Journal of the American Heart Association

ORIGINAL RESEARCH

Platelet Function Is Associated With Dementia Risk in the Framingham Heart Study

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BACKGROUND: Vascular function is compromised in Alzheimer disease (AD) years before amyloid and tau pathology are detected and a substantial body of work shows abnormal platelet activation states in patients with AD. The aim of our study was to investigate whether platelet function in middle age is independently associated with future risk of AD.

METHODS AND RESULTS: We examined associations of baseline platelet function with incident dementia risk in the community-based FHS (Framingham Heart Study) longitudinal cohorts. The association between platelet function and risk of dementia was evaluated using the cumulative incidence function and inverse probability weighted Cox proportional cause-specific hazards regression models, with adjustment for demographic and clinical covariates. Platelet aggregation response was measured by light transmission aggregometry. The final study sample included 1847 FHS participants (average age, 53.0 years; 57.5% women). During follow-up (median, 20.5 years), we observed 154 cases of incident dementia, of which 121 were AD cases. Results from weighted models indicated that platelet aggregation response to adenosine diphosphate 1.0 µmol/L was independently and positively associated with dementia risk, and it was preceded in importance only by age and hypertension. Sensitivity analyses showed associations with the same directionality for participants defined as adenosine diphosphate hyper-responders, as well as the platelet response to 0.1 µmol/L epinephrine.

CONCLUSIONS: Our study shows individuals free of antiplatelet therapy with a higher platelet response are at higher risk of dementia in late life during a 20-year follow-up, reinforcing the role of platelet function in AD risk. This suggests that platelet phenotypes may be associated with the rate of dementia and potentially have prognostic value.

Key Words: aggregation ■ Alzheimer's disease ■ dementia ■ Framingham ■ LTA ■ platelet function ■ risk prediction

Alzheimer disease (AD) is the most important form of dementia and its growing prevalence requires biomarkers that can identify AD risk as early as middle age, when preventive interventions will be more effective.[1,2] Recent studies suggest that vascular function is compromised in AD years before amyloid-beta (Aβ) and tau abnormalities can be detected,[3–5] and platelet activation is one of the earliest events observed in capillary dysfunction.[6] Prior work also suggests that platelets play a functional role in amyloid plaque formation in experimental animal models,[7–9] and both abnormal platelet activation and fibrinogen-amyloid

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Supplemental Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.121.023918

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Using one of the largest platelet function repositories available at the Framingham Heart Study, we explored associations of the platelet aggregation response measured by light transmission aggregometry and the risk of developing subsequent dementia during a 20-year follow-up.
- Our study shows middle-aged individuals free of antiplatelet therapy with a higher platelet aggregation response to 1.0 μmol/L adenosine diphosphate are at higher risk of developing dementia.
- Associations with the same directionality were observed for the response to both adenosine diphosphate and epinephrine, suggesting that the associations are not agonist-specific.

What Are the Clinical Implications?

- Prior research has shown that platelets participate in beta-amyloid and tau physiology, potentially contributing to Alzheimer disease pathology, however, whether abnormalities in platelet function are associated with the risk of developing Alzheimer disease later in life is unknown.
- Our study reinforces a role of platelet function in Alzheimer disease, suggesting that platelet phenotypes may be associated with the rates of developing dementia and potentially have prognostic value.
- Future methodological innovations for large-scale exploration of platelet function in at-risk populations are needed.

Non-standard Abbreviations and Acronyms

FHS	Framingham Heart Study
LTA	light transmission aggregometry

hemostasis interactions have been reported in patients with AD.[10–14]

The central role of platelets in cardiovascular disease (CVD) is well established, and platelet function is the target of drug treatments to prevent arterial thrombosis. Longitudinal studies have consistently demonstrated an association between platelet function and CVD events in patients with established coronary artery disease,[15–17] and the role of platelet function in predicting incident CVD events and mortality, remaining independently significant after adjusting for traditional risk factors, has been described in a healthy population from the FHS (Framingham Heart Study).[18] Recently, investigators using the FHS demonstrated an independent role of cardiovascular risk profiles on the risk of incident dementia when combined with genetic risk profiles.[19] Whether an abnormal platelet activation state is associated with a heightened risk of AD later in life is still unknown.

The FHS is a longitudinal, prospective, community-based cohort with long-term surveillance that contains a large number of individuals who are free of antiplatelet therapy and with laboratory-based measures of platelet function. The aim of this study was to examine whether baseline platelet function was independently associated with incident dementia in the FHS.

METHODS

All participants provided written informed consent. Study protocols and consent forms were approved by the institutional review board at the Boston University Medical Center. All data and materials have been made publicly available at the BioLINCC repository and can be accessed at https://biolincc.nhlbi.nih.gov/home/. Code used for analysis is available upon reasonable request and for collaboration and reproducibility purposes.

Framingham Heart Study

The FHS is one of the oldest active longitudinal cohort studies in the United States, initiated in 1948 and with over 70 years of follow-up in the baseline cohort. The original cohort had 5209 residents of Framingham, MA, who after recruitment underwent up to 32 examinations, every 2 years, where a variety of clinical and laboratory data were collected.[20] In 1971, a total of 5124 offspring of the original cohort and their spouses were enrolled in the 'offspring' cohort. A total of 9 examinations are available in the offspring cohort, with the latest completed examination performed in 2011 to 2014.[21] Offspring cohort participants attending the fifth examination cycle (1991–1995), during which platelet function was assayed, were eligible for the present investigation.

Platelet Aggregation Data

Platelet aggregation was previously characterized, and the methods have been described.[18] A total of 3799 individuals attending the fifth examination cycle of the offspring cohort were considered. Excluded observations were those when the use of aspirin was reported at the time of platelet function analyses, as determined by lack of platelet response to arachidonic acid. Briefly, platelet aggregation was evaluated in fresh citrated blood samples in isolated platelet rich plasma in

response to adenosine diphosphate (ADP) and epinephrine by light transmission aggregometry (LTA) with a 4-channel PAP-4 aggregometer (Bio/Data, Horsham, PA). Our analysis primarily focused on the platelet aggregation response to ADP 1.0 µmol/L because of its association with CVD outcomes in our previous study[18] and larger number of observations available. As a secondary analysis, the aggregation response to ADP 3.0 and 5.0 µmol/L, and epinephrine at 0.1, 0.5, 1.0, and 3.0 µmol/L were also explored. Participants who responded (≥50% maximal aggregation) at least at 1 low dose of ADP (0.05, 0.1, 0.5, and/or 1.0 µmol/L) were considered hyper-responders for ADP. Similarly, hyperreactivity to epinephrine was defined as ≥50% maximal aggregation with at least 1 low dose of epinephrine (0.01, 0.03, 0.05, 0.1, 0.5, or 1.0 µmol/L).

Dementia Surveillance

Dementia characterization methods in the FHS were made in accordance with Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria and FHS methods for continuous surveillance of dementia have been previously described.[22–25] Cognition was examined every cycle with the use of the Mini-Mental State Examination scale.[26] The Mini-Mental State Examination was used to identify participants for dementia screening when performance fell below education-based cutoff scores at any examination; there was a decline of >3 points between consecutive examinations, or >5 points from the participant's highest obtained Mini-Mental State Examination score. Participants were also flagged for further evaluation in response to referrals or concerns from participants themselves, relatives, or other professionals. Flagged participants were offered a full neuropsychological test battery and a neurological examination, which were reviewed to refer for dementia review. The dementia review panel, which includes neurologists and neuropsychologists, reviews possible cognitive decline and dementia and determines whether a participant had dementia, the dementia subtype, and the date of diagnosis using data from multiple sources. After a participant dies, a medical panel manually reviews medical records up to the date of death and includes an assessment of whether the participant might have had cognitive decline since his or her last examination. This medical panel refers any participants who might have had cognitive decline to the dementia review panel for postmortem review. The main outcome of our study was incident dementia using continuous surveillance with clinician diagnosis at the end of the follow-up period up to 2018. A sensitivity analysis for confirmed diagnosis of AD was also conducted.

Covariates

Baseline was defined as the time of clinic examination corresponding to the platelet function detection (examination cycle 5). Smoking was defined based on smoking status the year preceding baseline. We defined hypertension as a systolic blood pressure ≥130 mm Hg and/or use of antihypertensive drugs.[27] Diabetes was identified by fasting glucose levels >126 mg/dL (7.0 mmol/L) and/or use of diabetes treatments. Levels of all cardiovascular risk variables including body mass index, total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined from examination cycle 5. Years of education were included for each participant. History of cardiovascular disease (CVD) events at the time of clinical examination included: reported history of coronary heart disease, congestive heart failure, myocardial infarct, intermittent claudication, ischemic stroke, intracerebral hemorrhage, or transient ischemic attack.

