Received: 20 March 2024 | Accepted: 5 April 2024

https://doi.org/10.1016/j.rpth.2024.102406

ORIGINAL ARTICLE

Factors that modulate platelet reactivity as measured by 5 assay platforms in 3429 individuals

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Handling Editor: Vania M. Morelli

Abstract

Background: Assessment of platelet function is key in diagnosing bleeding disorders and evaluating antiplatelet drug efficacy. However, there is a prevailing "one-size-fits-all" approach in the interpretation of measures of platelet reactivity, with arbitrary cutoffs often derived from healthy volunteer responses.

Objectives: Our aim was to compare well-used platelet reactivity assays.

Methods: Blood and platelet-rich plasma obtained from the Framingham Heart Study (N = 3429) were assayed using a range of agonists in 5 platelet assays: light transmission aggregometry, Optimul aggregometry, Multiplate impedance aggregometry (Roche Diagnostics), Total Thrombus-Formation Analysis System, and flow cytometry. Using linear mixed-effect models, we determined the contribution of preanalytical and technical factors that modulated platelet reactivity traits.

Results: A strong intra-assay correlation of platelet traits was seen in all assays, particularly Multiplate velocity (r = 0.740; ristocetin vs arachidonic acid). In contrast, only moderate interassay correlations were observed (r = 0.375; adenosine diphosphate Optimul E_{max} vs light transmission aggregometry large area under the curve). As expected, antiplatelet drugs strongly reduced platelet responses, with aspirin use primarily targeting arachidonic acid–induced aggregation, and explained substantial variance ($\beta = -1.735$; $P = 4.59 \times 10^{-780}$; variance proportion = 46.2%) and $P2Y_{12}$ antagonists blocking adenosine diphosphate responses ($\beta = -1.612$; $P = 6.75 \times 10^{-27}$; variance proportion = 2.1%). Notably, female sex and older age were associated with enhanced platelet reactivity. Fasting status and deviations from standard venipuncture practices did not alter platelet reactivity significantly. Finally, the agonist batch, phlebotomist, and assay technician (more so for assays that require additional sample manipulation) had a moderate to large effect on measured platelet reactivity.

Conclusion: Caution must be exercised when extrapolating findings between assays, and the use of standard ranges must be medication-specific and sex-specific at a

minimum. Researchers should also consider preanalytical and technical variables when designing experiments and interpreting platelet reactivity measures.

KEYWORDS
data correlation, platelet, population, platelet function tests, platelet activation

Essentials

- Platelet function testing in large populations is rare.
- We performed 5 types of platelet tests on 3429 participants of the Framingham Heart Study.
- Different tests are unique, and so one cannot be substituted for another.
- Sex, age, and aspirin use significantly affect results, so care must be taken in interpreting reference ranges.

1 | INTRODUCTION

Cardiovascular diseases are the primary cause of mortality worldwide [1,2]. It is therefore critical to understand their pathophysiology in order to prevent cardiovascular events, including myocardial infarction and stroke [3]. Platelets are the lynchpin of hemostasis and thrombosis, and platelet activation is known to be caused by a myriad of factors [4,5]. However, the assessment of platelet reactivity using the gold standard light transmission aggregometry (LTA) is generally time-consuming and requires fresh blood samples, experienced personnel, and dedicated equipment to generate reproducible data [6].

As a result, though there are many studies assessing platelet reactivity in patients, large-scale population studies are rare [7–12]. Among the largest studies with platelet reactivity data is the Framingham Heart Study (FHS) Generation 2 cohort (N = 2604) in which our group previously identified genetic variants related to increased platelet function, which was replicated in the Genetic Study of Atherosclerosis Risk participants with European ancestry (N = 1060) [13,14]. An additional study in 825 African Americans of Genetic Study of Atherosclerosis Risk cohort similarly identified novel loci associated with platelet aggregation, which were replicated in 1221 European Americans [15]. However, these studies were limited to LTA using a narrow concentration range of particular platelet agonists (arachidonic acid [AA], adenosine diphosphate [ADP], collagen, and epinephrine) in platelet-rich plasma (PRP) [16]. Indeed, despite a recent study of LTA and Optimul aggregometry that was performed in 338 elderly participants of the Bruneck Study [17], there is still a paucity of even small-scale comparative studies on platelet assays, with most focused on the effects of aspirin and $P2Y_{12}$ receptor antagonists in patients [9,18,19].

Here, we describe a comprehensive study of platelet reactivity in participants of the FHS Third Generation, New Offspring Spouse (NOS), and Omni 2 cohorts using 5 different platelet function assays in a range of agonists. These included traditional LTA, Optimul aggregometry [20], Multiplate impedance aggregometry (MP), thrombus formation under flow (Total Thrombus-Formation Analysis System [T-TAS]) [21], and flow cytometry [22]. We report standard ranges and assay comparisons and discuss preanalytical and technical aspects that moderate these platelet reactivity assays, in some cases explaining large variances in the assay outputs.

2 | METHODS

2.1 | Study participants

FHS is a community-based, prospective, longitudinal study following 3 generations of participants [23]. The participants of the sample population studied here represent the third examination of the Third Generation and NOS cohorts of primarily European ancestry (N = 3140) and the Omni 2 cohort of African, Hispanic, Asian, and mixed ancestry (N = 289), with examinations conducted between 2016 and 2019. Self-reported race and ethnicity questionnaires based on the commonly used race/ethnicity forms of the respective time periods were administered at FHS examinations between 1994 and 2011. The participants in the present study were given the questionnaires at examinations 1 (2002-2005) and/or 2 (2008-2011). Self-reported race and ethnicity in this study was based on a cumulative race and ethnicity dataset created to record participants' responses to race and ethnicity questions over time. Participants were allowed to select more than 1 race and could vary their response over time.

Participants attended a ~4.5-hour examination, and detailed concurrent and historic anthropometric measurements, disease event surveillance data, and other clinical and subclinical measures were collected. Participants stated medication use at the time of blood draw and were also asked to bring medications used to the examination. Formulations were noted and categorized with the World Health Organization's Anatomical Therapeutic Chemical classifications. This study was approved by the Boston University Medical Center institutional review board, and participants gave written informed consent [24].

2.2 | Blood processing

Participants fasted overnight, and blood was drawn the following morning in a supine position from the antecubital vein with a 21-gauge

butterfly collection set with the aid of a tourniquet [25,26]. Blood was collected into glass sodium citrate (3.2%; Becton Dickinson) and hirudin (whole blood [WB]; Roche Diagnostics, DiaPharma) vacutainers. The workflow is summarized in Figure 1.

Any deviations from these standard phlebotomy procedures were noted, as well as other relevant factors including self-reported last antiplatelet medications, known diabetes, and time of last meal. Participants were considered fasting after a minimum 10-hour fast.

Blood samples were processed at room temperature for platelet function testing in accordance with the International Society on Thrombosis and Haemostasis guidelines [27]. Briefly, blood was left to stand for >15 minutes after blood taking before being processed. Sodium citrate tubes were centrifuged (200 × *g*, 10 minutes; Sorvall ST8 centrifuge, Thermo Fischer Scientific 75003181 rotor) to isolate PRP and pooled. Samples were further centrifuged (1500 × *g*, 15 minutes) for isolation of platelet-poor plasma.

2.3 | Platelet function testing

Platelet function tests were conducted using the MP system (Roche Diagnostics) and T-TAS automated microchip flow chamber (PL-chip; Zacros, Fujimori Kogyo) in WB, LTA (PAP-8E, Bio/Data Corporation) and Optimul aggregometry were conducted in PRP, and flow cytometry (Accuri C6, BD Biosciences) was conducted in both WB and PRP.

All agonists, except AA/ASPItest (Roche Diagnostics), U46619 (Cayman Chemical), and the agonists in a limited number of Optimul plates, were obtained from Bio/Data Corporation in large, dedicated batches (∼1 batch/y) to minimize variation in this study. Further assay testing details can be found in the Supplementary Materials.

