9), and cardiogenic shock on admission (n = 1). For repeated enrolments, only the first observation was used to preserve the assumption of independence of observations.

### 2.2. Procedures

Coronary angiography was performed on all patients and reviewed by one to two experienced interventional cardiologists. CAD was defined as stenosis of 50% or more of the vessel diameter of any main coronary arteries of >2 mm in diameter or a history of previous percutaneous coronary intervention (PCI) for a diseased coronary vessel, according to the American College of Cardiology/American Heart Association (ACC/AHA) lesion classifications [23]. AMI was defined as the clinical presentations of ischemia along with the elevation in Troponin I and ischemic-type ECG changes, unless a different diagnosis was more likely.

Hypertension was defined as repeated blood pressure (BP) measurements with systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg, or previously diagnosed hypertension. Pulse pressure was calculated as the difference between systolic and diastolic BP. Diabetes mellitus was defined as fasting blood glucose ≥7.0 mmol/L on multiple measurements or the use of anti-diabetic medication. Patients were considered to have a positive history of smoking if they were current smokers or ex-smokers. Central obesity was defined as either elevated body mass index (BMI) ≥30 kg/m$^2$ or elevated waist circumference (≥102 cm in men and ≥88 cm in women) by the International Diabetes Federation's definition [24].

Venous blood samples were taken when patients initially presented to the emergency department or the coronary care unit prior to angiography. Complete blood counts were obtained from an ethylenediaminetetraacetate (ETDA) tube by an automated cell analyzer. Lipid studies, liver function tests, urea, electrolytes and creatinine were automatically analyzed from a serum separator tube from the same venipuncture. PLR was calculated by dividing platelet count by lymphocyte count.

The study population was stratified into a younger group, aged < 55 years old (n = 197), and an older group, aged 55 years old and over (n = 625). Patients were divided into tertiles based on admission or pre-procedural PLR values. A high and low PLR group was defined as patients having values in the highest tertile (≥146.7) and the lower two tertiles (<146.7) respectively.

The study complied with the Declaration of Helsinki and the protocol was approved by the Human Research Ethics Committee of St. Vincent's Hospital, Melbourne. All participants provided written informed consent prior to participation.

### 2.3. Statistical methods

Statistical analyses were performed using STATA version 13.1 (StataCorp, Texas, USA). Continuous data were expressed as mean ± standard deviation if they were normally distributed or as median and interquartile range (IQR) for non-normally distributed data. Categorical data were presented as number of patients and percentage. Differences in baseline characteristics between the two age groups were evaluated using the Wilcoxon rank-sum test for continuous variables, and the Chi-square test or Fisher's exact test for categorical variables. Univariate and multivariate logistic regression analyses assessed PLR as an independent predictor of angiographically demonstrated CAD. The Kolmogorov-Smirnov test was used to assess the normality of distribution for continuous variables. Some variables were log-transformed to attain a normal distribution required for logistic regression. HbA1c was divided into quartiles. Important traditional cardiovascular risk factors were included in the multivariate logistic models; sex, central obesity, hypertension, lipid profiles, history of smoking, HbA1C, eGFR MDRD, aspirin use, and AMI presentation. Missing data were excluded from the adjusted analyses and a two-tailed $P$-value < 0.05 was considered statistically significant.

## 3. Results

The final analysis included 822 patients; 214 patients had normal coronary arteries on angiography and 608 had demonstrated CAD. 70% were male and the median age was 64 [55-71]. 197 patients were aged under 55 (CAD n = 127; control n = 70) and 625 patients were aged 55 or above (CAD n = 481; control n = 144).

### 3.1. Baseline characteristics

Baseline characteristics of patients are outlined in Table 1. The two age groups were comparable in terms of sex distribution, obesity status and dyslipidemia. Compared with younger patients, older patients had increased prevalence of hypertension, widened pulse pressure, diabetes and raised HbA1c. There was a significantly higher use of statins and angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB) in the older group. Among hematological and biochemical parameters, low platelet count, high creatinine, high total triglyceride and low HDL-C were associated with CAD in younger patients. In older patients, reduced lymphocyte count, high creatinine, high urea, low HDL-C, and low LDL-C were associated with CAD. History of smoking was associated with CAD in both groups of patients. In younger patients, those with CAD had a significantly lower median [IQR] admission PLR (112.9 [92.3–135.2]) than those with normal coronary arteries (126.3 [106.3–150.0]) ($P = 0.022$). In contrast, there was no significant difference in median [IQR] admission PLR between older patients with CAD (129.1 [97.1–159.2]) compared with the control group (122.3 [98.3–151.2]) ($P = 0.350$).

History of PVD and stroke were evaluated as possible confounders of PLR. However, the levels of PLR were not statistically different in patients with and without PVD (age < 55, $P = 0.092$; age ≥ 55, $P = 0.487$) or history of stroke (age < 55, $P = 0.553$; age ≥ 55, $P = 0.643$), and the presence of these were not included as covariates in the final regression models.

### 3.2. Patients under 55 years old

Univariate logistic regression analyses between CAD and multiple parameters were performed (Table 2). On univariate logistic regression, high PLR showed significant negative association with premature CAD (OR 0.45, 95% CI 0.23–0.87, $P = 0.017$). Male sex, hypertension, positive smoking history, HDL-C, LDL-C, triglyceride, total cholesterol and HbA1c were also significantly associated with premature CAD. Independent associations between the presence of CAD and PLR were assessed using multivariate logistic regression models adjusting for traditional cardiac risk factors and AMI presentation (Table 3). On multivariate regression,

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