Statistical Analysis

Variables used in the analyses were: (A) platelet function (% maximal aggregation) in response to ADP at 1.0 µmol/L; (B) outcome: clinical dementia diagnosis; and (C) basic demographic and clinical parameters (continuous or categorical), which included age, sex, years of education, body mass index, smoking, high-density lipoprotein cholesterol, low-density-lipoprotein cholesterol, total cholesterol, triglycerides, hypertension, diabetes, and history of CVD. Demographic and clinical differences between study groups were assessed in univariate analyses using t tests for continuous variables and χ^2 tests for nominal variables. When non-normal distributions were detected, Kruskal–Wallis tests were used. Spearman correlations were used to test the association between continuous variables. In general, statistical significance was defined by $P<0.05$, and tendencies by $P\leq0.1$.

R 3.6 and Python 3.7 were used for statistical analysis and visualization. Duration of follow-up was calculated from the date of platelet function characterization until the latest clinical diagnosis available before the end of follow-up in 2018. In observational studies like the FHS where there is no random assignment to treatment groups (or variable of interest like the platelet function response in our case), the unadjusted comparison between treatment groups may be misleading because of confounding. One method to adjust for measured confounders is inverse probability of treatment weighting (IPW).[28,29] To identify potential independent associations between platelet aggregation and incident dementia, we fit Cox proportional hazard models with IPW as described previously.[30] The IPW approach weighted each subject by the inverse of the probability of each subject's observed platelet function level using the median as a

cutoff, adjusting for non-random selection of subjects into high versus low platelet function groups. These probabilities were estimated from a logistic regression model for high versus low platelet function, with adjustment for age, sex, high school education, body mass index, hypertension, diabetes, current smoking status, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides, and history of CVD. The Cox models were then weighted by the estimated probabilities of platelet function level, with adjustment for the same covariates. The proportional hazards assumption was validated using the Schoenfeld residual test included in the cox.zph function of the coxph package.[31,32] To help interpretability and visualization of forest plots, we computed hazard ratio corresponding to an increase in LTA of 10 units (LTA=LTA/10). Spline terms on the platelet aggregation response were used to estimate the relationship between the platelet aggregation response and risk of dementia in the weighted Cox models. Next, we estimated the cumulative incidence function for dementia for the ADP hyper-responder groups (yes/no) using propensity scores and the causalCmprsk package.[28] Because of the competing risk of death, the models are interpretable as cause-specific hazard models, ie, for the risk of dementia among those still alive. Finally, in an exploratory approach we applied the survival data implementation of Breiman random-forest models in the randomForestSRC[33] to estimate the relative importance of each of the covariates in dementia risk. The variable importance of each predictor is estimated by using variable selection methods of random forest survival models. The variable selection method uses a prediction error approach by "noising-up" each variable in turn. The variable importance of a variable Xi is the difference in prediction error when Xi is randomly permuted, compared with the prediction error under the true values. The package ggRandomForests was used for visualization.

RESULTS

Study Sample

The final study sample consisted of 1847 participants from FHS and included the combination of platelet aggregation (ADP 1.0 µmol/L) and clinical and demographic covariates (Figure 1) described previously. Baseline characteristics for all participants at the time of platelet function characterization are shown in Table 1. The average age of participants in the study at baseline was 53 years (interquartile range, 47–61); 57.5% were women (1062).

Platelet Aggregation

Platelet aggregation response to 1.0 µmol/L ADP followed a bimodal distribution and the median response was 11.0. Participants showing a higher response to ADP (above median) were older, predominantly women, with higher high-density-lipoprotein cholesterol and total cholesterol, and more likely to have a history of hypertension (P<0.05) (Table 1).

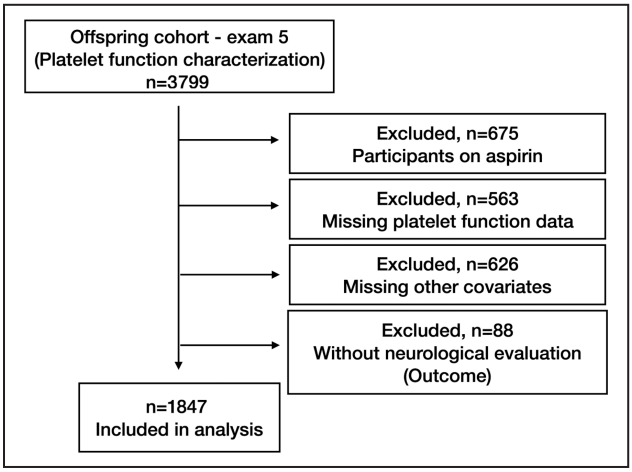

Figure 1. Inclusion diagram.

Incident Dementia

During follow-up (median, 20.5 years [interquartile range, 14.9–25.0]), 154 cases of incident dementia (102 AD cases without vascular dementia, 19 mixed dementia cases (AD+ vascular dementia), 5 vascular dementia cases (non-AD), and 28 other dementias that include frontotemporal, Lewi body, and other dementias of unknown subtype), and 269 deaths without incident dementia were observed. Logistic models for the estimation of the IPW weights indicated that age and sex were strongly associated with the platelet function response to 1.0 µmol/L ADP (Table S1). Associations with the same directionality were found when estimating weights for the other agonists and concentrations. Hazard ratios for the univariate and fully adjusted IPW multivariate Cox proportional hazard models for incident dementia are summarized in Table 2. The platelet aggregation response to 1.0 µmol/L ADP was independently associated with dementia risk (Figure 2, Table 2). Results indicated a 7% increase in dementia risk for a 10-unit increase in the response to 1.0 µmol/L ADP in the fully adjusted models. A sensitivity analysis for confirmed AD suggested results of the same directionality with the platelet aggregation response associated to higher rates of AD (Table S2). Cubic spline analysis suggested a continuous linear trajectory for the association of the response to ADP and the risk of dementia and AD both in unadjusted and adjusted models (Figure 3). Ranked feature importance of

Table 1. Baseline Demographic and Clinical Characteristics of the 1845 Men and Women in the Study Sample Grouped by Platelet Aggregation Response to 1.0 µmol/L ADP

	Overall (n=1847)	Platelet response to ADP-1 µmol/L, below median=11.0 (n=925)	Platelet response to ADP-1 µmol/L, above median=11.0 (n=922)	*P* value
Age, median [Q1, Q3], y	53.0 [47.0, 61.0]	51.0 [46.0, 60.0]	55.0 [48.0, 62.0]	<0.001
Women, n (%)	1062 (57.5)	452 (48.9)	610 (66.2)	<0.001
Years of education, mean (SD)	14.1 (2.6)	14.2 (2.6)	14.0 (2.6)	0.114
BMI, median [Q1, Q3]	26.3 [23.6, 29.3]	26.4 [23.8, 29.4]	26.2 [23.5, 29.2]	0.197
LDL cholesterol , median [Q1, Q3]	125.0 [103.5, 146.0]	124.0 [103.0, 143.0]	126.0 [104.0, 150.0]	0.11
HDL cholesterol, median [Q1, Q3]	49.0 [40.0, 60.0]	48.0 [38.0, 58.0]	50.0 [42.0, 62.0]	<0.001
Total cholesterol , median [Q1, Q3]	203.0 [179.0, 226.0]	202.0 [178.0, 221.0]	204.0 [180.0, 230.0]	0.004
Triglycerides, median [Q1, Q3]	113.0 [82.0, 163.0]	115.0 [82.0, 166.0]	112.0 [83.0, 160.0]	0.619
Smoker, n (%)	365 (19.8)	186 (20.1)	179 (19.4)	0.752
Diabetic, n (%)	48 (2.6)	20 (2.2)	28 (3.0)	0.301
Hypertense, n (%)	667 (36.1)	313 (33.8)	354 (38.4)	0.047
CVD history, n (%)	104 (5.6)	55 (5.9)	49 (5.3)	0.626
Platelet function response to ADP-1.0 µmol/L, median [Q1, Q3]	11.0 [6.0, 20.0]	6.0 [4.0, 8.0]	20.0 [15.0, 39.0]	<0.001

Definitions described in Methods. ADP indicates adenosine diphosphate; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

random forest competing risk models assigned highest priority to age, followed by hypertension, as a distant second, immediately followed by the platelet response to 1.0 µmol/L ADP, education, and triglycerides (Figure S1). Variables with lower importance included low-density lipoprotein cholesterol, history of CVD, total cholesterol, body mass index, sex, diabetes, and smoking status. No associations were found for the higher concentrations of ADP (3.0 and 5.0 µmol/L) (Table 2). An association of ADP hyper-responders (yes/no) and higher rates of dementia was suggested for dementia and AD (Table 2, Table S2). Cumulative incidence function curves suggested that the incidence of dementia was higher in ADP hyper-responders (Figure S2), although the CIs are overlapping and there is insufficient power to conclude that the curves are different. For example, the probability of dementia before death occurring within 20 years for those non-ADP hyper-responders at baseline was 8.57% (95% CI, 6.34–8.75), while for those who are ADP hyper-responders it was 13.96% (95% CI, 8.60–16.06). An association was also found for the platelet aggregation response to 0.1 µmol/L epinephrine and dementia risk although the number of dementia cases was smaller (Table 2). An association with incident AD was also suggested for low-dose stimulation with 0.1 and 0.5 µmol/L epinephrine (Table S2). No associations were found for epinephrine hyper-responders or any of the higher concentrations of epinephrine (Table 2, Table S2). A sensitivity analysis for subgroups with AD and non-AD dementia (with and without vascular dementia) suggested a general association of platelet aggregation with any type of dementia, and not an association with AD specifically (Figures S3 and S4).