Aspirin use was defined as a final aggregation response of <40% to AA (500 μg/mL) in LTA. If this was not available, it was determined by MP area under the curve (AUC) of <40 units to ASPItest. Using this definition, our study consisted of 736 aspirin users.

2.4 | Exclusion criteria

Data were excluded from 92 participants who did not have platelet assays (total *N* = 3429/3521). In addition, participant data from PRP assays (LTA, Optimul, and PRP flow cytometry) were excluded due to hemolytic (*n* = 5) and lipidemic samples (*n* = 25) but retained for the WB assays (MP, T-TAS, and WB flow cytometry). Due to instrument downtime, reagent availability, and the addition of T-TAS later in the examination, the sample size was variable for each instrument (Supplementary Figure S1).

2.5 | Statistical analysis

All platelet traits and technical factors were treated as continuous except for sex, aspirin use, $P2Y_{12}$ antagonist use (determined by prescribed medications brought to the examination), fasting status, deviation in blood draw, short blood draw, multiple venipuncture, tourniquet use, phlebotomist, assay technician, and assay batch, which were categorical.

Correlation coefficients were calculated using the Pearson's correlation method. Linearity was not tested prior to calculation.

Inverse normal transformation was applied to platelet traits, and linear mixed-effect models were used to determine the association between these transformed traits (dependent variables) and technical factors (independent variables) including age, sex, and aspirin [28]. All models accounted for family relatedness, and for each trait were specifically tailored for sample preparation (PRP or WB), assays, and agonists used (Supplementary Table S1). A *P* value was computed for a global test for those technical factors with 3 or more levels, such as agonist batch, technician, or phlebotomist. This tests whether there is association between a platelet trait and a technical factor with 3 or more levels, ie, H_0: all levels have the same platelet mean vs H_1: at least 1 level has different platelet trait mean from other(s). Differences were considered significant at $P < .0000467$ after Bonferroni correction for multiple testing for 63 platelet traits and 17 technical factors.

All analyses and figures were conducted or produced using the R language and environment (R Core Team) [29].

3 | RESULTS

3.1 | Study participants

The study consisted of 3429 participants who had at least 1 platelet function measure. Of these, 53.7% (*n* = 1842) were female, the mean age was 54.4 ± 9.3 years, and the average body mass index was 28.5 ± 5.8 kg/m^2. Overall, 351 individuals were diagnosed with diabetes (10.2%) and 995 with hypertension (29.0%; Table 1). The Third Generation and NOS cohorts were of primarily European ancestry, and the Omni 2 cohort included other ancestries (White, 0.7%; Black, 18.0%; Hispanic, 9.7%; Asian, 23.9%; Mixed or Other, 47.8%; where 57.2% of Mixed or Other were Hispanic-White and Other; Supplementary Table S2).

We observed 19.9% (*n* = 683) self-reported aspirin users. This was superseded by the 21.5% (*n* = 736) who we identified as aspirin takers using our criteria. There was good agreement between self-report and LTA (kappa = 0.68) and MP (kappa = 0.64), while agreement between self-report and Optimul (kappa = 0.3) and T-TAS (kappa = 0.28) was fair (Supplementary Table S3). In addition, there was a clear separation of LTA responses to AA in the aspirin (7.0 ± 6.1%) group vs the nonaspirin group (74.9 ± 8.0%; $P < .0001$).

Since sex and aspirin status can be easily determined and have a large effect on platelet reactivity, reference ranges for each assay were derived based on aspirin use and sex (Tables 2–6).

3.2 | Correlation between assays

We constructed correlation matrices for the 5 platelet assays in all participants included in the study (Figure 2A). In particular, AA-

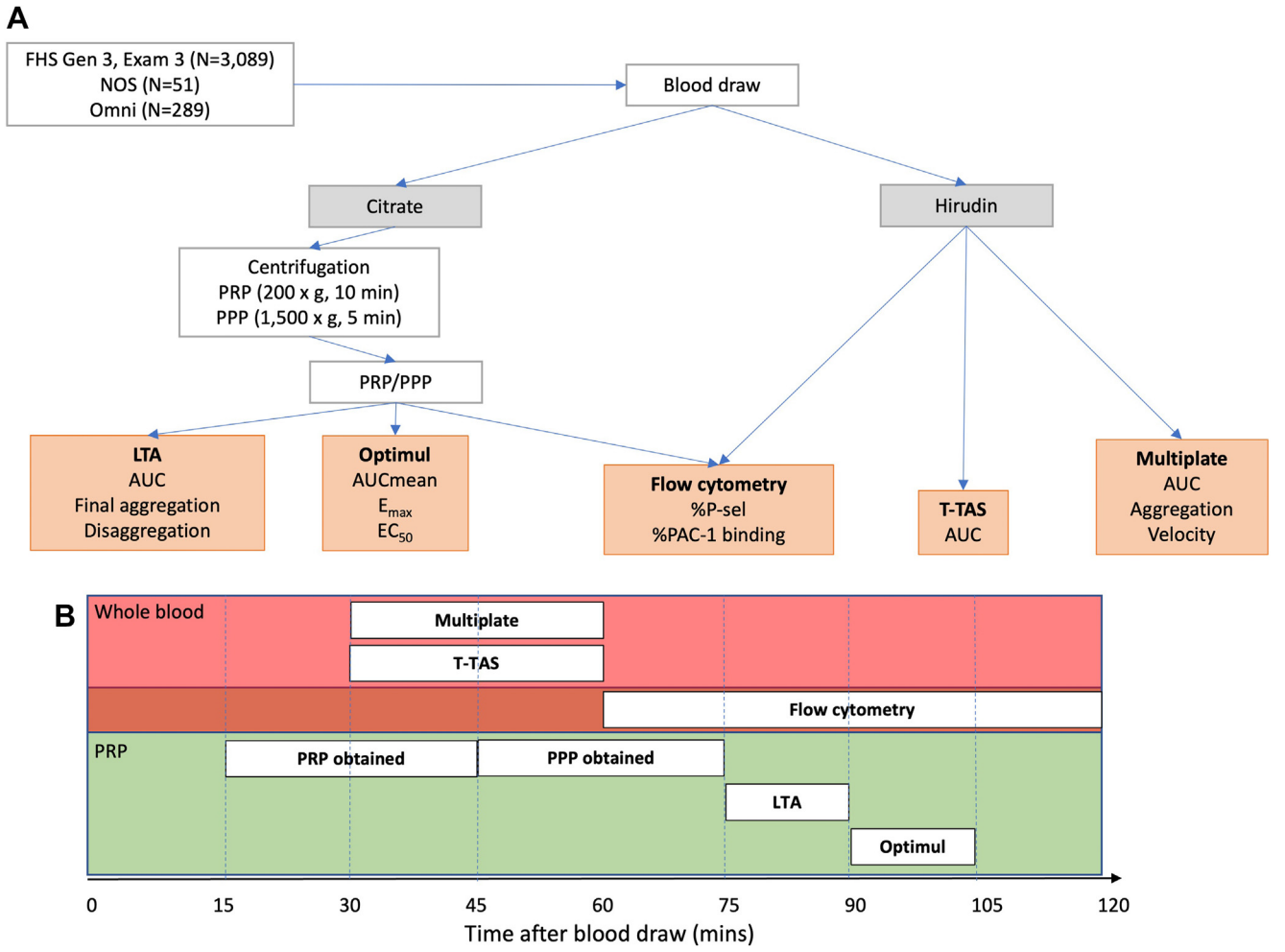

FIGURE 1 Daily workflow by (A) assay and (B) timeline. For all experiments, blood was drawn and either kept as whole blood or processed by centrifugation for platelet-rich plasma (PRP) and platelet-poor plasma (PPP). Light transmission aggregometry (LTA), Optimul aggregometry, Multiplate impedance aggregometry, Total Thrombus-Formation Analysis System (T-TAS), and flow assays were then conducted as soon as possible and within a 4-hour timeframe. AUC, area under the curve; EC_{50}, half maximal effective concentration; E_{max}, maximum effect; FHS Gen 3, Framingham Heart Study Generation 3; NOS, New Offspring Spouse; PAC-1, procaspase-activating compound-1; P-sel, P-selectin.

mediated responses in LTA and the MP aspirin tests were strongly correlated (r = 0.793; $P = 4.34 \times 10^{-687}$). Since these responses were principally driven by aspirin users (n = 736), we repeated these correlations in the individuals not on aspirin and found that most of these correlations were significantly reduced (Figure 2B). We found intra-assay correlations, particularly with LTA and MP (ristocetin vs AA velocity; r = 0.740; $P = 9.84 \times 10^{-561}$), even using different agonists.

