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Clinical and Laboratory Factors Associated with Shear-dependent Platelet Hyper-reactivity in Patients on Chronic Aspirin Therapy

Susanna M. Nazarian^b, Jason B. Thompson^a, Tyler J. Gluckman^a, Katherine Laws^a, Jayesh T. Jani^c, Thomas S. Kickler^c, and Jeffrey J. Rade^a

^aDepartment of Medicine, Baltimore, MD, USA.

^bDepartment of Surgery, Baltimore, MD, USA.

^cDepartment of Pathology of the Johns Hopkins School of Medicine, Baltimore, MD, USA.

Keywords

platelet hyper-reactivity; PFA-100; coronary artery disease; vonWillebrand factor

Introduction

The Platelet Function Analyzer-100[®] (PFA-100) assesses agonist-induced platelet aggregation under high shear by measuring the closure time (CT) of a membrane aperture by the formation of a platelet plug [1]. The two agonist cartridges currently available are designed to screen patients for defects in primary hemostasis: The collagen/epinephrine (CEPI) agonist cartridge detects the antiplatelet effects of aspirin and the collagen/ADP (CADP) agonist cartridge, which is not influenced by aspirin, is useful in identifying patients with acquired and congenital platelet disorders such as von Willebrand disease [2]. With both agonist cartridges, prolongation of the CT beyond the normal range indicates inhibited or impaired platelet function. Recent evidence also suggests that the PFA-100 has utility in identifying patients with platelet hyper-reactivity, indicated by an abnormally low CT. In patients with cardiovascular disease, a CEPI CT in the normal range despite aspirin therapy has been associated with an increased risk of recurrent cardiovascular events [3,4]. A CADP CT below the normal range, indicative of enhanced global platelet reactivity, has been observed in patients with acute coronary syndromes and found to correlate both with the degree of myocardial damage and with recurrent ischemic events [5–7].

In addition to intrinsic platelet reactivity, the PFA-100 CT is also influenced by a complex interplay of rheologic and plasma factors that support platelet aggregation. As this assay quantifies shear-dependent platelet aggregation in calcium-depleted blood samples, both CEPI and CADP CT are known to be influenced by the plasma concentration and activity of von Willebrand factor (vWF) and the citrate concentration in which the blood is collected [8–11]. Other factors that have been reported to variably influence CT include age, gender, tobacco

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Correspondence to: Jeffrey J. Rade, MD, Division of Cardiology, Johns Hopkins School of Medicine, Carnegie 568, 600 N. Wolfe St., Baltimore, MD 21287, USA. Phone: +1 410 614-1361. Fax: +1 410 614-0096. jjrade@jhmi.edu.

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use, leukocyte count, hematocrit, platelet count, non-aspirin antiplatelet therapy and inflammation [1,8,12]. Understanding the relative contribution of these variables to low CTs in patients with cardiovascular disease is difficult to ascertain, as many were assessed in isolation and often in normal patient populations.

The goal of the present study was to comprehensively investigate the relative influences of patient and pre-analytic variables on low CEPI and CADP CTs in patients with known cardiovascular disease on chronic aspirin therapy.

Materials and Methods

Subjects

This study was a prospectively planned analysis of subjects enrolled in the Reduction in Graft Occlusion Rates (RIGOR) Study, a multicenter observation study designed to identify novel risk factors for early vein graft thrombosis after coronary artery bypass (CABG) surgery [13]. All patients were provided 325 mg tablets of enteric-coated aspirin and directed to take one tablet daily until follow-up 6 months after surgery at which time vein graft patency was assessed by multidetector computed tomography coronary angiography. The dosage of aspirin was permitted to be modified at the discretion of the patient's physicians.

Blood and Urine Collection

Morning blood and urine samples were obtained from 288 fasted patients on chronic aspirin therapy a median of 189 days (interquartile range 182–202 days) after CABG surgery. Blood was drawn into siliconized glass vacutainers containing EDTA (for complete blood counts), 3.2% (0.106 mol/L) and 3.8% (0.129 mol/L) sodium citrate (for platelet function testing and measurement of fibrinogen and vWF antigen) or plain glass vacutainers (for serum CRP determination). Platelet function analyses were performed within 1 hour of blood collection after being hand-carried to the laboratory and maintained at room temperature. Aliquots of centrifuged platelet-poor plasma, serum and urine were stored at -70°C until batch analyzed.