DISCUSSION

In addition to their primary role in thrombosis and hemostasis, platelets are immune cells with important inflammatory roles in both health and disease. The measurement of platelet function has gained interest as a biomarker of AD because of its potential mechanistic role. Our study in the community-based longitudinal FHS offspring cohort demonstrates that individuals with a higher response to ADP appear to be at a higher risk of dementia during a 20-year follow-up period. These findings are significant even after adjustment for a number of covariates that could play a confounding role in the association of platelet function with dementia risk.

The role of platelet function as a biomarker in AD is of interest and it has been extensively studied (reviewed in Plagg et al[34]). Platelets are cells that initiate and accelerate vascular inflammatory processes that are crucial in cerebrovascular health, but are also associated with tau and Aβ physiology, both hallmarks of AD pathology. Platelets are carriers of tau and Aβ species[35–38] and previous studies have proposed platelet-derived tau as a biomarker for AD.[39–41] Platelets are 300 to 500 times more concentrated in blood clots, compared with non-clotted blood; hence, allowing for a massive release of Aβ (either directly or as a byproduct of released amyloid-beta precursor protein [APP]) at the site of clot formation.[42] Research in experimental animal models of AD shows that platelets play a crucial

Table 2. Full List of Univariate and Fully Adjusted Hazards Ratios for the Association of Platelet Function with Incident Clinical Diagnosis of Dementia

Outcome: clinical dementia diagnosis			Univariate model		Fully adjusted model	
Platelet function measure	No. at risk	No. cases	HR (95% CI)	*P* value	HR (95% CI)	*P* value
ADP, µmol/L						
1.0	1847	154	1.10 (1.04–1.17)*	<0.001*	1.07 (1.01–1.15)*	0.03*
3.0	1847	154	1.10 (1.02–1.18)*	0.02*	1.04 (0.95–1.14)	0.36
5.0	1304	96	1.04 (0.91–1.19)	0.57	1.03 (0.87–1.21)	0.74
Epinephrine, µmol/L						
0.1	1038	98	1.10 (1.03–1.18)*	0.004*	1.09 (1.01–1.17)*	0.02*
0.5	1590	127	1.06 (1.00–1.12)*	0.07*	1.04 (0.98–1.11)	0.20
1.0	1667	124	1.02 (0.96–1.08)	0.56	1.00 (0.94–1.07)	0.93
3.0	936	61	1.01 (0.92–1.11)	0.83	1.01 (0.91–1.12)	0.80
Hyper-responders to ADP (yes/no)	1847	154	1.67 (1.06–2.61)*	0.03*	1.54 (0.94–2.52)*	0.08*
Hyper-responders to epinephrine (yes/no)	1847	154	1.25 (0.75–2.06)	0.39	1.35 (0.81–2.25)	0.25

Univariate and multivariate adjusted cytochrome C oxidase models with inverse probability weighting. Median follow-up was 20.5 years. Fully adjusted models included age, sex, high school education, body mass index, hypertension, diabetes, LDH, low-density lipoprotein, total cholesterol, triglycerides, current smoking status, and history of cardiovascular disease. Analysis excluded participants on aspirin at the time of platelet function determination. ADP indicates adenosine diphosphate; and HR, hazard ratio.

*Results with $P \leq 0.1$ are highlighted.

role in Aβ brain accumulation and vascular damage at early stages.[8,9,43] Furthermore, studies have shown platelets are responsible for the accumulation of Aβ in blood clots inside and around cerebral blood vessels in mouse models.[42] In addition, platelets induce the conversion of soluble Aβ to toxic aggregated species.[43,44] There is also commonality between the proteomic signature of the human brain with cerebral atherosclerosis, which can produce platelet activation, and AD pathology.[45] It is therefore feasible that

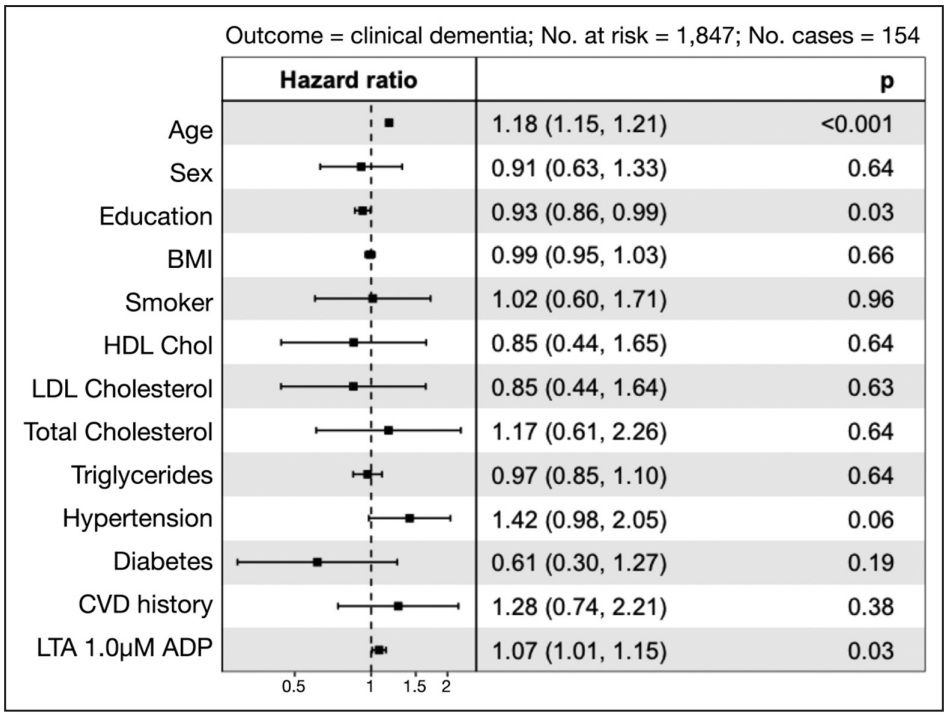

Figure 2. Forest plot for the association of platelet aggregation response to 1.0 µmol/L adenosine diphosphate and dementia risk in the FHS (Framingham Heart Study) using inverse probability of treatment weighting Cox proportional hazards regression.
ADP indicates adenosine diphosphate; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and LTA, light transmission aggregometry.