There was varying interassay correlation across the same agonist in different assays, with weak to moderate correlations between PRP and WB assays. For example, Optimul and LTA were weakly correlated in shared agonists (eg, ristocetin LTA final aggregation vs Optimul E_{max}, r = 0.378; $P = 7.46 \times 10^{-82}$), and collagen MP AUC and T-TAS AUC responses were moderately correlated (r = 0.467; $P = 1.84 \times 10^{-50}$), as well as ADP measures across all assays in the nonaspirin sample (ADP LTA AUC vs Optimul E_{max}, r = 0.375; $P = 1.68 \times 10^{-80}$). However, there was no correlation between LTA and MP aggregation after stimulation with ristocetin or thrombin receptor activating peptide 6 (TRAP-6) amide (ristocetin: r = 0.037; P = .06; TRAP-6: r = −0.007; P = .73).

P values and correlation coefficients for each assay in the presence and absence of aspirin, as defined by our criteria, are provided in Supplementary Tables 4–7.

3.3 | Technical factors

We used the significance (Figure 3A) in multivariable regression models as well as the proportion of explained variance (Figure 3B) to determine the contribution of each technical, preanalytical, or medication factor to each platelet trait (all results shown in Supplementary Table S8). In the text below, we highlight results for those factors that account for the highest explained variance proportion (VP) in platelet reactivity traits.

TABLE 1 Participant demographics summary of the Framingham Heart Study samples in Generation 3, New Offspring Spouse, and Omni 2 cohorts.

	No aspirin (male)	No aspirin (female)	Aspirin (male)	Aspirin (female)
Number of participants	1141	1552	446	290
Age (y)	52.76 ± 8.85 (32-87)	53.47 ± 9.13 (32-93)	59.44 ± 8.72 (33-89)	58.34 ± 8.94 (34-87)
Body mass index (kg/m^2)	29.29 ± 5.08 (17.29-51.13)	27.52 ± 6.24 (15.45-54.64)	30.17 ± 5.11 (18.52-53.01)	28.67 ± 6.5 (17.01-59.22)
Total cholesterol (mg/dL)	187.13 ± 33.97 (91-380)	195.64 ± 35.97 (101-386)	171.07 ± 39.28 (68-382)	191.85 ± 35.34 (108-333)
High-density lipoprotein (mg/dL)	50.6 ± 15.18 (11-175)	67.86 ± 19.29 (7-161)	49.68 ± 14.78 (13-107)	64.64 ± 19.21 (18-134)
Current smoker, *n* (%)	80 (7)	104 (6.7)	21 (4.7)	19 (6.6)
Diagnosed hypertension, *n* (%)	311 (27.3)	321 (20.7)	240 (53.9)	123 (42.6)
Diagnosed diabetes, *n* (%)	101 (8.9)	114 (7.3)	95 (21.3)	41 (14.2)
Fasting at blood draw, *n* (%)	1101 (96.5)	1515 (97.6)	420 (94.2)	281 (96.9)
Self-reported aspirin use at time of blood draw, *n* (%)	82 (7.2)	69 (4.4)	349 (78.3)	183 (63.1)
$P2Y_{12}$ antagonist use determined by medications brought to examination, *n* (%)	4 (0.4)	7 (0.5)	18 (4)	4 (1.4)
Light transmission aggregometry, *n* (%)	1105 (96.8)	1517 (97.7)	431 (96.6)	288 (99.3)
Optimul aggregometry, *n* (%)	1056 (92.6)	1424 (91.8)	414 (92.8)	275 (94.8)
Multiplate impedance aggregometry, *n* (%)	1137 (99.6)	1547 (99.7)	446 (100)	290 (100)
Total Thrombus-Formation Analysis System, *n* (%)	384 (33.7)	530 (34.1)	126 (28.3)	89 (30.7)
Flow cytometry, *n* (%)	986 (86.4)	1266 (81.6)	373 (83.6)	237 (81.7)

Data are presented as mean ± SD, unless stated otherwise.

3.3.1 | Age and sex

Age was directly associated with ADP (0.95 μmol/L, VP = 2.9%; 1.82 μmol/L, VP = 0.8%) and ristocetin-induced platelet final aggregation (VP = 0.6%) in LTA. Similarly, ADP responses in Optimul increased with age, as well as epinephrine-induced reactivity. Interestingly, platelet responses were counter-directional in MP with reduced reactivity to collagen with increased age. Age did not contribute to >0.5% of explained variance in T-TAS or flow assays, but, generally, greater age was associated with a

TABLE 2 Reference means and ranges for light transmission aggregometry assay performed in the Framingham Heart Study participants by aspirin use and sex.

