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Comparison of Platelet Activity Measurements using Arterial and Venous Blood Sampling

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Pathological and clinical studies consistently demonstrate that platelets play a significant role in the pathophysiology of atherothrombosis, and measurement of platelet activity can identify individuals at increased cardiovascular risk. While light transmission aggregation is the historical "gold standard" for platelet activity measurement, the large sample volume and significant sampling time preclude this measurement from being widely used. Other more convenient markers of platelet activity are increasingly being investigated as data demonstrating their association with clinical outcomes emerge [1]. Monocyte (MPA) and leukocyte platelet aggregates (LPA) are robust markers of platelet adhesion and activation measured by flow cytometry, the advantages of which include the use of low sample volumes, standardization of technique, and allowance for whole blood to be immediately fixed and processed at a more suitable time [2–3]. Mean platelet volume (MPV), measured on routine automated hemograms, reflects platelet size. Larger platelets are metabolically and enzymatically more active, with greater prothrombotic potential, and associated with clinical outcomes [4–5]. Finally, soluble markers of activation, such as p-selectin, can be evaluated anytime from stored frozen plasma [6]. Collectively, these data make it pertinent for us to understand these markers of platelet activity, including the effect of blood source sampling on their measurements. In this study, we aim to assess the relationship between arterial and venous sources of different markers of platelet activity.

Patients in this study were part of the platelet substudy of a randomized trial evaluating effects of glucose-lowering medications in patients with diabetes mellitus, which included non-diabetic controls [7]. All patients underwent coronary angiography at the Manhattan

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Addendum

B. Shah contributed to study design and concept, analysis of data and interpretation of data, critical writing, and final approval of the version submitted for publication. S. P. Sedlis and J. Berger contributed to study design and concept, interpretation of data, critical revision of intellectual content, and final approval of the version submitted for publication. X. Mai, N. S. Amoroso, and J. Lorin contributed to collection and interpretation of data, critical revision of intellectual content, and final approval of the version submitted for publication. Y. Guo contributed to analysis and interpretation of data, critical revision of intellectual content, and final approval of the version submitted for publication. J. Berger and B. Shah had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure of Conflict of Interests

The authors state that they have no conflicts of interest.

Veterans Affairs Hospital. Of the 75 patients enrolled in this substudy measuring markers of platelet activity on procedural access, 70 had simultaneous measurements from arterial and venous blood sources. All patients signed informed consent, and the institutional review board approved the study.

At the time of coronary angiography, blood samples were collected after an initial 2cc discard simultaneously from the antecubital vein using a minimum 21-guage needle and the radial (n=7) or femoral (n=63) artery using a minimum 5 french sheath. Blood was collected in a 7.2mg K2 ethylenediaminetetraacetic (EDTA) acid tube (BD Vacutainer 4.0mL, BD Franklin Lakes, NJ, USA) and processed within 60 minutes on a Sysmex XE-2100 hematology analyzer (Mundelein, Illinois, USA) for platelet count (reported coefficient of variation (CV) <4.0%), MPV (CV <3.0%), and immature platelet fraction (IPF) (CV not reported) measurements. Additional blood was collected in a 3.2% (0.109 moles/L) sodium citrate tube (BD Vacutainer 2.7mL, BD Franklin Lakes, NJ, USA) and processed within 25 minutes for measurement of MPA, LPA, and sP-selectin (sP-selectin) levels. MPA and LPA were measured via an Accuri C6 flow cytometer using directly conjugated CD14-PE or CD45-PE and CD42a-FITC antibodies. Citrate-anticoagulated blood was centrifuged at 8°C for 10 min at 2500×g, plasma was stored at −80°C, and measurements of sP-selectin were made using commercial enzyme-linked immunosorbent assay (ELISA) (eBioscience, CV 7.8%).

Measures of platelet activity are presented as median [interquartile range] given their skewed distribution (Shapiro-Wilk). Reproducibility and agreement of each platelet measure between the arterial and venous source was assessed by intraclass correlation coefficient (ICC) and the Bland-Altman analysis of agreement, respectively. ICC was defined as a variance ratio $\left(\rho=\frac{{\sigma_b}^2}{{\sigma_b}^2+{\sigma_w}^2}\right)$, the proportion of between subject variation, which was estimated using the differences of between subject variance and within subject variance over the total variation $\left(\frac{{S_b}^2-{S_w}^2}{{S_b}^2+{S_w}^2}\right)$, where S_b^2 represents the between subject variance and S_w^2 represents within subjects variance [8]. The corresponding variance components were estimated in the framework of one-way ANOVA model. Plots are presented with 95% limits of agreement (precision ± 2 standard deviations) and bias (mean difference) [9]. Correlation of platelet measures between the arterial and venous source was performed using the Spearman's test [10]. Statistical analysis was conducted using the R program for Scientific Computing (available at www.r-project.org).