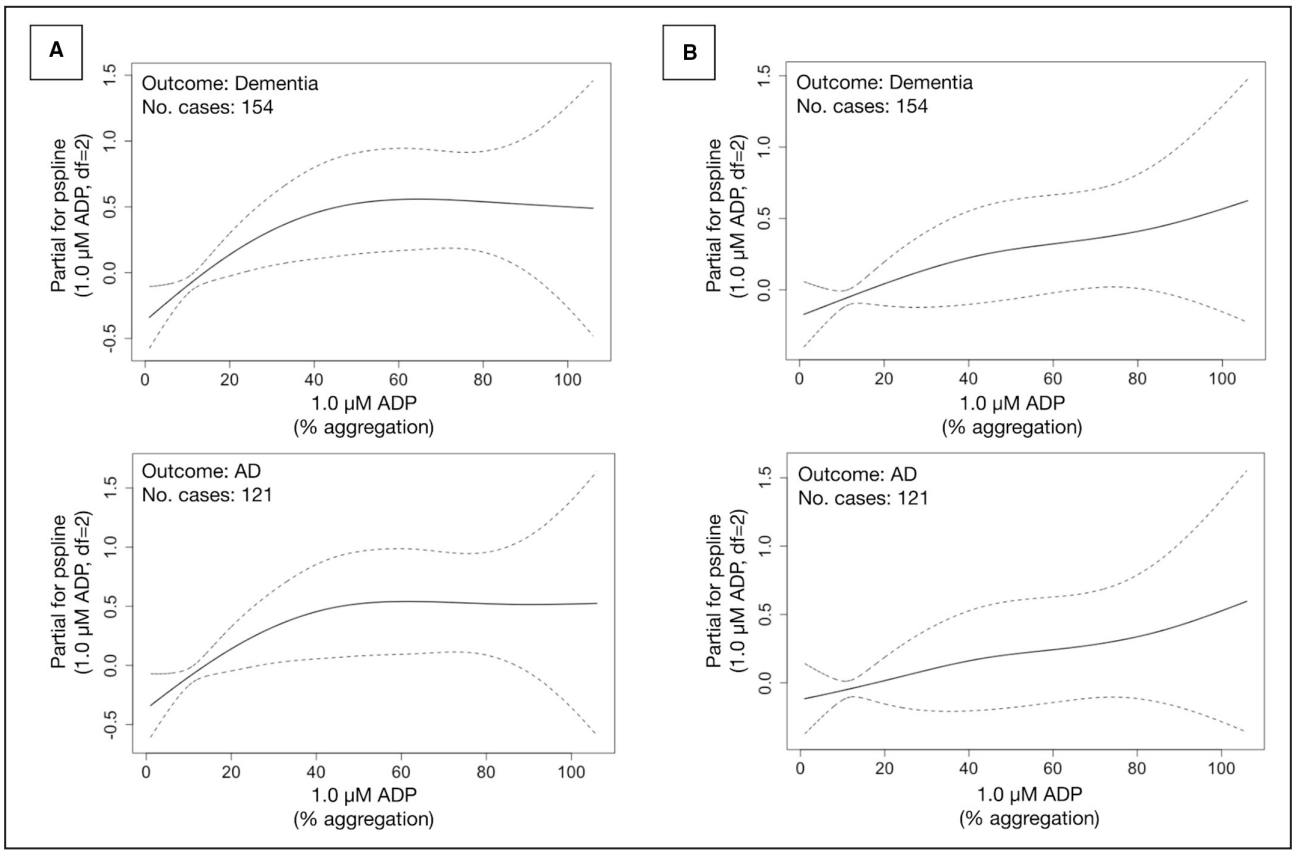

Figure 3. Unadjusted (A) and fully adjusted (B) splines of the platelet aggregation response to 1.0 µmol/L adenosine diphosphate against hazard ratio (with 95% confidence limits) for dementia and Alzheimer disease.
AD indicates Alzheimer disease; ADP, adenosine diphosphate; df, degrees of freedom; and pspline, penalised smoothing spline.

platelets could be relevant contributors to AD pathology and that an early abnormal platelet activation state precedes AD-vascular dysfunction.

Our study in 1847 participants of a well characterized community-based cohort shows an independent association of platelet function with future dementia, suggesting that platelet phenotypes may be associated with the rates of dementia and have prognostic value. Platelet aggregation can be initiated via various pathways and therefore several agonists can be used to detect platelet aggregation in functional assays, one of the most common being ADP.[46] Our results identified higher dementia risk with greater responses to ADP that was further validated with the response to epinephrine indicating that abnormal platelet phenotypes in a community-based sample who are free of antiplatelet therapy are associated with dementia risk and this deserves further study. Of special note is that the associations were significant for low-dose stimulation (1.0 µmol/L ADP and 0.1 µmol/L epinephrine) which have been previously shown to efficiently identify hyper-reactive phenotypes and outcomes.[18,47] The results of sensitivity analysis for subgroups with AD and non-AD dementia suggested a general association of platelet aggregation with any type of dementia, and not an association with AD specifically that should be evaluated in the future when more cases become available.

Discrepant results have prevented establishing a robust platelet-derived AD biomarker, probably because of technical challenges associated to platelet function detection, as well as differences in study design and measurements.[48–50] Data suggestthat peripheral platelets could be abnormally activated in early and/or preclinical stages of AD. Maccioni et al. has repeatedly proposed platelet-tau as a biomarker for AD indicating that platelet tau/p-tau ratio correlates with cognitive impairment,[39] and that the ratio of high molecular weight tau/low molecular weight tau in platelets correlates with regional brain atrophy.[51] Previous research by Ahn et al, using flow cytometry identified a heightened platelet activation state in AD compared with controls.[10] These results were later validated by the group of Laske et al, who additionally observed that activated glycoprotein IIb-IIIa complex and P-selectin were higher in patients with AD with fast cognitive decline compared with patients with AD with slow cognitive decline during a 1-year follow-up period.[52] Interestingly, it has also

been shown that Aβ binds the activated glycoprotein IIb-IIIa complex through its RHDS sequence, which causes integrin outside-in signaling and downstream activation of Syk and PLCr2, that ultimately promotes the release of the chaperone clusterin and ADP from platelets.[9] A recent study in an AD transgenic mouse model has also shown that the major contribution of atherosclerosis, to the risk of developing AD pathology, is via its effects on blood coagulation and the formation of platelet-mediated Aβ aggregates which compromise cerebral blood flow and therefore neuronal function.[43]

Meanwhile, other studies explored potential proxies of platelet function such as mean platelet volume, or markers of platelet enzymatic activity such as APP expression, the APP ratios, BACE1, ADAM-10, or cytochrome C oxidase, among others.[34] Still, to our knowledge, no studies have explored whether platelet function is associated with future risk of dementia. Several limitations may have prevented this, including technical challenges associated with platelet function detection methods since most assays cannot be done using frozen samples. Despite these limitations, a recent study explored the integration of LTA data in machine-learning models for the classification of AD versus healthy controls and reported a sensitivity of 96.6% and specificity of 80% for models that included a combination of LTA, clinical markers, and micro-RNA data. Although the results suggested that platelet function data may contribute to AD biomarker panels,[14] the authors found a higher platelet response to 0.5 μmol/L ADP in healthy controls compared with AD cases, which contradicts our hypothesis that a heightened platelet function in middle age is associated with a higher risk of incident AD. Three important differences may help explain these differences. First, the cross-sectional design and use of a case control approach with a small number of observations may limit the generalizability of the findings to larger populations. Second, potential sex differences may have influenced the results, since the authors had a higher number of women in their control population (55%) compared with the AD group (45%). Because female sex has such a large effect on all platelet assays (also evidenced in our FHS study) this may explain why they identified higher platelet function in controls. Finally, about 20% to 22% adults use aspirin regularly and it is unclear if participants on aspirin were excluded, which may have significantly affected the results. Nonetheless, the same study identified higher PAC-1 (activated GP IIb/IIIa) binding to ADP stimulation in AD cases compared with controls, in agreement with our findings, suggesting that future studies will need to delineate the role of platelet function both in preclinical and clinical stages of AD, in combination with AD biomarkers and carefully account for potential sex-effects in the above associations.

Our study has some limitations. First, we observed associations for 1.0 μmol/L ADP and for 0.1 μmol/L epinephrine, however, we were unable to explore associations with even lower stimulation doses that may facilitate the identification of abnormal platelet phenotypes. Platelet aggregation assays are known to vary across laboratories because of a lack of standardization of the concentration of agonists used, thus making comparison of studies challenging. Despite the large community-based sample and long follow-up times, the modest number of incident dementia cases did not allow for the exploration of sex-specific or ethnic-specific subgroup analyses that should be considered as additional data become available.

In conclusion, platelet function in middle age in participants of the FHS who are free of antiplatelet therapy was independently associated with future incidence of clinical dementia during a 20-year follow-up. Given these associations remained significant after adjusting for a relatively high number of covariates, our study suggests that platelet phenotypes may be associated with the rates of incident dementia and thus potentially have prognostic value. Future methodological innovations for large-scale exploration of platelet function in at risk populations are needed.

ARTICLE INFORMATION

Received September 9, 2021; accepted February 1, 2022.

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Acknowledgments

We thank the participants of the FHS.

Sources of Funding

J.R.C. is supported by an intergovernmental Personnel Act (IPA) agreement with the Veterans Affairs (VA) Cooperative Studies Program. A.D.J. is supported by National Heart, Lung, and Blood Institute (NHLBI) intramural funds. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the NHLBI; the National Institutes of Health (NIH); or the US Department of Health and Human Services; A.B. is supported by R01NS017950, NIH/National Institute on

Aging (NIA) R01AG054076, NIH UH2 NS100605, NIH RF1AG063507, NIH/NHLBI 2018-AARG-591645, Alzheimer's Association 75N92019D00031, NIH/NHLBI RF1AG059421-01, NIH/NIA R01AG059725, NIH/NIA R01AG062531-01A1, and NIH AARG-D 2020; S.S. is supported by NIH 75N92019D00031 and AG054076; J.S. is supported by K23AG057760; J.S.B. is supported by NCT02106429, NCT01897103; N.R.F. is supported by VA Cooperative Studies Program, NIH, and American Heart Association; O.M.B. is supported by NIH K23AG068534, R25HL105444, P30AG059303, P30AG066512, L30-AG064670, and AASMF BS-231-20; A.P. is supported by NIH K25HL151912 and Itamar Medical Ltd; R.A.B. is supported by NIH 5R01NS094610-05, 1R25AG067931-01, 5P30DK040561-24, P30AG066512-01, 2P01AG036694-11, R01DA054990-01, and NSF 2034022; T.M.W. is supported by P30 AG066512, and P01 AG060882; R.S.O. is supported by R01AG056531, R01AG056031, and P30 AG066512. The FHS is funded by Boston University contract 75N92019D00031 and the NIH Dementia RO1 grant AG054076.

Disclosures

A.B. has received compensation from textbook royalties unrelated to the present work and participated as advisor for the South Texas Alzheimer's Center; S.S. has received consulting fees from Biogen Inc; J.S. has received honoraria from Eisai, and the AAN; J.S.B. has received consulting fees from Janssen and Amgen, compensation for expert testimony from Saxton and Stump, and has participated as advisor on NIH funded studies and for Amgen; B.A.A. has received consulting fees from Rutgers University; O.M.B. has participated as advisor for a Resource Center in Minority Aging Research; A.C. has participated as advisor for the Jacinto Convit World Health Organization without compensation; R.A.B. has received consulting fees for service on data safety monitoring boards for Apotex, Reata, Biogen, PTI, Alexion, and the National Cancer Institute Board of Epidemiology and Clinical Sciences. She has also received honoraria from Einstein Biostatistics, and received payment for expert testimony in plaintiffs in vena cava litigation, and patent cases for Teva, Amarin, and Amazon. The remaining authors have no disclosures to report.