Agonist (concentration)	Measurement	No aspirin (male)		No aspirin (female)		Aspirin (male)		Aspirin (female)	
		Mean ± SD (range)	*N*	Mean ± SD (range)	*N*	Mean ± SD (range)	*N*	Mean ± SD (range)	*N*
AA (500 μg/mL)	AUC	0.647 ± 0.083 (0.24-0.97)	1101	0.665 ± 0.083 (0.17-0.96)	1511	0.069 ± 0.047 (0-0.3)	429	0.082 ± 0.051 (0-0.39)	288
Collagen (0.19 mg/mL)	AUC	0.54 ± 0.075 (0-0.87)	1104	0.561 ± 0.064 (0.29-0.85)	1517	0.393 ± 0.1 (0.01-0.63)	429	0.428 ± 0.085 (0.03-0.67)	287
ADP (0.95 μmol/L)	AUC	0.168 ± 0.17 (0-0.9)	1047	0.267 ± 0.22 (0-0.92)	1400	0.108 ± 0.083 (0-0.7)	417	0.124 ± 0.076 (0.01-0.46)	269
ADP (1.82 μmol/L)	AUC	0.413 ± 0.232 (0-0.98)	1102	0.544 ± 0.209 (0.02-0.97)	1510	0.246 ± 0.142 (0-0.84)	431	0.294 ± 0.131 (0.03-0.77)	288
ADP (5.71 μmol/L)	AUC	0.667 ± 0.104 (0.08-0.98)	1102	0.698 ± 0.087 (0.14-0.97)	1511	0.525 ± 0.143 (0.01-0.97)	429	0.579 ± 0.112 (0.1-0.95)	287
Epinephrine (0.1 mmol/L)	AUC	0.54 ± 0.139 (0.01-0.97)	1102	0.576 ± 0.126 (0.01-0.94)	1516	0.249 ± 0.133 (0-0.73)	431	0.266 ± 0.128 (0.02-0.7)	288
Ristocetin (1.5 mg/mL)	AUC	0.73 ± 0.078 (0.03-0.98)	1090	0.747 ± 0.073 (0.01-0.97)	1494	0.715 ± 0.1 (0.03-0.97)	430	0.736 ± 0.087 (0.21-0.96)	286
TRAP-6 amide (670 μmol/L)	AUC	0.723 ± 0.078 (0.42-1)	1103	0.737 ± 0.076 (0.37-0.99)	1517	0.72 ± 0.085 (0.37-0.98)	429	0.738 ± 0.073 (0.5-0.97)	287
AA (500 μg/mL)	Final aggregation (%)	74.136 ± 8.085 (40-100)	1102	75.498 ± 7.962 (43-100)	1511	6.415 ± 5.922 (0-36)	431	7.938 ± 6.224 (0-36)	288
Collagen (0.19 mg/mL)	Final aggregation (%)	73.47 ± 8.863 (0-100)	1105	74.729 ± 7.845 (45-100)	1517	68.406 ± 11.666 (0-100)	431	70.573 ± 8.412 (11-100)	288
ADP (0.95 μmol/L)	Final aggregation (%)	21.89 ± 26.079 (0-100)	1047	33.886 ± 31.258 (0-100)	1400	9.662 ± 9.122 (0-78)	417	10.309 ± 7.824 (0-51)	269
ADP (1.82 μmol/L)	Final aggregation (%)	47.45 ± 30.876 (0-100)	1102	62.079 ± 27.47 (0-100)	1510	19.93 ± 15.189 (0-95)	431	23.142 ± 14.039 (0-70)	288
ADP (5.71 μmol/L)	Final aggregation (%)	73.73 ± 12.997 (0-100)	1103	76.317 ± 10.816 (4-100)	1512	48.832 ± 18.421 (0-100)	429	54.767 ± 15.295 (5-100)	287
Epinephrine (0.1 mmol/L)	Final aggregation (%)	71.064 ± 14.404 (4-100)	1103	73.637 ± 13.132 (0-100)	1517	33.619 ± 19.639 (0-100)	431	35.542 ± 18.825 (0-93)	288
Ristocetin (1.5 mg/mL)	Final aggregation (%)	78.481 ± 8.36 (0-100)	1091	80.248 ± 7.9 (0-100)	1494	78.667 ± 10.869 (4-100)	430	80.64 ± 8.981 (14-100)	286
TRAP-6 amide (670 μmol/L)	Final aggregation (%)	78.514 ± 8.517 (46-100)	1103	79.933 ± 8.295 (42-100)	1517	77.54 ± 9.009 (40-100)	430	79.39 ± 7.91 (54-100)	287
AA (500 μg/mL)	Lag time	4.24 ± 10.066 (0-79)	1102	3.014 ± 9.042 (0-79)	1511	30.053 ± 22.813 (0-187)	431	28.219 ± 18.804 (0-105)	288
Collagen (0.19 mg/mL)	Lag time	58.056 ± 21.837 (0-291)	1105	53.075 ± 19.424 (0-125)	1517	99.148 ± 37.116 (0-301)	431	91.885 ± 32.557 (0-218)	287
ADP (0.95 μmol/L)	Disaggregation (%)	3.945 ± 4.379 (0-25)	1047	4.544 ± 5.653 (0-38)	1400	7.482 ± 4.646 (0-22)	417	10.74±5.348 (0-26)	269
ADP (1.82 μmol/L)	Disaggregation (%)	5.287 ± 7.312 (0-34)	1102	3.957 ± 8.078 (0-43)	1510	15.659 ± 7.09 (0-38)	431	18.26±8.223 (0-47)	288
ADP (5.71 μmol/L)	Disaggregation (%)	0.995 ± 3.78 (0-33)	1103	0.855 ± 3.367 (0-35)	1512	11.925±8.606 (0-49)	429	10.948±7.732 (0-37)	287

AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; TRAP-6, thrombin activating peptide 6.

TABLE 3 Reference means and ranges for Optimul aggregometry performed in the Framingham Heart Study participants by aspirin use and sex.

		No aspirin (male)		No aspirin (female)		Aspirin (male)		Aspirin (female)	
Agonist	**Measurement**	**Mean ± SD (range)**	***N***	**Mean ± SD (range)**	***N***	**Mean ± SD (range)**	***N***	**Mean ± SD (range)**	***N***
AA	AUC	0.75 ± 0.28 (0.1-1.5)	963	0.81 ± 0.29 (0.1-1.5)	1335	0.28 ± 0.14 (0-0.8)	323	0.27 ± 0.15 (0-1.1)	222
ADP	AUC	1.58 ± 0.36 (0.5-3.4)	1010	1.69 ± 0.38 (0.7-3.6)	1384	1.53 ± 0.31 (0.3-2.7)	398	1.6 ± 0.31 (0.8-2.7)	271
Collagen	AUC	1.3 ± 0.54 (0.2-3.2)	997	1.45 ± 0.59 (0.3-3.2)	1373	1.03 ± 0.47 (0.1-2.5)	388	1.07 ± 0.49 (0.3-2.4)	263
Epinephrine	AUC	1.9 ± 0.55 (0.4-3.9)	1018	2 ± 0.55 (0.2-3.8)	1391	1.53 ± 0.46 (0.2-3.3)	393	1.64 ± 0.45 (0.6-2.7)	267
Ristocetin	AUC	0.81 ± 0.17 (0.1-1.4)	988	0.82 ± 0.17 (0.2-1.3)	1377	0.69 ± 0.12 (0.2-1.2)	384	0.68 ± 0.11 (0.3-1.2)	265
TRAP-6 amide	AUC	2.68 ± 0.16 (0.6-3.1)	941	2.7 ± 0.14 (0.4-3.1)	1222	2.65 ± 0.25 (0-3)	361	2.69 ± 0.1 (2-3.1)	238
U46619	AUC	2.18 ± 0.39 (1.3-3.7)	980	2.27 ± 0.42 (0.6-3.5)	1393	2.11 ± 0.37 (0.4-3.2)	386	2.2 ± 0.38 (1-3.2)	272
AA	E_{max}	85.7 ± 11.06 (10.5-100)	982	87.31 ± 7.41 (20-100)	1356	51.98 ± 21.82 (6.9-100)	339	50.43 ± 20.25 (4.6-98.7)	228
ADP	E_{max}	87.57 ± 4.43 (19.8-100)	1020	88.17 ± 2.96 (66.3-100)	1385	87.02 ± 6.16 (25.5-99.9)	399	88.01 ± 4.06 (48.8-99.9)	271
Collagen	E_{max}	84.48 ± 7.06 (13-100)	1008	85.13 ± 5.64 (32.7-100)	1378	77.46 ± 13.03 (18.1-99)	392	78.95 ± 10.8 (25.7-98.7)	263
Epinephrine	E_{max}	85.16 ± 8.09 (14.6-100)	1027	86.19 ± 7.29 (33.3-99.9)	1394	77.44 ± 13.37 (15.5-99.9)	397	80.45 ± 11.27 (26.2-97.8)	269
Ristocetin	E_{max}	90.77 ± 3.27 (52.1-100)	1004	90.87 ± 2.76 (55.6-100)	1387	90.07 ± 5.09 (29.5-100)	389	90.38 ± 3.61 (48.5-100)	266
TRAP-6 amide	E_{max}	89.94 ± 3.52 (38.8-100)	941	90.06 ± 2.7 (54.1-100)	1222	89.74 ± 4.35 (27.2-99.3)	361	90.09 ± 2.21 (80.4-99.9)	238
U46619	E_{max}	89.77 ± 3.65 (41.4-100)	998	89.6 ± 3.46 (30.1-100)	1398	89.5 ± 5.1 (38.2-100)	393	89.78 ± 2.82 (65.6-99.7)	272

AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; Emax, maximum effect (% aggregation); TRAP-6, thrombin activating peptide 6.

TABLE 4 Reference means and ranges for Multiplate impedance aggregometry performed in the Framingham Heart Study participants by aspirin use and sex.