Platelet Function and Other Analyses

Complete blood counts, reticulocyte counts and immature platelet fraction were measured with an XE-2100 automated analyzer (Sysmex Corporation, Kobe, Japan). Platelet rich plasma was prepared from blood collected in 3.2% citrate by centrifugation at 100 rpm for 10 minutes. The platelet count was adjusted to $180,000/\text{mm}^3$ by the addition of platelet-poor plasma and impedance aggregometry was performed after stimulation with 0.5 mM arachidonic acid using a Chrono-Log Model 560CA aggregometer (Chrono-Log, Havertown, PA).[14] Platelets were considered non-responsive to arachidonic acid stimulation if aggregation was ≤ 1 ohms.

Shear-dependent platelet aggregation was performed on whole blood collected in both 3.2% and 3.8% citrate using a Platelet Function Analyzer-100[®] (Siemens Healthcare Diagnostics, Newark, DE) with both CEPI and CADP agonist cartridges according to the manufacturer's instructions. For statistical analysis, samples with non-closure were assigned a CT value of 300 seconds, the maximal measurable CT by the device. Because the coefficients of variance for CEPI and CADP CT performed on quality control samples were $\leq 10\%$, a single determination was performed on experimental samples unless there was non-closure or there was a significant discrepancy between CTs in 3.2% and 3.8% citrate. In the case of repeat determinations, the average CT was used for statistical analysis. The normal ranges used by our laboratory for CEPI and CADP CTs are 94–193 and 71–118 seconds, respectively.

Plasma fibrinogen was measured by a modified Clauss method using the Multifibren U kit (Siemens) and expressed in mg/dL (coefficients of variance $<3\%$, lower limit of detection = 35

mg/dL). Plasma vWF antigen was measured by an immunoturbometric assay using the STA®-Liatest®VWF:Ag kit (Diagnostica Stago, Asnieres, France) and expressed as percent of normal control plasma (coefficients of variance <10%, lower limit of detection =5% of normal plasma). Both coagulation tests were performed on a Siemens BCS Coagulation Analyzer. Serum CRP was measured by high-sensitivity immunoturbometric assay (Tina-quant CRP (Latex); Roche Diagnostics, Mannheim, Germany) and expressed as mg/L (coefficients of variance ≤10%, lower limit of detection =0.1 mg/L). Measurement of 11-dehydro-thromboxane B₂ in urine samples was performed in duplicate by Esoterix Laboratory Services, Inc. (Austin, TX) by ELISA and expressed as pg/mg of urine creatinine (coefficients of variance ≤10%, lower limit of detection =25 pg/mL). Measurement of 8-isoprostane-prostaglandin F_{2α} in urine samples was performed in duplicate by Corgenix Medical Corporation (Broomfield, CO) by ELISA and expressed as pg/mg of urine creatinine (coefficients of variance ≤20%, lower limit of detection =2.0 pg/mL).

Statistical Analysis

Only subjects on chronic aspirin therapy with non-responsiveness to arachidonic acid-induced platelet aggregation were included in the analysis. A logistic regression model was employed to determine odds ratios for low CEPI and CADP CTs performed in both 3.2% and 3.8% citrate. A low CEPI CT was defined as ≤193 seconds based general acceptance in the literature as the lower limit of aspirin responsiveness for both 3.2% and 3.8% citrate [15]. A low CADP CT was defined as ≤71 seconds based on the lower limit of the normal range in our laboratory. Normality assumptions for categorical variables were tested using quantile-quantile plots and the Shapiro-Francia test for normality. Skewed data were log-transformed; those that remained skewed after log transformation were treated dichotomously or categorically. Category values were chosen based on the reference ranges established by the Johns Hopkins Medical Laboratory. As no normal range has been established for urine 8-iso-prostaglandin F_{2α}, the median value of 1073 pg/mg creatinine was chosen as the dichotomizing point for this variable.