Supplemental Material

Table S1–S2
Figure S1–S4

REFERENCES

1. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol*. 2011;10:819–828. doi: 10.1016/S1474-4422(11)70072-2
2. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol*. 2014;13:788–794. doi: 10.1016/S1474-4422(14)70136-X
3. Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC.; Alzheimer's Disease Neuroimaging I. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun*. 2016;7:11934. doi: 10.1038/ncomms11934
4. Nortley R, Korte N, Izquierdo P, Hirunpattarasilp C, Mishra A, Jaunmuktane Z, Kyrargyri V, Pfeiffer T, Khennouf L, Madry C, et al. Amyloid beta oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. *Science*. 2019;365.
5. Love S, Miners JS. Cerebrovascular disease in ageing and Alzheimer's disease. *Acta Neuropathol*. 2016;131:645–658. doi: 10.1007/s00401-015-1522-0
6. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol*. 2011;11:264–274. doi: 10.1038/nri2956
7. Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood*. 2012;120:e73–e82. doi: 10.1182/blood-2012-04-416594
8. Kniewallner KM, Foidl BM, Humpel C. Platelets isolated from an Alzheimer mouse damage healthy cortical vessels and cause inflammation in an organotypic ex vivo brain slice model. *Sci Rep*. 2018;8:15483. doi: 10.1038/s41598-018-33768-2
9. Donner L, Falker K, Gremer L, Klinker S, Pagani G, Ljungberg LU, Lothmann K, Rizzi F, Schaller M, Gohlke H, et al. Platelets contribute to amyloid-beta aggregation in cerebral vessels through integrin alphaiibbeta3-induced outside-in signaling and clusterin release. *Sci Signal*. 2016;9:ra52.
10. Sevush S, Jy W, Horstman LL, Mao WW, Kolodny L, Ahn YS. Platelet activation in Alzheimer disease. *Arch Neurol*. 1998;55:530–536. doi: 10.1001/archneur.55.4.530
11. Cortes-Canteli M, Zamolodchikov D, Ahn HJ, Strickland S, Norris EH. Fibrinogen and altered hemostasis in Alzheimer's disease. *J Alzheimers Dis*. 2012;32:599–608. doi: 10.3233/JAD-2012-120820
12. Zamolodchikov D, Berk-Rauch HE, Oren DA, Stor DS, Singh PK, Kawasaki M, Aso K, Strickland S, Ahn HJ. Biochemical and structural analysis of the interaction between beta-amyloid and fibrinogen. *Blood*. 2016;128:1144–1151.
13. Zamolodchikov D, Renne T, Strickland S. The Alzheimer's disease peptide beta-amyloid promotes thrombin generation through activation of coagulation factor xii. *J Thromb Haemost*. 2016;14:995–1007.
14. Wiest I, Wiemers T, Kraus MJ, Neeb H, Strasser EF, Hausner L, Frolich L, Bugert P. Multivariate platelet analysis differentiates between patients with Alzheimer's disease and healthy controls at first clinical diagnosis. *J Alzheimers Dis*. 2019;71:993–1004. doi: 10.3233/JAD-190574
15. Trip MD, Cats VM, van Capelle FJ, Vreeken J. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. *N Engl J Med*. 1990;322:1549–1554. doi: 10.1056/NEJM199005313222201
16. Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, Mohler ER, Reilly MP, Berger JS. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. *J Thromb Haemost*. 2010;8:148–156. doi: 10.1111/j.1538-7836.2009.03584.x
17. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. *J Am Coll Cardiol*. 2007;50:1822–1834. doi: 10.1016/j.jacc.2007.07.051
18. Puurunen MK, Hwang SJ, Larson MG, Vasan RS, O'Donnell CJ, Tofler G, Johnson AD. ADP platelet hyperreactivity predicts cardiovascular disease in the FHS (Framingham heart study). *J Am Heart Assoc*. 2018;7:e008522. doi: 10.1161/JAHA.118.008522
19. Peloso GM, Beiser AS, Satizabal CL, Xanthakis V, Vasan RS, Pase MP, Destefano AL, Seshadri S. Cardiovascular health, genetic risk, and risk of dementia in the Framingham heart study. *Neurology*. 2020;95:e1341–e1350. doi: 10.1212/WNL.0000000000010306
20. Dawber TR, Kannel WB, Lyell LP. An approach to longitudinal studies in a community: the Framingham study. *Ann N Y Acad Sci*. 1963;107:539–556. doi: 10.1111/j.1749-6632.1963.tb13299.x
21. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol*. 1979;110:281–290. doi: 10.1093/oxfordjournals.aje.a112813
22. Satizabal CL, Beiser AS, Chouraki V, Chene G, Dufouil C, Seshadri S. Incidence of dementia over three decades in the Framingham heart study. *N Engl J Med*. 2016;374:523–532. doi: 10.1056/NEJMoa1504327
23. Seshadri S, Beiser A, Au R, Wolf PA, Evans DA, Wilson RS, Petersen RC, Knopman DS, Rocca WA, Kawas CH, et al. Operationalizing diagnostic criteria for Alzheimer's disease and other age-related cognitive impairment-part 2. *Alzheimer's Dement*. 2011;7:35–52. doi: 10.1016/j.jalz.2010.12.002
24. Seshadri S, Wolf PA, Beiser A, Au R, McNulty K, White R, D'Agostino RB. Lifetime risk of dementia and Alzheimer's disease. The impact of mortality on risk estimates in the Framingham study. *Neurology*. 1997;49:1498–1504. doi: 10.1212/WNL.49.6.1498
25. Au R, Seshadri S, Knox K, Beiser A, Himali JJ, Cabral HJ, Auerbach S, Green RC, Wolf PA, McKee AC. The Framingham brain donation program: neuropathology along the cognitive continuum. *Curr Alzheimer Res*. 2012;9:673–686.
26. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189–198. doi: 10.1016/0022-3956(75)90026-6
27. Reboussin DM, Carey RM, Whelton PK. Evidence supporting the blood pressure treatment goal of less than 130/80 mm hg. *Hypertension*. 2019;73:972–974.
28. Vakulenko-Lagun B, Mandel M, Betensky RA. Inverse probability weighting methods for cox regression with right-truncated data. *Biometrics*. 2020;76:484–495. doi: 10.1111/biom.13162