The median age (n=70) was 65 years (interquartile range 63−72), 99% male, and 80% White. Medical history was significant for known coronary artery disease in 59%, diabetes mellitus in 69%, peripheral artery disease in 14%, and tobacco use in 76%. Measures of platelet activity using an arterial versus venous blood source were reproducible by varying degrees (Table 1). The limits of agreement for arterial versus venous measurement of MPV, IPF, MPA, LPA, and sP-selectin were 0.38 fL to −0.59 fL, 2.0% to −2.39%, −19.0% to −22.2%, 5.7% to −6.5%, and 36.5 ng/mL to −56.6 ng/mL, respectively (Figure S1). The bias for the hematology analysis markers ranged from −0.11 to −0.17, the flow cytometric markers ranged from −0.4% to −1.6%, and sP-selectin was −10.1 ng/mL.

Platelet activity is increasingly investigated as a surrogate end-point in the setting of percutaneous coronary intervention (PCI) [11]. Arterial access obtained during PCI provides for a convenient source of blood sampling, whereas venous sampling is more convenient outside the procedural time period. Therefore, when there is a need to compare time-related changes in platelet activity in the PCI population, it is necessary to understand whether venous samples obtained pre- or post-procedure can be compared with arterial samples

obtained during the procedure. In the current study, markers of platelet size and activity measured on an automated hematology analyzer and using flow cytometry demonstrated excellent to good agreement between arterial and venous samples, while plasma markers measured by ELISA less so.

Jaumdally and colleagues demonstrated no difference in sP-selectin levels from the coronary ostium, aorta, coronary sinus and right femoral vein [12]. They did note a difference in MPV measurements between the coronary ostium and coronary sinus or femoral vein. However, the flow patterns between the aorta and the coronary ostium are markedly different, and the difference in MPV measurements was attenuated when those from the aorta and femoral vein were compared. Furthermore, the study did not provide measures of reliability. Similarly, Chen and colleagues demonstrated significantly higher levels of sP-selectin in the left atrium of 16 patients compared with levels from the right atrium, femoral vein and femoral artery, but similar levels between the femoral vein and artery [13]. In both studies, arterial and venous samples were collected from introducer sheaths, whereas in our study, venous samples were collected from the antecubital vein, a common source of blood sampling post-procedure. Some of the limitations of the current study include the predominately male cohort and small sample size, although this cohort represents one of the larger studies published and is one of the very few studies to evaluate the agreement and correlation between samples obtained via an arterial versus venous blood source.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Markers of platelet activity measured using arterial and venous blood sources

	Source of blood		ICC**	95%CI	Spearman's rho	p-value
	Artery*	Vein*				
Hematology analysis						
Platelet count, × 10^9/L	172 [144–225]	175 [142–225]	0.95	0.92–0.97	0.92	<0.001
MPV, fL	10.4 [9.8–11.0]	10.6 [10.0–11.0]	0.98	0.97–0.99	0.95	<0.001
IPF, %	4.7 [3.1–6.1]	4.9 [3.1–6.0]	0.93	0.89–0.96	0.89	<0.001
Flow cytometric markers						
MPA, %	18.1 [13.9–24.4]	19.9[12.3–31.2]	0.71	0.57–0.81	0.65	<0.001
LPA, %	9.0[7.2–12.2]	10.0[7.1–12.2]	0.76	0.64–0.84	0.75	<0.001
Plasma markers						
p-selectin, ng/mL	61.4 [47.3–76.1]	69.3[56.3–89.5]	0.57	0.39–0.71	0.68	<0.001

*data presented as median [interquartile range]

**all p-values <0.001

CI = confidence interval, ICC = intraclass correlation coefficient, IPF = immature platelet fraction, LPA = leukocyte platelet aggregation, MPA = monocyte platelet aggregation, MPV = mean platelet volume

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