Univariate logistic regression for odds of low CT was performed using clinically- and historically-relevant candidate predictors. Variables with $P \leq 0.2$ were entered into the four initial multivariate models for prediction of odds of low closure time (CEPI and CADP using both 3.2% and 3.8% citrate). Thereafter, model fit was optimized using the Akaike Information Criteria (AIC), by testing stepwise removal of each variable. Collinearity among dependent variables was considered using chi-squared tests of independence for categorical or binary covariates, or Pearson's correlation coefficients for continuous variables. Potential relations between binary and continuous explanatory variables were examined using logistic regression. For pairs of collinear variables that were non-significant when together in the model, the variable with the greatest explanatory power per AIC was retained. Random effects logistic regression models were explored for the four outcomes, by including a random intercept for surgeon or hospital or both.

All statistical analysis was performed using Stata/MP 10.0 for Macintosh (StataCorp, College Station, TX, USA). Unless otherwise specified, all tests were 2-sided with significance set at $\alpha = 0.05$.

Results

Of the 288 patients on chronic aspirin therapy in the study cohort, 285 (99%) were non-responsive to arachidonic acid-induced platelet aggregation and thus included in the analysis. Subject demographics, medications and comorbidities are shown in Table 1. Figure 1A shows the distribution of CEPI and CADP CTs for blood collected in 3.2% and 3.8% citrate. CEPI and CADP CTs were lower by an average of 44.9 and 10.3 seconds, respectively, in blood collected in 3.2% compared to 3.8% citrate, resulting in significantly higher percentages of

patients with defined low CTs (Figure 1B). The percentage of patients with both a low CEPI and CADP CT was significantly higher when measured in blood collected in 3.2% compared to 3.8% citrate (19.2% versus 2.8%, respectively, $P < 0.0001$; Figure 1C). Table 2 shows the degree of concordance between low CEPI and CADP CTs stratified by citrate concentration. Although average CTs were higher with 3.8% citrate, there were 21 instances (6 with CADP and 15 with CEPI cartridges) where patients with low CTs in 3.8% citrate did not have low CTs in 3.2% citrate. The correlation coefficients for low CTs between citrate concentrations were 0.39 and 0.57 for CEPI and CADP agonist cartridges, respectively.

Univariate logistic regression was performed to predict odds of low CEPI and CADP CTs in both 3.2% and 3.8% citrate (Table 3). An elevated vWF antigen level $>150\%$ (the upper limit of normal in our laboratory) was the only significant predictor of a low CT consistently identified in all four combinations of agonist cartridge and citrate concentration. Multivariate analysis identified several factors that differentially correlated with odds of low CADP and CEPI CTs depending on whether the blood was collected in 3.2% vs. 3.8% citrate (Tables 4 and 5). For both agonist cartridges, collection of blood in 3.8% rather than 3.2% citrate was associated with fewer variables correlating with low CT. A vWF antigen level $>150\%$ was the only significant predictor of low CEPI and CADP CT in blood samples collected in 3.8% citrate.

Discussion

The major findings of this study are: 1) Platelet hyper-reactivity, defined by a low CEPI and/or CADP CT, occurred frequently in patients taking aspirin 6 months after CABG surgery; 2) Collection of blood in 3.2% compared to 3.8% citrate significantly increased the prevalence of a low CADP and/or CEPI CT, and; 3) In blood collected in 3.8% citrate, the only variable significantly associated with low CEPI and CADP CTs was an elevated vWF antigen level, whereas in blood collected in 3.2% citrate, multiple patient-specific variables were differentially associated with low CTs.