29. Neumann A, Billionnet C. Covariate adjustment of cumulative incidence functions for competing risks data using inverse probability of treatment weighting. *Comput Methods Programs Biomed*. 2016;129:63–70. doi: 10.1016/j.cmpb.2016.03.008
30. Ramos-Cejudo J, Johnson AD, Beiser A, Seshadri S, Salinas J, Berger JS, Fillmore NR, Do N, Zheng C, Kovbasyuk Z, et al. The neutrophil to lymphocyte ratio is associated with the risk of subsequent dementia in the Framingham heart study. *Front Aging Neurosci*. 2021;13:773984. doi: 10.3389/fnagi.2021.773984
31. Grambsch PM. Goodness-of-fit and diagnostics for proportional hazards regression models. *Cancer Treat Res*. 1995;75:95–112.
32. In J, Lee DK. Survival analysis: part II - applied clinical data analysis. *Korean J Anesthesiol*. 2019;72:441–457. doi: 10.4097/kja.19183
33. O'Brien RC, Ishwaran H, Szczotka-Flynn LB, Lass JH.; Cornea Preservation Time Study G. Random survival forests analysis of intraoperative complications as predictors of descemet stripping automated endothelial keratoplasty graft failure in the cornea preservation time study. *JAMA Ophthalmol*. 2021;139:191–197. doi: 10.1001/jamaophthalmol.2020.5743
34. Plagg B, Humpel C. *Platelets in Alzheimer's Disease, on the Non-Thrombotic Role of Platelets in Health and Disease*. IntechOpen; 2015.
35. Veitinger M, Varga B, Guterres SB, Zellner M. Platelets, a reliable source for peripheral Alzheimer's disease biomarkers? *Acta Neuropathol Commun*. 2014;2:65. doi: 10.1186/2051-5960-2-65
36. Daniele S, Pietrobono D, Fusi J, Lo Gerfo A, Cerri E, Chico L, Iofrida C, Petrozzi L, Baldacci F, Giacomelli C, et al. Alpha-synuclein aggregated with tau and beta-amyloid in human platelets from healthy subjects: correlation with physical exercise. *Front Aging Neurosci*. 2018;10:17.
37. Pomara N, Murali DP. Does increased platelet release of abeta peptide contribute to brain abnormalities in individuals with depression? *Med Hypotheses*. 2003;60:640–643.
38. Shimizu T, Uchida C, Shimizu R, Motohashi H, Uchida T. Prolyl isomerase pin1 promotes proplatelet formation of megakaryocytes via tau. *Biochem Biophys Res Commun*. 2017;493:946–951. doi: 10.1016/j.bbrc.2017.09.115
39. Neumann K, Farias G, Slachevsky A, Perez P, Maccioni RB. Human platelets tau: a potential peripheral marker for Alzheimer's disease. *J Alzheimers Dis*. 2011;25:103–109. doi: 10.3233/JAD-2011-101641
40. Akingbade OES, Gibson C, Kalaria RN, Mukaetova-Ladinska EB. Platelets: peripheral biomarkers of dementia? *J Alzheimers Dis*. 2018;63:1235–1259. doi: 10.3233/JAD-180181
41. Mukaetova-Ladinska EB, Abdell-All Z, Andrade J, da Silva JA, Boksha I, Burbaeva G, Kalaria RN, T.O'Brien J. Platelet tau protein as a potential peripheral biomarker in Alzheimer's disease: an explorative study. *Curr Alzheimer Res*. 2018;15:800–808. doi: 10.2174/1567205015666180404165915
42. Kucheryavykh LY, Davila-Rodriguez J, Rivera-Aponte DE, Zueva LV, Washington AV, Sanabria P, Inyushin MY. Platelets are responsible for the accumulation of beta-amyloid in blood clots inside and around blood vessels in mouse brain after thrombosis. *Brain Res Bull*. 2017;128:98–105.
43. Wang M, Lv J, Huang X, Wisniewski T, Zhang W. High-fat diet-induced atherosclerosis promotes neurodegeneration in the triple transgenic (3 x tg) mouse model of Alzheimer's disease associated with chronic platelet activation. *Alzheimer's Res Ther*. 2021;13:144.
44. Gowert NS, Donner L, Chatterjee M, Eisele YS, Towhid ST, Münzer P, Walker B, Ogorek I, Borst O, Grandoch M, et al. Blood platelets in the progression of Alzheimer's disease. *PLoS One*. 2014;9:e90523. doi: 10.1371/journal.pone.0090523
45. Wingo AP, Fan W, Duong DM, Gerasimov ES, Dammer EB, Liu Y, Harerimana NV, White B, Thambisetty M, Troncoso JC, et al. Shared proteomic effects of cerebral atherosclerosis and Alzheimer's disease on the human brain. *Nat Neurosci*. 2020;23:696–700. doi: 10.1038/s41593-020-0635-5
46. Cattaneo M, Cerletti C, Harrison P, Hayward CPM, Kenny D, Nugent D, Nurden P, Rao AK, Schmaier AH, Watson SP, et al. Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH. *J Thromb Haemost*. 2013;11:1183–1189. doi: 10.1111/jth.12231
47. Berger JS, Becker RC, Kuhn C, Helms MJ, Ortel TL, Williams R. Hyperreactive platelet phenotypes: relationship to altered serotonin transporter number, transport kinetics and intrinsic response to adrenergic co-stimulation. *Thromb Haemost*. 2013;109:85–92.
48. Nylander S, Johansson K, Van Giezen JJ, Lindahl TL. Evaluation of platelet function, a method comparison. *Platelets*. 2006;17:49–55. doi: 10.1080/09537100500197448
49. Fuchs G, Berg N, Broman LM, Prahl WL. Modeling sensitivity and uncertainties in platelet activation models applied on centrifugal pumps for extracorporeal life support. *Sci Rep*. 2019;9:8809. doi: 10.1038/s41598-019-45121-2
50. Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thromb J*. 2016;14:49. doi: 10.1186/s12959-016-0123-z
51. Slachevsky A, Guzman-Martinez L, Delgado C, Reyes P, Farias GA, Munoz-Neira C, Bravo E, Farias M, Flores P, Garrido C, et al. Tau platelets correlate with regional brain atrophy in patients with Alzheimer's disease. *J Alzheimers Dis*. 2017;55:1595–1603.
52. Stellos K, Panagiota V, Kogel A, Leyhe T, Gawaz M, Laske C. Predictive value of platelet activation for the rate of cognitive decline in Alzheimer's disease patients. *J Cereb Blood Flow Metab*. 2010;30:1817–1820. doi: 10.1038/jcbfm.2010.140

Supplemental Material

Data S1.

Supplemental Methods

Image analysis

Images were analysed using 4D LV-Analysis© software (TomTec Imaging Systems GmbH, Germany, 2015) by a single experienced reader. For the experimental studies, analysis of 3DE LV datasets was performed in all datasets obtained per participant (i.e. 4 analyses/participant). For the observational study, the analysis was performed according to a pre-specified protocol, and image quality was defined as follows:

1) **Good(score-1)**=clear visualization of endocardium in all 16 segments in both ED and ES frames.
2) **Fair(score-2)=**unclear visualization of endocardium in ≤2 segments or presence of minor artefacts e.g. apical noise.
3) **Adequate(score-3)**=unclear visualization of endocardium in ≤6 segments.
4) **Poor(score-4)**=unclear visualization of endocardium in >6 segments in ED or ES frames, but the endocardium can still be tracked with confidence throughout the cardiac-cycle using the adjacent segments as a reference.
5) Unacceptable image quality was defined as presence of major stitching artefacts preventing reliable tracking of the endocardium, unacceptable visualization of the LV endocardial boundaries, or ≥4 segments of the LV wall being outside of the image sector.

The software automatically selected and displayed three standard apical views and one short-axis view. Alignment of the longitudinal axis of the LV in all apical views were further modified manually if needed using two anatomical landmarks at both ends (the mitral valve annulus and the apex). The endocardial borders were then defined automatically by the software in all apical views at end-diastole. Manual adjustments could be made but these were kept as minimal as possible to enhance reproducibility. The software then tracked the endocardium throughout the cardiac cycle in 3D space from which the 3D LV endocardial shell was constructed. The tracing of LV endocardial boundaries was further adjusted manually when needed in ED and ES frames. The software then divided the LV into 16 segments and generated curves and maps of global and segmental volumetric and deformation indices.

Table S1. Feasibility of 2D-guided M-mode LV linear dimensions* in 1438 SABRE participants.

LVIDd	1354(94%)
LVIDs	1352(94%)
IVSd	1354(94%)
IVSs	1352(94%)
PWd	1354(94%)
PWs	1353(94%)

*LV volumes from conventional 2D-echocardiography were calculated by the Teichholz formula using the linear dimensions from which 2D LV ejection fraction was derived.

IVSd, diastolic interventricular septal thickness; IVSs, systolic interventricular septal thickness; LV, left ventricle; LVIDd, diastolic left ventricular internal diameter; LVIDs, systolic left ventricular internal diameter; PWTd, diastolic posterior wall thickness; PWTs, systolic posterior wall thickness.

Table S2. Baseline characteristics of SABRE participants with and without 3DE LV analysis.

	+ TomTec 3DE LV analysis (n=529)	- TomTec 3DE LV analysis (n=878)	P value
Age, y	69.1±6.1	70.0±6.1	0.009
Male, n(%)	405(76.6)	664(75.6)	0.69
Ethnicity, European/South Asian/African Caribbean(%)	51.6/28.5/20.0	45.1/40.9/14.0	<0.0001
Systolic blood pressure, mmHg	140.2±17.9	140.1±17.8	0.96
Diastolic blood pressure, mmHg	76.5±9.6	77.3±9.8	0.14
Heart rate	67.2±11.4	68.9±12.7	0.008
Body mass index, kg/m^2	26.1±3.5	28.5±5.2	<0.0001
Waist: hip ratio	0.96±0.07	0.99±0.08	<0.0001
Hypertension, n(%)	301(56.9)	642(73.1)	<0.0001
Known diabetes, n(%)	118(22.3)	322(36.7)	<0.0001
Prior coronary heart diseases, n(%)	89(16.8)	266(30.3)	<0.0001
Smoking status, never/ex/current(%)	54.1/38.1/7.8	58.7/36.0/5.3	0.09

Data are mean±SD or n(%).

Table S3. Relationships with image quality for 3D-EF and 3D-GLS in the SABRE study(n=529).

2015 ASE/EACVI guidelines-based image-quality score			
	coefficient(95% CI), *p*		
	Unadjusted	**Adjusted**	**Absolute standardized bias (%)***
3D-EF, %	-2.9(-3.9, -1.8), <0.0001	-2.5(-3.6, -1.5), <0.0001	4.6%
3D-GLS, %	-0.6(-1.1, 0.0), 0.058	-0.7(-1.2, -0.1), 0.018	3.7%
Poor image-quality segments score			
	coefficient(95% CI), *p* (per 1-point increment in score)		
	Unadjusted	**Adjusted**	**Absolute standardized bias (%)**
3D-EF, %	-1.3(-1.8, -0.8), <0.0001	-1.2(-1.7, -0.7), <0.0001	2.2%
3D-GLS, %	-0.5(-0.8, -0.2), <0.0001	-0.5(-0.7, -0.2), <0.0001	2.6%
SABRE image-quality score			
	coefficient(95% CI), *p* (per 1-point increment in score)		
	Unadjusted	**Adjusted**	**Absolute standardized bias (%)**
3D-EF, %	-2.1(-2.8, -1.3), <0.0001	-2.0(-2.7, -1.3), <0.0001	3.7%
3D-GLS, %	-0.4(-0.8, -0.1), 0.025	-0.4(-0.8, -0.0), 0.030	2.1%

Coefficients are unstandardized coefficients of regression. Adjustment was performed for age, sex, ethnicity, height, weight, heart rate, history of percutaneous coronary intervention and/or coronary artery bypass graft and/or history of chronic obstructive pulmonary disease. *The extent of adjusted bias represented in standardized terms relative to the overall mean. Abbreviations: CI, confidence interval; EF, ejection fraction; and GLS, global longitudinal strain.