Agonist (concentration)	Measurement	No aspirin (male)		No aspirin (female)		Aspirin (male)		Aspirin (female)	
		Mean ± SD (range)	*N*	Mean ± SD (range)	*N*	Mean ± SD (range)	*N*	Mean ± SD (range)	*N*
AA (0.5 mmol/L)	AUC	87.52 ± 21.91 (10.2-162.8)	1098	91.51 ± 23.92 (1.1-164.8)	1467	26.49 ± 16.41 (1.3-118.3)	434	23.9 ± 15.2 (2.3-135.6)	274
ADP (3.19 μmol/L)	AUC	66.19 ± 18.52 (22.2-141.8)	1137	77.17 ± 18.84 (18.9-164.9)	1542	64.82 ± 22.28 (10.2-160)	445	77.06 ± 22.19 (9.1-142.2)	289
Collagen (0.061 mg/mL)	AUC	55.06 ± 13.97 (7.7-112.2)	1137	57.52 ± 14.74 (9.9-116.1)	1545	42.9 ± 14.4 (4.9-104.5)	444	44.3 ± 14.28 (6-101)	289
Ristocetin (1.15 mg/mL)	AUC	102.37 ± 25.71 (32.9-259.8)	1126	117 ± 28.24 (24.6-236.6)	1519	73.1 ± 26.68 (3.3-162.3)	444	82.28 ± 29.88 (9.1-178.7)	286
TRAP-6 amide (216 μmol/L)	AUC	114.69 ± 21.51 (13.2-207.1)	1137	117.91 ± 21.06 (31-187.1)	1545	113.25 ± 23.63 (26-183.1)	444	122.35 ± 24.37 (33.4-201.7)	288
AA (0.5 mmol/L)	Aggregation (%)	163.38 ± 34.65 (22.1-287.2)	1098	166.37 ± 37.25 (4.7-297.4)	1467	60.24 ± 34.29 (5.1-235.1)	434	54.49 ± 32.53 (5.8-238.9)	274
ADP (3.19 μmol/L)	Aggregation (%)	127.78 ± 34.72 (41.6-255.6)	1137	143.02 ± 33.27 (37.7-308.1)	1542	123.88 ± 41.5 (22.7-277.3)	445	143.01 ± 40.56 (20.4-265.9)	289
Collagen (0.061 mg/mL)	Aggregation (%)	142.46 ± 26.99 (39.9-239.2)	1137	145.87 ± 27.67 (27.2-261.2)	1545	116.77 ± 29.31 (13.3-215.2)	444	121.33 ± 30.21 (36.6-229.5)	289
Ristocetin (1.15 mg/mL)	Aggregation (%)	207.82 ± 44.99 (67.3-381)	1126	230.17 ± 49.66 (63.4-423)	1519	161.45 ± 58 (12.8-334.7)	444	177.98 ± 65.99 (29.6-358.9)	286
TRAP-6 amide (216 μmol/L)	Aggregation (%)	196.34 ± 35.41 (26.8-329.6)	1137	198.51 ± 33.96 (53.6-300.6)	1545	192.37 ± 38.76 (48.8-303.2)	444	204.21 ± 39.11 (59.1-320.4)	288
AA (0.5 mmol/L)	Velocity	19.17 ± 4.75 (3.1-45.5)	1098	20.83 ± 5.44 (1.6-44.3)	1467	7.03 ± 3.44 (1.9-23.9)	434	6.68 ± 3.47 (2-30.1)	274
ADP (3.19 μmol/L)	Velocity	14.04 ± 3.7 (5.3-35.4)	1137	16.51 ± 3.9 (5.3-36.2)	1542	13.87 ± 4.45 (3.6-42)	445	16 ± 4.07 (4-26.2)	289
Collagen (0.061 mg/mL)	Velocity	16.37 ± 2.98 (5.5-32)	1137	17.26 ± 3.22 (4.4-30.4)	1545	13.46 ± 2.97 (3.3-27.9)	444	14.33 ± 3.19 (4.9-27.4)	289
Ristocetin (1.15 mg/mL)	Velocity	32.34 ± 9.24 (7.6-88.2)	1126	36.89 ± 10.31 (8.4-80.4)	1519	18.59 ± 7.85 (3.2-57.2)	444	20.82 ± 8.5 (4.9-46.4)	286
TRAP-6 amide (216 μmol/L)	Velocity	25.81 ± 5.37 (4-51.4)	1137	27.68 ± 5.65 (6.9-48.8)	1545	25.42 ± 5.82 (6.3-45.9)	444	28.2 ± 6.27 (8.4-47.8)	288

AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; TRAP-6, thrombin activating peptide 6.

TABLE 5 Reference means and ranges for Total Thrombus-Formation Analysis System performed in the Framingham Heart Study participants by aspirin use and sex.

		No aspirin (male)		No aspirin (female)		Aspirin (male)		Aspirin (female)	
Agonist (concentration)	Measurement	Mean ± SD (range)	N	Mean ± SD (range)	N	Mean ± SD (range)	N	Mean ± SD (range)	N
Collagen (PL-chip)	AUC	307.35 ± 82.73 (7.4-458.5)	384	312.61 ± 93.34 (2.7-481.9)	530	174.34 ± 102.94 (3.2-395.3)	126	202.69 ± 99.11 (5.2-447.1)	89

AUC, area under the curve.

reduction in MP traits and an increase in flow cytometry–related traits.

All assays except T-TAS were profoundly influenced by sex, even though T-TAS platelet thrombus formation tended to be higher in women. Female sex was associated with an increase in platelet reactivity in all agonists studied in the MP WB assay. Lower concentrations of ADP were associated with increased reactivity in females in LTA (0.95 μmol/L, VP = 0.7%; 1.82 μmol/L, VP = 1.0%). In addition, epinephrine (AUC, VP = 0.6%) and U46619 (AUC, VP = 0.8%) responses in Optimul were elevated in females. ADP stimulation in PRP (P-selectin, VP = 2.0%; procaspase-activating compound-1 [PAC-1] binding, VP = 0.8%; double positivity, VP = 1.0%) and WB (P-selectin, VP = 3.9%; PAC-1 binding, VP = 1.8%; double positivity, VP = 3.0%) samples also resulted in greater expression of activation markers in flow cytometry in females. Furthermore, females had a higher estimated platelet count than males in both WB (mean, 368,770 ± 130,037/μL vs 330,272 ± 119,164/μL) and PRP (521,022 ± 179,432/μL vs 510,310 ± 192,079/μL) as estimated by flow cytometry direct volume counting.

Once centrifuged, the PRP volume from each tube of citrated WB was noted (0.422 ± 0.069 mL citrate per milliliter WB; range, 0.178-0.741 mL citrate per milliliter WB). Greater PRP volume (per milliliter WB), which is linked to hematocrit and, by extension, being female, enhanced platelet reactivity as measured in PRP-based assays.

3.3.2 | Aspirin and $P2Y_{12}$ antagonist use

As expected, aspirin strongly attenuated AA traits in the LTA (VP = 46.2%) and Optimul (VP = 30.4%) assays. Additionally, aspirin reduced platelet reactivity to several other agonists in PRP assays, including collagen (LTA, VP = 28.8%; Optimul, VP = 9.1%), epinephrine (LTA, VP = 34.3%; Optimul, VP = 7.0%), and ristocetin (LTA, VP = 0.7%; Optimul, VP = 11.1%; Figure 3A, B; Supplementary Table S8). Interestingly, aspirin also significantly dampened ADP responses in these assays (LTA, VP = 18.7%; Optimul, VP = 0.7%). Similarly, since T-TAS uses collagen as an agonist, these responses were blunted with aspirin use (VP = 13.9%). MP responses to ASPItest (VP = 38.7%), collagen (VP = 9.8%), and ristocetin (VP = 23.2%) were attenuated. Aspirin use had no effect on thromboxane (Tx) receptor agonism by the TxA_2 mimetic U46619 and very little effect on TRAP-6 amide (thrombin protease-activated receptor 1 agonist) assays.

$P2Y_{12}$ antagonists, as noted from medications being taken by participants, elicited an expected reduction in ADP aggregation traits in the LTA (VP = 2.1%) and Optimul (VP = 2.0%) assays. Of note, $P2Y_{12}$ antagonist use was also associated with reduced epinephrine, TRAP-6 amide, and U46619 responses in the Optimul assay as well as a reduced T-TAS. Similarly, $P2Y_{12}$ drug use was associated with lower expression of platelet activation markers in flow cytometry after the addition of ADP. MP ADP responses were blunted with $P2Y_{12}$ antagonist use but unaffected by aspirin ($P > .05$; Supplementary Table S8).

TABLE 6 Reference means and ranges for flow cytometry performed in the Framingham Heart Study participants by aspirin use and sex.