PFA-100 CTs have long been recognized to be higher in blood samples collected in 3.8% compared to 3.2% citrate, with greater prolongation observed for the CEPI than the CADP agonist cartridge [10,11]. Our findings confirm and extend these earlier observations by demonstrating that at the lower citrate concentration, multiple patient-specific and extrinsic platelet factors appear to differentially influence low CTs, while at the higher citrate concentration, an elevated vWF level was the only extrinsic platelet factor associated with a low CEPI and CADP CT. This finding may be explained by a greater degree of calcium chelation achieved with 3.8% compared to 3.2% citrate. The concentration of extracellular ionized calcium is recognized to modulate platelet reactivity with a particular effect on the interaction between platelets and vWF. For example, vWF potentiates ADP-induced platelet aggregation at low, but not at physiologic, levels of extracellular calcium [16]. Platelet attachment to immobilized vWF under high shear is also enhanced by the addition of a metal ion chelator, an effect thought to be due to reduced calcium-dependent ADAMTS13 activity [17].

In patients on aspirin therapy, a low CEPI CT has been frequently used to define those that are aspirin resistant [15]. The major physiologic effect of aspirin on platelets is to irreversibly inhibit the COX-1 enzyme. All patients included in our analysis had documented inhibition of arachidonic acid-induced platelet aggregation, indicative of suppressed platelet COX-1 activity. Despite this, a relatively high proportion of patients had low CEPI CTs (39.1% and 13.5%, when assayed in blood collected in 3.2% and 3.8% citrate, respectively). Several prior studies have demonstrated that the incidence of aspirin resistance is much higher when defined by a low CEPI CT than when defined by suppressed arachidonic acid-induced platelet

aggregation [9,18]. This suggests that factors other than incomplete platelet COX-1 inhibition may contribute to a low CEPI CT. By restricting our analysis only to patients non-responsive to arachidonic acid-induced platelet aggregation, we convincingly demonstrate that factors other than the failure of aspirin to inhibit the platelet COX-1 enzyme are associated with a low CEPI CT in the vast majority of patients on chronic aspirin therapy. This finding calls into question the specificity of the CEPI cartridge for assessing aspirin resistance.

Our results are also notable for what was not found to be associated with low CTs. Previous studies found that changes in the platelet count and hematocrit alter PFA-100 CT, with the manufacturer recommending against its use in patients with $<150 \times 10^3$ platelets/cubic mm or with a hematocrit $<35\%$ [1]. We did not find platelet count to be a significant effector of low CT for any agonist cartridge/citrate concentration combination on multivariate analysis. Although we found no association between hematocrit and low CT for CEPI and CADP agonist cartridges in blood collected in 3.8% citrate, we did find that an increasing hematocrit was associated with a lower CT for both cartridges in blood collected in 3.2% citrate. Prior studies have also described prolonged CEPI and CADP CTs for patients with blood group type O, perhaps due to the higher levels of vWF associated with this blood group [8,19]. We only found blood group type O to be associated with smaller odds of low CTs for CEPI/3.2% citrate combination.

A weakness of our study lies with the discordance revealed by comparing the numbers of patients identified as having low CTs at either citrate concentration (Table 2). While 3.8% citrate is generally felt to prolong the CT, 21 patients paradoxically had low CTs in blood collected in 3.8% citrate but high CTs for blood collected in 3.2% citrate. This unexpected occurrence of higher CTs in samples anticoagulated with 3.2% citrate raises concerns over the internal validity of the PFA-100 assay, concerns that have been echoed by previous investigators [8,20].

In summary, patient-specific variables differentially influence the incidence of low CEPI and CADP CTs in patients with cardiovascular disease on chronic aspirin therapy depending on the citrate concentration into which the blood is collected. Collection of blood in 3.8% citrate is associated with fewer variables associated with low CT for either agonist cartridge than blood collected in 3.2% citrate. These findings may have implications for the design and interpretation of clinical studies correlating low PFA-100 CT and adverse outcome.