Table S4. 3DE derived LV myocardial indices by poor image-quality segments score in the SABRE study(n=529).

	None-segment	1-segment	2-sgements	≥3-segments	P value
n(%)	**63(11.9)**	**115(21.7)**	**219(41.4)**	**132(25.0)**	
EDV, ml/m^2					
Mean±SD	58.4±11.8	58.6±14.4	57.9±13.9	54.7±11.4	0.067
Mean Δ(95% CI)	Reference	0.2(-3.9, 4.2)	-0.5(-4.2, 3.2)	-3.7(-7.7, 0.2)	
ESV, ml/m^2					
Mean±SD	25.7±7.1	26.4±8.4	27.6±9.3	25.9±7.1	0.179
Mean Δ(95% CI)	Reference	0.7(-1.9, 3.3)	1.9(-0.4, 4.3)	0.2(-2.3, 2.7)	
SV, ml					
Mean±SD	57.7±13.2	59.7±15.6	56.1±13.7	53.7±13.2	0.009
Mean Δ(95% CI)	Reference	2.0(-2.3, 6.3)	-1.6(-5.5, 2.3)	-3.9(-8.1, 0.3)	
n(%)	**63(11.9)**	**103(19.5)**	**212(40.1)**	**151(28.5)**	
GCS, %					
Mean±SD	27.7±3.3	26.8±4.4	25.2±4.0	24.5±3.8	<0.0001
Mean Δ(95% CI)	Reference	-1.0(-2.2, 0.3)	-2.5(-3.6, -1.4)	-3.2(-4.3, -2.0)	
Peak averaged CS, %					
Mean±SD	27.9±3.3	27.0±4.6	25.4±4.0	24.6±3.7	<0.0001
Mean Δ(95% CI)	Reference	-0.9(-2.2, 0.3)	-2.6(-3.7, -1.5)	-3.4(-4.5, -2.2)	
Peak averaged LS, %					
Mean±SD	19.1±2.7	18.9±2.8	18.0±2.9	18.1±3.2	0.008
Mean Δ(95% CI)	Reference	-0.2(-1.1, 0.7)	-1.1(-1.9, -0.2)	-1.0(-1.9, -0.2)	
Peak averaged PTS, %					
Mean±SD)	32.8±3.4	32.3± 4.4	30.8±4.0	30.2±3.9	<0.0001
Mean Δ(95% CI)	Reference	-0.5(-1.8, 0.7)	-2.0(-3.1, -0.9)	-2.6(-3.8, -1.5)	
Peak averaged RS, %					
Mean±SD	39.4±4.4	38.5±5.5	36.4±4.9	35.8±5.1	<0.0001
Mean Δ(95% CI)	Reference	-0.9(-2.5, 0.7)	-3.0(-4.4, -1.6)	-3.6(-5.1, -2.1)	
Peak basal rotation, °					
Mean±SD	6.2±3.4	6.0±3.3	5.3± 3.2	5.0±3.3	0.014
Mean Δ(95% CI)	Reference	-0.2(-1.2, 0.9)	-0.9(-1.8, 0.01)	-1.2(-2.2, -0.3)	
Peak apical rotation, °					
Mean±SD	9.4±4.2	9.5±4.3	8.0±4.3	7.3±4.5	0.0002
Mean Δ(95% CI)	Reference	0.1(-1.3, 1.4)	-1.4(-2.6, -0.2)	-2.1(-3.4, -0.8)	
Peak twist, °					
Mean±SD	15.2±6.9	15.2±6.9	13.0±6.5	11.8±7.1	0.0001
ean Δ(95% CI)	Reference	-0.0(-2.1, 2.1)	-2.2(-4.1, -0.3)	-3.5(-5.5, -1.5)	
Peak torsion, °/cm					
Mean±SD	1.9±0.9	1.9±0.9	1.6±0.8	1.5±0.9	0.0001
Mean Δ(95% CI)	Reference	-0.0(-0.3, 0.2)	-0.3(-0.6, -0.1)	-0.5(-0.7, -0.2)	

Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; ESV, end-systolic volume; GCS, global circumferential strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume.

Table S5. 3DE derived LV myocardial indices by SABRE image-quality score in the SABRE study(n=529).

	Good	Fair	Adequate	Poor	P value
n(%)	**19(3.6)**	**235(44.4)**	**239(45.2)**	**36(6.8)**	
EDV, ml/m^2					
Mean±SD	58.1±10.0	58.5±13.3	56.4±13.9	55.3±9.5	0.260
Mean Δ(95% CI)	Reference	0.4(-5.8, 6.6)	-1.7(-7.9, 4.5)	-2.8(-10.2, 4.5)	
ESV, ml/m^2					
Mean±SD	25.1[21.4-27.7]*	25.2[21.6-29.9]*	26.1[21.9-29.8]*	25.8[21.7-30.0]*	0.808[#]
Mean Δ(95% CI)	Reference	0.9(-3.0, 4.8)	1.8(-2.2, 5.7)	0.8(-3.9, 5.5)	
SV, ml					
Mean±SD	57.0±11.9	59.1±14.9	54.2±13.2	54.5±12.4	0.002
Mean Δ(95% CI)	Reference	2.0(-4.5, 8.5)	-2.9(-9.4, 3.7)	-2.5(-10.2, 5.2)	
GCS, %					
Mean±SD	27.9±3.2	26.7±4.1	24.5±3.9	24.3±3.1	<0.0001
Mean Δ(95% CI)	Reference	-1.2(-3.0, 0.7)	-3.3(-5.2, -1.5)	-3.6(-5.8, -1.4)	
Peak averaged CS, %					
Mean±SD	28.2±3.2	26.9±4.1	24.7±3.9	24.2±3.1	<0.0001
Mean Δ(95% CI)	Reference	-1.3(-3.1, 0.6)	-3.5(-5.3, 1.7)	-3.9(-6.1, -1.8)	
Peak averaged LS, %					
Mean±SD	19.1±2.0	18.6±2.7	17.9±3.1	19.2±3.3	0.005
Mean Δ(95% CI)	Reference	-0.5(-1.9, 0.9)	-1.3(-2.6, -0.1)	0.1(-1.6, 1.7)	
Peak averaged PTS, %					
Mean±SD	33.0±3.3	32.0±4.1	30.2±4.0	30.5±3.2	<0.0001
Mean Δ(95% CI)	Reference	-1.0(-2.9, 0.8)	-2.8(-4.7, -0.9)	-2.5(-4.7, -0.3)	
Peak averaged RS, %					
Mean±SD	39.7±3.9	38.2±5.1	35.7±5.2	36.5±4.6	<0.0001
Mean Δ(95% CI)	Reference	-1.5(-3.8, 0.9)	-4.0(-6.3, -1.6)	-3.2(-6.0, -0.4)	
Peak basal rotation, °					
Mean±SD	7.8±2.8	5.7±3.3	5.1± 3.1	4.4±3.6	0.0007
Mean Δ(95% CI)	Reference	-2.0(-3.5, -0.5)	-2.6(-4.1, -1.1)	-3.3(-5.1, -1.5)	
Peak apical rotation, °					
Mean±SD	10.2[8.6-11.9]*	8.5[5.7-11.7]*	7.3[4.6-10.4]*	6.5[3.3-9.8]*	0.0001[#]
Mean Δ(95% CI)	Reference	-1.4(-3.4, 0.7)	-2.7(-4.7, -0.6)	-3.6(-6.0, -1.1)	
Peak twist, °					
Mean±SD	17.8±6.3	14.4±6.8	12.4±6.7	10.7±7.4	<0.0001
Mean Δ(95% CI)	Reference	-3.4(-6.6, -0.2)	-5.4(-8.6, -2.2)	-7.1(-10.9, -3.3)	
Peak torsion, °/cm					
Mean±SD	2.2±0.8	1.8±0.9	1.5±0.8	1.3±1.0	<0.0001
Mean Δ(95% CI)	Reference	-0.4(-0.8, -0.0)	-0.7(-1.1, -0.3)	-0.9(-1.4, -0.4)	

Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; ESV, end-systolic volume; GCS, global circumferential strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume. [#] by Kruskal-Wallis. *Data are median[interquartile range].

Table S6. Relationships with image quality for other LV myocardial indcies using SABRE image-quality score in the SABRE study(n=529).