Agonist (concentration)	Measurement	No aspirin (male)		No aspirin (female)		Aspirin (male)		Aspirin (female)	
		Mean ± SD (range)	N	Mean ± SD (range)	N	Mean ± SD (range)	N	Mean ± SD (range)	N
ADP (20 µmol/L)	PAC-1 % positivity in whole blood	20.25 ± 15.22 (0-87.1)	983	23.85 ± 16.41 (0-87)	1277	18.63 ± 14.98 (0-71.6)	371	23.65 ± 16.84 (0-76.2)	237
ADP (20 µmol/L)	P-selectin % positivity in whole blood	9.45 ± 6.44 (0-44.9)	983	12.8 ± 8.45 (0-54.3)	1277	9.32 ± 7.1 (0-54.7)	371	12.4 ± 8 (0-36.4)	237
ADP (20 µmol/L)	PAC-1 and P-selectin % double positivity in whole blood	9.02 ± 6.81 (0-47.1)	983	11.97 ± 8.78 (0-61.3)	1277	9.01 ± 7.6 (0-42.9)	371	11.63 ± 8.5 (0-45.1)	237
ADP (20 µmol/L)	PAC-1 % positivity in PRP	14.38 ± 12.46 (0-69.7)	974	15.8 ± 13.59 (0-73.5)	1254	13.61 ± 12.87 (0-61.6)	365	15.38 ± 13.71 (0-57.2)	238
ADP (20 µmol/L)	P-selectin % positivity in PRP	31.27 ± 12.54 (0.9-71.9)	974	35.74 ± 13.12 (0-75.2)	1254	31.04 ± 12.68 (0-60.7)	365	33.77 ± 13.17 (0-66.2)	238
ADP (20 µmol/L)	PAC-1 and P-selectin % double positivity in PRP	10.39 ± 8.55 (0-46.3)	974	11.87 ± 9.83 (0-51.7)	1254	9.95 ± 8.68 (0-39.8)	365	11.49 ± 9.75 (0-41.2)	238

ADP, adenosine diphosphate; PAC-1, procaspase-activating compound-1; PRP, platelet-rich plasma.

3.3.3 | Timing

Day in the year was not associated with platelet reactivity in LTA or T-TAS. However, as the time of year advanced, collagen responses were attenuated in the MP assay, and ADP-stimulated PAC-1 binding and P-selectin were reduced in flow cytometry. Conversely, responses in the Optimul assay to collagen were enhanced (Supplementary Table S8).

The majority of blood draws (93.5%) were completed between 7 AM and 10 AM, with only 14 conducted after 11 AM. Mean draw time was 8:48 AM ± 54 minutes (SD). Interestingly, later draw times in the day significantly blunted LTA responses to AA, collagen, ristocetin, and P-selectin expression in flow. Conversely, a longer time from blood draw to test blunted responses in only the MP assay.

The time in the morning in which blood was centrifuged to obtain PRP (PRP spin time) was inversely correlated with ADP-, collagen-, ristocetin-, and TRAP-6-induced aggregation in LTA and a later PRP spin time was associated with increased P-selectin expression and enhanced PAC-1 binding. Conversely, a later platelet-poor plasma spin time was associated with the opposite effect (Supplementary Table S8).

When we defined the time of year as seasons: spring (March 1 to May 31), summer (June 1 to August 31), fall (September 1 to November 30), and winter (December 1 to February 28), we saw a reduction in PAC-1 expression in summer and fall (Supplementary Figure S2).

3.3.4 | Fasting status and draw issues

Fasting status (defined as >10 hours of fasting) had no effect on platelet traits. Fasting time in individuals using this criterion (n = 3317) ranged from 600 to 1470 minutes (775 ± 89 minutes), with 93% of fasting participants fasting for between 10 and 15 hours. Similarly, any deviations from the protocol regarding blood draw, such as a short draw (a less than complete number of tubes drawn due to early discontinuation of phlebotomy), more than 1 attempt at obtaining blood, or the requirement of the tourniquet throughout the draw, did not seem to affect platelet traits (n = 443).

Individual phlebotomists, however, did have a moderately significant bearing on platelet reactivity induced by collagen and TRAP-6 in LTA. It is important to note, however, that certain highly experienced phlebotomists were called upon for difficult draws and older participants, which may have skewed results.

3.3.5 | Agonist batch

We identified a major effect of the place of manufacture (London, United Kingdom, or Framingham, Massachusetts, United States) for the Optimul plates. This likely primarily results from different agonist origins, but it is also possible that there are effects due to different technicians manufacturing plates, different freeze-driers and other equipment, or changes in agonists with the extra transportation involved. Although efforts were made to reduce the number of agonist

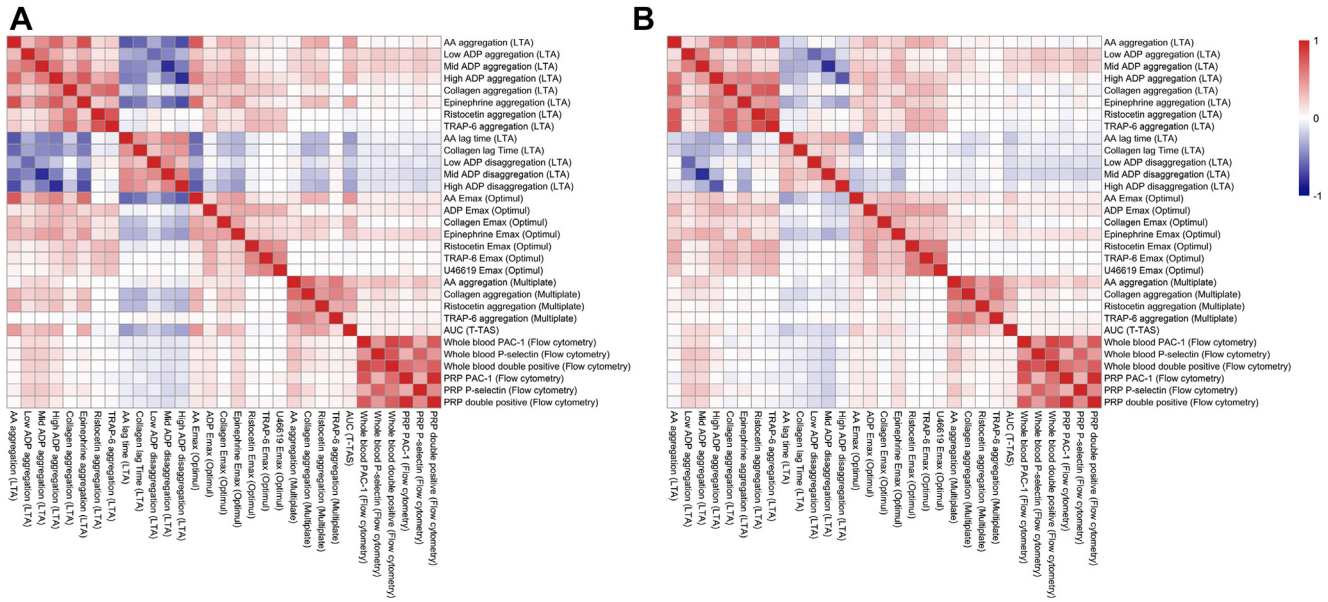

FIGURE 2 Correlation matrices of the 5 platelet assays in (A) all Framingham Heart Study participants (N = 3429) and in (B) participants without aspirin use (n =2693). Darker red shows high positive correlation, darker blue shows high negative correlation, and white shows no correlation. AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; E_{max}, maximum effect (% aggregation); LTA, light transmission aggregometry; PAC-1, procaspase-activating compound-1; PPP; platelet-poor plasma; PRP, platelet-rich plasma; TRAP-6, thrombin receptor activating peptide 6; T-TAS, Total Thrombus-Formation Analysis System.

batches across the entire examination, batch was associated with a few other variables, particularly in LTA (ADP, collagen, and TRAP-6 amide), MP (AA, collagen, and TRAP-6 amide), and flow cytometry (ADP; Figure 3; Supplementary Table S8).