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References

1. Hayward CP, Harrison P, Cattaneo M, Ortel TL, Rao AK. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemost* 2006;4:312–319. [PubMed: 16420557]
2. Favaloro EJ. Clinical application of the PFA-100. *Curr Opin Hematol* 2002;9:407–415. [PubMed: 12172459]
3. Reny JL, de Moerloose P, Dauzat M, Fontana P. Use of the PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a systematic review and meta-analysis. *J Thromb Haemost* 2008;6:444–450. [PubMed: 18194417]

4. Crescente M, Di Castelnuovo A, Iacoviello L, De Gaetano G, Cerletti C. PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a meta-analysis of 19 studies comprising 3,003 patients. *Thromb Haemost* 2008;99:1129–1131. [PubMed: 18521524]
5. Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H, et al. Platelet function predicts myocardial damage in patients with acute myocardial infarction. *Circulation* 2004;110:1392–1397. [PubMed: 15313953]
6. Harrison P, Mackie I, Mathur A, Robinson MS, Hong Y, Erusalimsky JD, et al. Platelet hyper-function in acute coronary syndromes. *Blood Coagul Fibrinolysis* 2005;16:557–562. [PubMed: 16269928]
7. Fuchs I, Frossard M, Spiel A, Riedmuller E, Laggner AN, Jilma B. Platelet function in patients with acute coronary syndrome (ACS) predicts recurrent ACS. *J Thromb Haemost* 2006;4:2547–2552. [PubMed: 17002662]
8. Haubelt H, Anders C, Vogt A, Hoerd P, Seyfert UT, Hellstern P. Variables influencing Platelet Function Analyzer-100 closure times in healthy individuals. *Br J Haematol* 2005;130:759–767. [PubMed: 16115134]
9. Chakroun T, Gerotziakas G, Robert F, Lecrubier C, Samama MM, Hatmi M, et al. In vitro aspirin resistance detected by PFA-100 closure time: pivotal role of plasma von Willebrand factor. *Br J Haematol* 2004;124:80–85. [PubMed: 14675411]
10. von Pape KW, Aland E, Bohner J. Platelet function analysis with PFA-100 in patients medicated with acetylsalicylic acid strongly depends on concentration of sodium citrate used for anticoagulation of blood sample. *Thromb Res* 2000;98:295–299. [PubMed: 10822076]
11. Heilmann EJ, Kundu SK, Sio R, Garcia C, Gomez R, Christie DJ. Comparison of four commercial citrate blood collection systems for platelet function analysis by the PFA-100 system. *Thromb Res* 1997;87:159–164. [PubMed: 9253811]
12. Favaloro EJ. Utility of the PFA-100 for assessing bleeding disorders and monitoring therapy: a review of analytical variables, benefits and limitations. *Haemophilia* 2001;7:170–179. [PubMed: 11260277]
13. Gluckman TJ, Segal JB, Schulman SP, Shapiro EP, Kickler TS, Prechel MM, et al. Effect of anti-platelet factor-4/heparin antibody induction on early saphenous vein graft occlusion after coronary artery bypass surgery. In Review. 2009
14. Clinical and Laboratory Standards Institute. Platelet Function Testing by Aggregometry: Approved Guideline. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2008. p. 17-19.H58-A[28 (31)]
15. Crescente M, Di Castelnuovo A, Iacoviello L, Vermeylen J, Cerletti C, De Gaetano G. Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100. A systematic review. *Thromb Haemost* 2008;99:14–26. [PubMed: 18217130]
16. Harfenist EJ, Packham MA, Kinlough-Rathbone RL, Cattaneo M, Mustard JF. Effect of calcium ion concentration on the ability of fibrinogen and von Willebrand factor to support the ADP-induced aggregation of human platelets. *Blood* 1987;70:827–827. [PubMed: 3497679]
17. Donadelli R, Orje JN, Capoferri C, Remuzzi G, Ruggeri ZM. Size regulation of von Willebrand factor-mediated platelet thrombi by ADAMTS13 in flowing blood. *Blood* 2006;107:1943–1950. [PubMed: 16293606]
18. Gurbel PA, Bliden KP, DiChiara J, Newcomer J, Weng W, Neerchal NK, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. *Circulation* 2007;115:3156–3164. [PubMed: 17562955]
19. Jilma-Stohlawetz P, Hergovich N, Homoncik M, Dzirlo L, Horvath M, Janisiw M, et al. Impaired platelet function among platelet donors. *Thromb Haemost* 2001;86:880–886. [PubMed: 11583322]
20. Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. *J Lab Clin Med* 2001;138:152–163. [PubMed: 11528368]

Figure 1.

