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A comparison of five platelet reactivity tests in over 3,000 participants of the Framingham Heart Study

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INTRODUCTION

- Extensive platelet reactivity testing requires dedicated equipment, personnel and time.
- As a result, large studies are rarely conducted with the largest limited to platelet rich plasma (PRP) light transmission aggregometry in a small range of agonists[1]
- Even fewer studies compare platelet assays.

AIM

To identify correlations between platelet function assays performed in the FHS.

METHOD

Sample population. Informed consent was obtained from FHS generation 3, exam 3 participants (N=3,140, 46.4% male, 54.5±9.0 years, European ancestry). The study was approved by the Boston University (BU) Medical Center IRB.

Blood collection. Fasting blood was drawn into citrate and hirudin vacutainers. PRP and platelet poor plasma were obtained by centrifugation following ISTH guidelines. Up to 5 platelet function assays were performed.

Multiplate. Impedence aggregometry (DiaPharma/Roche) was performed in whole blood (WB) stimulated with arachidonic acid (AA; 0.5mM), ADP (3.19µM), collagen (0.061mg/mL), ristocetin (1.15mg/mL) and TRAP-6 amide (4.48µM).

Total Thrombus formation Analysis System (T-TAS). WB was run through collagen-coated PL chips at 1500s^{-1} shear.

Flow cytometry. Leukocyte (CD45, CD14) and platelet (CD61) counts were performed on an Accuri C6 (BD Biosciences). WB and PRP were stimulated with ADP (20µM) and CD61, CD-62P (p-selectin), PAC-1 binding (activated integrin αIIbβ3) and CD63 (granule release)-positive events were measured in 10,000 platelets

Light transmission aggregometry (LTA). PRP was stimulated with AA (1.6mM), ADP (0.95, 1.82, 5.71µM), collagen (0.19mg/mL), epinephrine (100µM), ristocetin (1.5mg/mL) and TRAP-6 amide (15µM) using a PAP-8E aggregometer (BioData).

Optimul aggregometry. Optimul plates were manufactured in-house[2] and aggregation in response to AA (0.03-1mM), ADP (0.005-40µM), collagen (0.01-40µg/mL), epinephrine (0.0004-10µM), ristocetin (0.14-4mg/mL), TRAP-6 amide (0.03-40µM) and U46619 (0.005-40µM) was measured. Data was processed using the nplr package in R.

Analysis. Aspirin use was defined as AA final aggregation <40% in LTA. Platelet responses were ranked into quintiles and Cohen's Kappa (κ) test was performed to assess the correspondence between the lowest and highest responders for each assay.

RESULTS

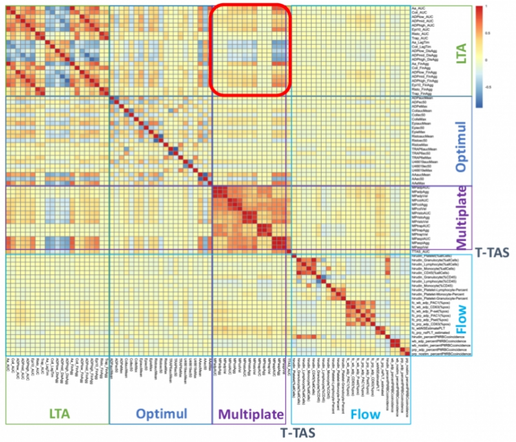

Figure 1A: A correlation matrix of the five platelet assays used in all FHS participants (N=3,410).

- Aspirin use was associated with a high correlation between AA-mediated responses in LTA and Multiplate (shown in red).
- When aspirin takers (N=681) were removed, this correlation was significantly reduced (Figure 1B).

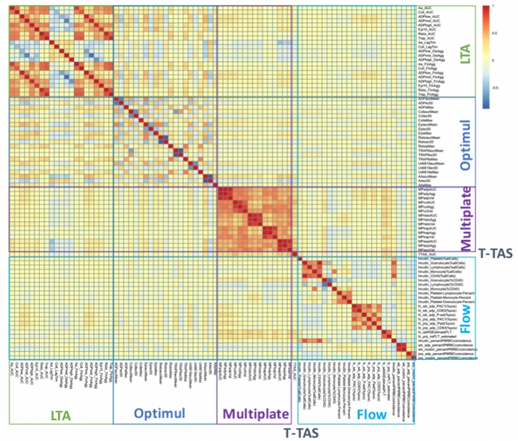

Figure 1B: A correlation matrix of the five platelet assays used in the non-aspirin taking FHS participants (N=2,459).

- There is strong correlation within assays using different agonists
- There is moderate correlation between assays (using PRP or WB) but, in general, low inter-assay correlation

Table 1: The correlation between platelet phenotypes and sex. The first 5 variables with the highest P values are presented.

Trait	Assay	r	P
ADP velocity	Multiplate	0.303	<2.22E-16
ADP AUC	Multiplate	0.281	<2.22E-16
ADP (1.82µM) AUC	LTA	0.280	<2.22E-16
Ristocetin AUC	Multiplate	0.246	<2.22E-16
ADP (1.82µM) final aggregation	LTA	0.241	<2.22E-16

- Female sex was associated with increased platelet reactivity in nearly all traits.

Note: P<2.22E-16 is the limit of the R package.

Table 2A: The correlation between the ranked top 20% responders using Cohen's Kappa test. The first 5 most correlated pairs are presented.

AssayA	AssayB	Total tests	Proportional agreement	Cohen's Kappa
Ristocetin AUC	Trap-6 amide AUC	2354	0.892	0.653
AA AUC	Ristocetin AUC	2354	0.890	0.648
AA AUC	Trap-6 amide AUC	2379	0.881	0.619
AA AUC	ADP (5.71µM) AUC	2375	0.873	0.594
ADP (5.71µM) AUC	Ristocetin AUC	2347	0.865	0.569

Table 2B: The correlation between the ranked lowest 20% responders using Cohen's Kappa test. The first 5 most correlated pairs are presented.

AssayA	AssayB	Total tests	Proportional agreement	Cohen's Kappa
Ristocetin AUC	Trap-6 amide AUC	2354	0.862	0.583
ADP (0.95µM) AUC	ADP (1.82µM) AUC	2212	0.858	0.563
AA AUC	ADP (5.71µM) AUC	2375	0.835	0.492
AA AUC	Collagen AUC	2382	0.832	0.488
Collagen AUC	ADP (5.71µM) AUC	2384	0.822	0.455

- The highest 20% of responders to ristocetin were also high responders to TRAP-6 amide.
- The lowest responders to these agonists also correlated strongly.

CONCLUSIONS

- This is the first large population study assessing multiple platelet function assays.
- Aspirin strongly affects platelet function in all assays.
- Female sex is strongly associated with increased platelet reactivity
- Participants who are high responders to one agonist are likely to be high responders to other agonists.
- One assay cannot be considered a surrogate for another and the dynamics of each assay should be considered when interpreting platelet function data.

REFERENCES

1 **Johnson AD** (2011) The genetics of common variation affecting platelet development, function and pharmaceutical targeting. J Thromb Haemost;9 Suppl 1:246-57.

2 **Chan MV, Warner TD** (2012) Standardised optical multichannel (optimul) platelet aggregometry using high-speed shaking and fixed time point readings. Platelets;23(5):404-408.

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CONTACT INFORMATION

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