3.3.6 | Technicians

The assay technician influenced some of the tests. In particular, the Optimul tests, T-TAS, and flow cytometry traits were most strongly associated with technician differences. Technician differences for these assays typically explained around 1% to 3% of variance. However, it was as high as 6.4% for Optimul ristocetin AUC, 5.2% for PRP 20 µmol/L ADP P-selectin response, and ~3% for Optimul ADP, collagen, and epinephrine assays (Figure 3; Supplementary Table S8). It is worth noting that there may have been some confounding factors, such as certain technicians working primarily in earlier periods of the 3-year examination when Optimul plates came from the United Kingdom or technicians that tended to run samples earlier or later in the day.

4 | DISCUSSION

4.1 | Overview of the study

In the present study, we used the FHS cohort to provide a previously unprecedented scale of population-level data on platelet function using 5 assay platforms, including the ubiquitous LTA and newer Optimul PRP assays, as well as the WB MP assay, flow cytometric analysis of platelet activation markers, and finally the recently developed T-TAS assay that flows platelets under shear stress over collagen-coated microvessels [25]. By directly comparing our assay outputs, we were able to determine that there are strong intra-assay correlations and that consistency across different assay modalities is relatively weak. In addition, we identified previously unexplored technical contributions to platelet reactivity measures.

4.2 | Assay correlations

The agonist is usually considered the driving factor of platelet reactivity, with "stronger" primary agonists such as collagen or thrombin eliciting greater aggregation. Although we observed interassay correlations for some agonists, they were not overwhelming and somewhat tied to sample preparation (eg, WB or PRP) [30,31]. Indeed, TRAP-6 amide LTA and Optimul responses correlated well with each other, though differences between these assays have been discussed previously [32,33]. Interestingly, TRAP-6 amide LTA responses did not correlate with MP responses at all. Previous studies have shown only a fair correlation of LTA and MP responses with an overall 85% concordance, and other studies concluded that MP, or impedance aggregometry generally, is less sensitive than LTA in detecting mild platelet function disorders [7,11,34,35]. We did show, however, that LTA and Optimul responses to AA corresponded strongly with MP ASPItest, confirming its application as a point-of-care test to detect aspirin use, though likely with less discriminative capacity than PRP-based measurements [36]. In line, ADP-stimulated responses in LTA and P-selectin expression in flow cytometry were moderately correlated, which have been demonstrated

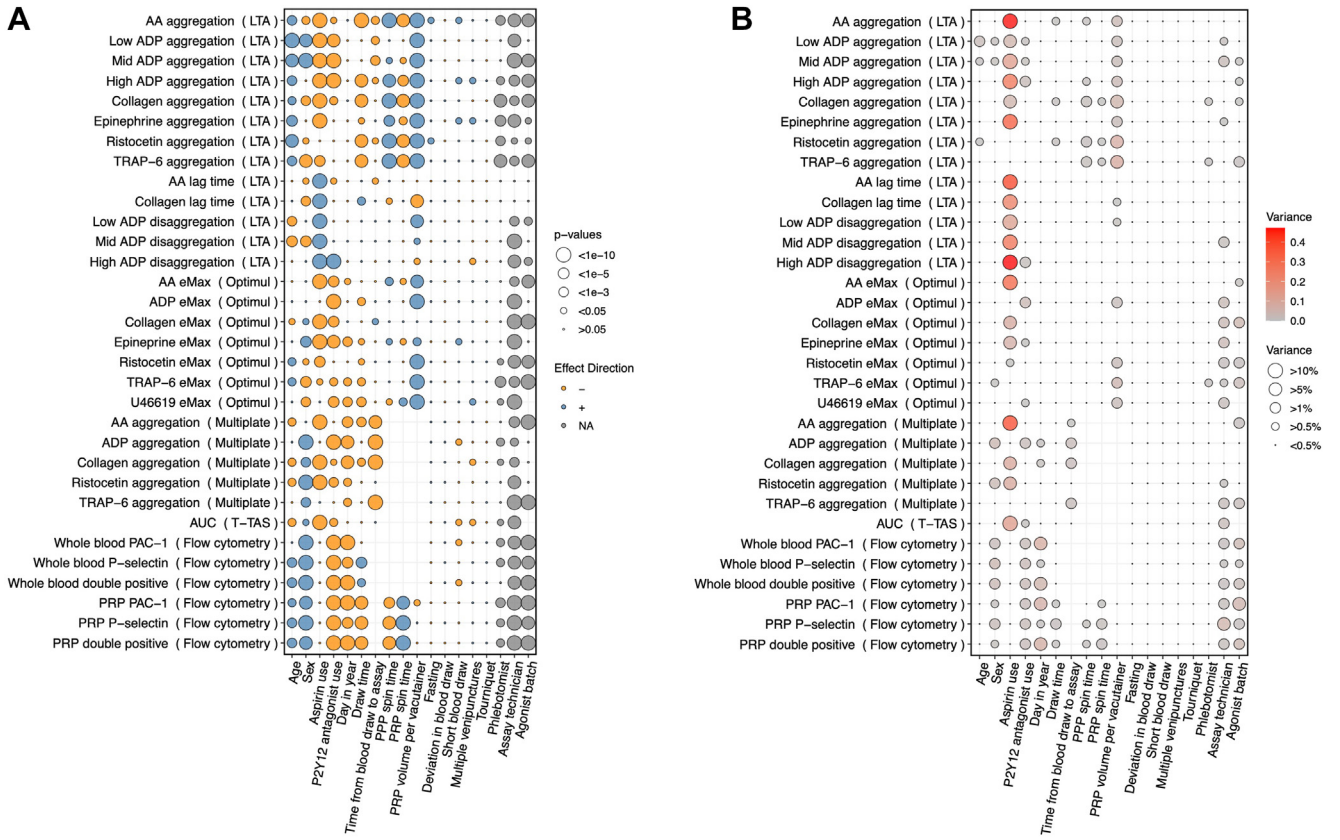

FIGURE 3 Graphical results of technical factor analysis against platelet traits of (A) *P* values and (B) explanation of variance in all assays. In panel A, the data were binned according to *P* value, with a smaller *P* value corresponding to a larger circle. Positive (blue) and negative (orange) effect direction was determined by the calculated β from analyses. No effect direction (gray) was reported when a global test was performed on 3 or more levels. In panel B, variance percentages were binned and colored red. Therefore, larger red circles represent a large portion of the trait variance attributed to this factor. AA, arachidonic acid; ADP, adenosine diphosphate; AUC, area under the curve; E_{max}, maximum effect (% aggregation); LTA, light transmission aggregometry; PAC-1, procaspase-activating compound-1; PPP; platelet-poor plasma; PRP, platelet-rich plasma; TRAP-6, thrombin receptor activating peptide 6; T-TAS, Total Thrombus-Formation Analysis System.

in patients with suspected platelet function defects and in the assessment of dual antiplatelet therapy efficacy [37,38].

4.3 | Technical factors

A unique aspect of our study is the addition of an analysis of the technical aspects of blood collection and performance of each assay using well–statistically powered multivariable models. Though there have been efforts to standardize blood collection and analysis of platelets using LTA or flow cytometry, to our knowledge, there are no large-scale studies that have systematically analyzed platelet function testing readouts with technical factors [27,39]. Here, we have confirmed previous factors that influence platelet reactivity and identified new ones that must be considered when interpreting results.