A. CTs for CEPI and CADP agonist cartridges stratified by citrate concentration. Median values with interquartile ranges (boxes), 5% and 95% confidence intervals (bars) and individual outliers are shown. B. Percent of patients with low CTs by CEPI, CADP and both agonist cartridges stratified by citrate concentration.

Table 1

Patient baseline characteristics (n=285).

Characteristic	Number
Age, years (mean $\pm$ SD)	63.1 $\pm$ 10.0
Male Gender	225 (79%)
Body Mass Index, kg/m ² (mean $\pm$ SD)	29.8 $\pm$ 6.2
Race	
White	247 (87%)
African American	28 (10%)
Asian American	8 (3%)
Hispanic Ethnicity	8 (3%)
Diabetes	100 (35%)
Hypertension	51 (18%)
Dyslipidemia	240 (84%)
Tobacco Use	69 (24%)
Myocardial Infarction	117 (41%)
Cerebral Vascular Disease	23 (8%)
Aspirin dose	
81 mg	28 (10%)
325 mg	257 (90%)
Clopidogrel	32 (11%)
Warfarin	14 (5%)

Table 2

Concordance of low CTs by cartridge and citrate concentration.

	CEPI CT ≤193 seconds 3.2% citrate	CEPI CT >193 seconds 3.2% citrate
CEPI CT ≤193 seconds 3.8% citrate	32	6
CEPI CT >193 seconds 3.8% citrate	79	163

	CADP CT ≤71 seconds 3.2% citrate	CADP CT >71 seconds 3.2% citrate
CADP CT ≤71 seconds 3.8% citrate	35	15
CADP CT >71 seconds 3.8% citrate	55	178

Table 3

Univariate analysis of potential factors associated with low CEPI and CADP CT.

	CEPI			CADP		
	3.2% Citrate	3.8% Citrate	3.2% Citrate	3.8% Citrate	3.8% Citrate	3.8% Citrate
	Odds Ratio	P-value	Odds Ratio	P-value	Odds Ratio	P-value
Gender (women vs. men)	0.68	0.207	1.00	0.993	0.66	0.212
Age (years)	1.00	0.693	1.00	0.933	1.00	0.742
Race (white vs. non-white)	1.56	0.250	1.29	0.651	3.49	0.012
Ethnicity (hispanic vs. nonhispanic)	0.92	0.913	2.19	0.347	0.30	0.264
Aspirin dose (325 mg vs. 81 mg)	1.00	0.993	0.53	0.203	0.42	0.032
Clopidogrel use	1.22	0.602	1.96	0.151	1.33	0.463
Warfarin use	1.16	0.784	1.07	0.932	4.20	0.012
WBC (≤ 4.5 vs. $4.5-1110^3/\text{mm}^3$)*	0.47	0.060	0.55	0.337	0.56	0.173
WBC (> 11 vs. $4.5-1110^3/\text{mm}^3$)*	3.63	0.128	2.47	0.291	1.53	0.585
Hematocrit (%)	1.10	0.009	1.03	0.567	1.11	0.007
MCV (< 80 vs. $80-100$ fL)	0.40	0.164	1.08	0.921	0.16	0.077
MCV (> 100 vs. $80-100$ fL)	0.36	0.370	1.62	0.670	1.36	0.736
RDW ($> 14.5\%$ vs. $\leq 14.5\%$)	0.72	0.199	1.56	0.207	0.96	0.887
Reticulocyte count (natural logtransformed %)	0.86	0.701	1.10	0.858	1.15	0.728
Platelet Count (≥ 150 vs. $< 150 \times 10^3/\text{mm}^3$)	1.58	0.140	1.93	0.192	1.55	0.181
MPV (natural log-transformed fL)	14.30	0.080	5.65	0.414	6.04	0.254
IPF (natural log-transformed %)	1.48	0.118	1.22	0.580	1.02	0.938
Blood group (O vs. non-O)	0.67	0.106	0.71	0.345	0.53	0.016
Rh factor (positive vs. negative)	1.06	0.872	1.65	0.428	0.84	0.641
CRP (< 5 vs. ≥ 5 mg/L)	0.84	0.551	1.16	0.771	1.34	0.314
Fibrinogen (> 450 vs. ≤ 450 mg/dL)	1.24	0.404	0.88	0.741	1.58	0.086
vWF antigen (> 150 vs. $\leq 150\%$)	1.85	0.014	4.14	0.001	3.11	0.000
Urine 11-dehydro-TXB ₂ (≥ 400 vs. < 400 pg/mg creatinine)	2.05	0.005	1.77	0.108	1.55	0.099
Urine 8-iso-PGF _{2a} (≥ 1072 vs. < 1072 pg/mg creatinine)	1.37	0.228	1.18	0.678	1.64	0.076

Variables included in subsequent multivariate models are italicized.

WBC = white blood cell count; MCV = mean corpuscular volume; RDW = red cell distribution width; MPV = mean platelet volume; IPF = immature platelet fraction; vWF = von Willebrand factor; 11-dehydro-TXB₂ = 11-dehydrothromboxane B₂; 8-iso-PGF₂ α = 8-isoprostane-prostaglandin F₂ α .

Table 4

Multivariate analysis of factors associated with low CEPI CT*

	3.2% Citrate			3.8% Citrate	
	<u>Odds Ratio</u>	<u>CI</u>	<u>P-value</u>	<u>CI</u>	<u>P-value</u>
vWF antigen (>150 vs. ≤150%)	NI	NI	NI	[1.75, 9.82]	0.000
Blood group (O vs. non-O)	0.53	[0.30, 0.94]	0.030	NI	NI
Hematocrit (%)	1.10	[1.01, 1.20]	0.036	NI	NI
IPF (natural log-transformed %)	1.68	[0.97, 2.92]	0.064	NI	NI
Urine 11-dehydro-TXB ₂ (≥400 vs. <400 pg/mg creatinine)	1.66	[0.92, 3.00]	0.093	NI	NI

* Shown in descending order of strength of association assessed by t-statistic and importance to model fit.

NI = not significant in univariate analysis and/or not included in the optimized model; vWF = von Willebrand factor; IPF = immature platelet fraction; 11-dehydro-TXB₂ = 11-dehydro-thromboxane B₂; CI = 95% confidence intervals.

Table 5

Multivariate analysis of factors associated with low CADP CT*

	3.2% Citrate			3.8% Citrate		
	<u>Odds Ratio</u>	<u>CI</u>	<u>P-value</u>	<u>Odds Ratio</u>	<u>CI</u>	<u>P-value</u>
vWF antigen (>150 vs. ≤150%)	2.99	[1.59, 5.60]	0.001	2.56	[1.19, 5.51]	0.016
Race (white vs. non-white)	3.69	[1.24, 11.03]	0.019	NI	NIM	NI
Warfarin use	6.11	[1.22, 30.66]	0.028	2.90	[0.81, 10.43]	0.103
Hematocrit (%)	1.11	[1.01, 1.23]	0.029	NI	NIM	NI
Urine 8-iso-PGF _{2α} (≥1072 vs. <1072 pg/mg creatinine)	1.79	[0.97, 3.32]	0.062	NI	NIM	NI
Platelet Count (≥150 vs. <150 × 10 ³ /mm ³)	2.03	[0.96, 4.31]	0.065	NI	NIM	NI
Gender (women vs. men)	0.52	[0.22, 1.23]	0.137	NI	NIM	NI
Blood group (O vs. non-O)	NI	NI	NI	0.55	[0.26, 1.14]	0.108

* Shown in descending order of strength of association assessed by t-statistic and importance to model fit.

NI = not significant in univariate analysis and/or not included in the optimized model; vWF = von Willebrand factor; 8-iso-PGF_{2α} = 8-isoprostane-prostaglandin F_{2α}; CI = 95% confidence intervals.