4.3.1 | Antiplatelet drugs

Although there was good concordance with self-reported aspirin use and aspirin use defined according to our criteria of AA response in LTA, followed by second-line use of MP ASPItest, it was not perfect due to possible significant platelet turnover since last aspirin dosing or reporting errors in participant recall or knowledge of over-the-counter drug formulations [40]. In our study, aspirin contributed to ~50% of the variation in the AA LTA aggregation response. We showed that aspirin also targets collagen- and epinephrine-induced platelet reactivity, indicating that TxA_2 is likely involved in the amplification of these responses. We replicated findings that aspirin attenuates collagen and AA MP responses in patients, including those with diabetes [19,41,42]. Aspirin also had a large effect on the collagen-based T-TAS assay, which demonstrates its ability to be used to discriminate the effects of this drug [43]. In concert, we showed that ADP responses in all assays were blunted in the presence of $P2Y_{12}$ receptor antagonists, which has also been demonstrated in healthy volunteers and patients [18,44]. Studies have assessed high platelet reactivity in the presence of $P2Y_{12}$ inhibitors in patients using LTA and MP stimulated by ADP, but 15% of patients were still misclassified compared with vasodilator-stimulated protein expression in flow cytometry [45]. Our findings underline the importance to any platelet study of having strict accounting of antiplatelet medications by questionnaire and reliable assays to determine this. Otherwise,

unaccounted medications could produce very strong outlier effects in nearly any study design format. Unfortunately, we were unable to discern the effects of aspirin (n = 714) or $P2Y_{12}$ antagonist (n = 11) monotherapy vs dual antiplatelet therapy (n = 22) due to low $P2Y_{12}$ antagonist use in this general middle-aged population, though we accounted for $P2Y_{12}$ antagonist use in our multivariable models.

4.3.2 | Age and sex

Reference ranges for assays are generally provided by the manufacturer or in-house laboratory measures but tend to be inferred from a limited number of healthy volunteers and not separated by demographic groups, in particular sex, which has a large effect on platelet reactivity. Here, we provided population reference ranges according to sex and aspirin use for each assay and have performed multivariable regression analyses between technical factors and platelet reactivity outcome measurements that support major group differences (Tables 2–6). In addition, we report that both age and sex affect platelet reactivity traits, with sex strongly affecting MP and flow cytometry traits and age dominating ADP and epinephrine PRP traits. We confirm the findings of others that female sex and increased age are associated with greater platelet reactivity in LTA [42,45,46]. This is in contrast to our findings in a population aged over 65 years, which showed no difference in platelet reactivity in LTA. However, in the present study, we assayed a broad range of ages from 32 to 93 years [17]. Furthermore, we saw strong effects of PRP volume per citrate tube. Lower PRP volume, which tends to be in males due to a higher hematocrit, was associated with lower platelet reactivity. Indeed, elevated hematocrit has been implicated in thrombosis, which may contribute to the elevated cardiovascular risk seen in males and could be due to increased platelet margination by increased red cells in central flow [47,48].

4.3.3 | Other factors

In addition to the major effects of aspirin, age, and sex, we identified several other factors that affected platelet reactivity measures. We saw a large batch effect in Optimul due to differences in origin of agonists (Supplementary Materials) on plates that were manufactured in the United Kingdom vs in-house, which was due to early startup phases in the United States or equipment downtime. Smaller but significant batch variations were seen in ADP batch used in flow cytometry, MP, and LTA. In agreement, P-selectin expression over time with the same donor has been shown to be consistent when the same batch of ADP was used [49]. Further, we noticed a reduction in PAC-1 expression in the summer and fall, which is likely linked to surface marker distribution since seasonal effects have been described, where platelet counts are lowest in the summer and mean platelet volume is highest in the spring [50,51]. A later morning draw time moderately corresponded with decreased platelet function [52–54]. The early morning rise in platelet function may have adaptive origins and has been suggested as one factor that may account for a higher prevalence of thrombosis in those hours [55]. Nearly all (99%) of our assays were conducted within 4 hours, which falls in line with the recommendations by various guidelines [27,56,57], and the time to assay did not greatly affect platelet function. With this, we conclude that recording draw time is likely more important than time from draw to test for most assays. However, calculating time until assay may still be a useful factor that can easily be acquired and may be most relevant in impedance aggregometry.

Finally, personnel accounted for some variation in assays. There were 4 phlebotomists used through the examination, and 2 performed 76% of all blood draws. Our results indicate that the phlebotomist had a moderate effect on some LTA traits (collagen and TRAP-6), accounting for 0.78% to 0.87% of the variance in these traits. This may have been due to the fact that some phlebotomists are called on for more difficult blood draws (older participants and participants with comorbidities), which may have skewed results. In addition, deviation in blood draw and fasting status did not affect platelet traits. The greatest variations seen in technician were demonstrated in assays with more complex sample manipulation. In particular, in flow cytometry, there are multiple centrifugation and liquid handling steps, including discarding supernatant around a cell pellet, which can be subjective and result in increased variability.

4.4 | Limitations/future studies

Though we described the first demonstration of in-depth platelet function across a variety of agonists and assays in a large population, this is still by no means comprehensive. Indeed, other agonists, combinations of agonists, concentrations, and assays, such as platelet function analyzer-100/200, vasodilator-stimulated protein, and VerifyNow point-of-care tests are utilized by researchers in the field. However, we were limited in time, cost, and blood volume available [58]. In addition, we did not directly measure platelet count by a traditional approach and, therefore, did not exclude participants with thrombocytopenia from our analysis [59]. Furthermore, since this was a population sample study, we did not exclude participants who reported common bleeding disorders including von Willebrand disease and immune thrombocytopenic purpura to reflect the prevalence of these in the population. Finally, reports of platelet reactivity are mostly limited to European ancestry, though some small studies have demonstrated attenuation of responses in African ancestries, particularly to ristocetin [45,60]. The influence of ethnicity on platelet reactivity is ill-explored, and the diversity of this cohort allows the exploration of this, which we hope to address in future studies [61].

5 | CONCLUSIONS

In this study, we identified several technical factors that should be considered when interpreting platelet reactivity outcomes. Though,

ideally, we would recommend that these sources of variabilities be limited, the practicalities of platelet reactivity testing often preclude this. As such, we encourage researchers to note these covariates and, where possible, match or exclude these variables when designing their studies. At the very least, age, sex, and aspirin use should be considered when interpreting platelet reactivity data rather than a "one-size-fits-all" approach. Here, we provide a road map of factors that researchers should mitigate if they have chosen a particular assay (Supplementary Table S8). By sorting according to their assay of choice, they can then identify which technical factors significantly modulate the data. For example, if they are already using Optimul, they should take care to note batches or perform all their experiments within one batch. Conversely, they should not be overly concerned if a blood draw is performed in the early vs late morning or a fasting period is within a precisely defined window as long as they exclude lipidemic samples in LTA. If they are choosing which assay would be the best for a long-term study, they could use LTA as it offers assay flexibility and reaction-time outputs and appears less reliant on effects like seasonality and technician variation. We recognize that each study, however, is highly context-dependent and that the dynamics of each assay, the sample preparation, and the agonist need to be reviewed. In addition, which assays are chosen must balance with the availability of equipment, blood volume, and the research question to be addressed.

We emphasize that platelet assays are not interchangeable and that one assay cannot be considered a surrogate for another due to different technologies and platelet preparations. Indeed, in order to provide more comprehensive studies of platelet function dynamics, researchers will be in the best position if they apply several testing modalities, agonists, and concentrations.

ACKNOWLEDGMENTS

We would like to thank the Boston University laboratory staff, and we are indebted to the generosity of the Framingham Heart Study participants. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute, the National Institutes of Health, or the US Department of Health and Human Services.

FUNDING

This work was supported by the US National Heart, Lung, and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195). This research was conducted in part using data and resources from the Framingham Heart Study of the National Heart, Lung, and Blood Institute of the National Institutes of Health (1ZIAHL006170) and Boston University School of Medicine.

ETHICS STATEMENT

This study was approved by the Boston University Medical Center institutional review board, and participants gave written informed consent.

AUTHOR CONTRIBUTIONS

M.V.C. contributed to the concept and design; collected, analyzed, and interpreted the data; and drafted the manuscript. M.-H.C., F.T., B.B.N., J.G., and J.E.H. analyzed and interpreted the data. A.R.L., A.D.J., Z.E.S., and C.W.d.M. collected the data. M.A.H., H.E.A., P.C.A., and T.D.W. contributed to the design and revised the intellectual content. A.D.J. contributed to the overall concept and design, analysis, and interpretation of the data. All authors approved the version to be published.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2024.102406