	SABRE image-quality score		
	coefficient(95% CI), *p* (per 1-point increment in score)		
	Unadjusted	**Adjusted**	**Absolute standardized bias (%)***
LV volumetric indices			
EDV, ml	-1.3(-4.8, 2.2), 0.464	-3.6(-6.6, -0.5), 0.021	3.4%
ESV, ml	1.6(-0.6, 3.7), 0.144	0.5(-1.4, 2.4), 0.622	1.0%
SV, ml	-2.9(-4.7, -1.1), 0.001	-4.0(-5.6, -2.5), <0.0001	7.1%
LV strain indices			
GCS, %	-1.6(-2.1, -1.1), <0.0001	-1.6(-2.1, -1.1), <0.0001	6.3%
Peak averaged LS, %	-0.3(-0.7, 0.1), 0.145	-0.3(-0.6, 0.1), 0.175	1.6%
Peak averaged CS, %	-1.7(-2.2, -1.2), <0.0001	-1.7(-2.1, -1.2), <0.0001	6.6%
Peak averaged RS, %	-1.6(-2.3, -1.0), <0.0001	-1.6(-2.2, -1.0), <0.0001	4.3%
Peak averaged PTS, %	-1.2(-1.7, -0.7), <0.0001	-1.2(-1.7, -0.7), <0.0001	3.9%
LV rotational indices			
Peak basal rotation, °	-0.8(-1.2, -0.4), <0.0001	-0.8(-1.3, -0.4), <0.0001	14.8%
Peak apical rotation, °	-1.2(-1.8, -0.7), <0.0001	-1.2(-1.8, -0.7), <0.0001	14.5%
Peak twist, °	-2.1(-3.0, -1.3), <0.0001	-2.2(-3.1, -1.3), <0.0001	16.4%
Peak torsion, °/cm	-0.3(-0.4, -0.2), <0.0001	-0.3(-0.4, -0.2), <0.0001	17.6%

Coefficients are unstandardized coefficients of regression. Adjustment was performed for age, sex, ethnicity, height, weight, heart rate, history of percutaneous coronary intervention and/or coronary artery bypass graft and/or history of chronic obstructive pulmonary disease. *The extent of adjusted bias represented in standardized terms relative to the overall mean. These results are shown for SABRE image-quality score only as other definitions of image quality differ between EF and GLS. Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; ESV, end-systolic volume; GCS, global circumferential strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; and SV, stroke volume.

Table S7. Relationships between image quality and 3D-EF and 3D-GLS according to 3D-EF in the SABRE study(n=529).

	≥50% EF (n=439)		<50% EF (n=90)	
2015 ASE/EACVI guidelines-based image-quality score				
	coefficient(95% CI), *p*			
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
3D-EF, %	-1.4(-2.2, -0.6), 0.001	-1.1(-1.9, -0.3), 0.005	2.4(-0.8, 5.6), 0.141	3.2(-0.3, 6.7), 0.076
3D-GLS, %	-0.2(-0.8, 0.3), 0.409	-0.3(-0.8, 0.3), 0.360	0.3(-1.0, 1.6), 0.640	0.1(-1.2, 1.4), 0.897
Poor image-quality segments score				
	coefficient(95% CI), *p* (per 1-point increment in score)			
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
3D-EF, %	-0.7(-1.1, -0.3), 0.001	-0.5(-0.9, -0.1), 0.009	1.1(-0.3, 2.6), 0.124	1.2(-0.3, 2.8), 0.125
3D-GLS, %	-0.3(-0.5, 0.0), 0.034	-0.2(-0.5, 0.0), 0.079	0.2(-0.6, 1.1), 0.604	-0.03(-0.9, 0.9), 0.942
SABRE image-quality score				
	coefficient(95% CI), *p* (per 1-point increment in score)			
	Unadjusted	**Adjusted**	**Unadjusted**	**Adjusted**
3D-EF, %	-1.2(-1.8, -0.7), <0.0001	-1.1(-1.7, -0.6), <0.0001	0.3(-1.5, 2.1), 0.738	0.1(-1.9, 2.0), 0.945
3D-GLS, %	-0.1(-0.5, 0.2), 0.424	-0.1(-0.5, 0.3), 0.564	0.4(-0.6, 1.5), 0.412	0.1(-1.0, 1.2), 0.805

Coefficients are unstandardized coefficients of regression. Adjustment was performed for age, sex, ethnicity, height, weight, heart rate, history of percutaneous coronary intervention and/or coronary artery bypass graft and/or history of chronic obstructive pulmonary disease. Abbreviations: CI, confidence interval; EF, ejection fraction; and GLS, global longitudinal strain.

Table S8. 3D-EF and 3D-GLS by image quality scores according to 3D-EF in the SABRE study(n=529).

2015 ASE/EACVI guidelines-based image-quality score					
	Good	**Poor**			**P value**
3D-EF, %	Mean±SD				
EF≥50%	(n=167)	(n=272)			
	56.5±4.5	55.1±3.8			0.0006
EF<50%	(n=11)	(n=79)			
	42.4±4.1	44.8±5.2			0.141
3D-GLS%	Mean±SD				
EF≥50%	(n=325)	(n=114)			
	19.7±2.5	19.5±2.9			0.409
EF<50%	(n=52)	(n=38)			
	15.7±3.1	16.0±2.8			0.639
Poor image-quality segments score					
	None-segment	**1-segment**	**2-segments**	**≥3-segments**	**P value**
3D-EF, %			Mean±SD		
EF≥50%	(n=60)	(n=107)	(n=171)	(n=101)	
	57.0±4.1	56.3±4.8	55.1±3.9	55.1±3.6	0.004
EF<50%	(n=3)	(n=8)	(n=48)	(n=31)	
	44.4*	41.6±4.6	44.3±5.6	45.5±4.5	0.285
3D-GLS%			Mean±SD		
EF≥50%	(n=60)	(n=96)	(n=170)	(n=113)	
	20.1±2.7	20.1± 2.3	19.4±2.5	19.5±2.9	0.009
EF<50%	(n=3)	(n=7)	(n=42)	(n=38)	
	17.2*	14.1±4.0	15.9±3.0	16.0±2.8	0.389
SABRE image-quality score					
	Good	**Fair**	**Adequate**	**Poor**	**P value**
3D-EF, %			Mean±SD		
EF≥50%	(n=18)	(n=212)	(n=181)	(n=28)	
	57.2±3.6	56.4±4.5	54.7±3.7	54.7±3.2	0.0001
EF<50%	(n=1)	(m=23)	(n=58)	(n=8)	
	43.1*	44.3±4.2	44.5±5.6	44.9±3.7	0.985
3D-GLS%			Mean±SD		
EF≥50%	(n=18)	(n=212)	(n=181)	(n=28)	
	20.1±2.0	19.8±2.4	19.3±2.8)	20.4±2.9	0.093
EF<50%	(n=1)	(m=23)	(n=58)	(n=8)	
	17.3*	15.4±3.1	15.9±2.9	16.6±3.1	0.722

*Standard deviation has not been presented for data where n ≤ 3, only the mean value is shown. Abbreviations: EF, ejection fraction; GLS, global longitudinal strain; and SD, standard deviation.

Table S9. Relationships between frames per cycle and 3DE derive LV myocardial indcies in the SABRE study(n=529).

	coefficient(95% CI), *p* (per frames/cycle)	
	Unadjusted	**Adjusted**
LV volumetric indices		
3D-EF, %	0.4(0.2, 0.5), <0.0001	0.4(0.2, 0.6), 0.001
EDV, ml	0.6(-0.1, 1.3), 0.117	0.5(-0.4, 1.4), 0.277
ESV, ml	-0.2(-0.7, 0.2), 0.336	-0.2(-0.8, 0.4), 0.436
SV, ml	0.8(0.4, 1.1), <0.0001	0.7(0.3, 1.2), 0.003
LV strain indices		
3D-GLS, %	0.1(0.0, 0.2), 0.012	0.1(0.0, 0.2), 0.029
GCS, %	0.2(0.1, 0.3), <0.0001	0.2(0.1, 0.4), 0.001
Peak averaged LS, %	0.1(0.0, 0.2), 0.021	0.1(0.0, 0.2), 0.051
Peak averaged CS, %	0.2(0.1, 0.3), <0.0001	0.2(0.1, 0.4), 0.002
Peak averaged RS, %	0.2(0.1, 0.4), 0.001	0.3(0.1, 0.5), 0.003
Peak averaged PTS, %	0.1(0.0, 0.2), 0.010	0.2(0.1, 0.4), 0.010
LV rotational indices		
Peak basal rotation, °	0.0(-0.1, 0.1), 0.509	0.0(-0.1, 0.2), 0.762
Peak apical rotation, °	0.0(-0.1, 0.1), 0.913	0.1(-0.1, 0.3), 0.193
Peak twist, °	0.0(-0.2, 0.1), 0.664	0.1(-0.1, 0.4), 0.298
Peak torsion, °/cm	0.0(-0.0, 0.0), 0.876	0.0(-0.0, 0.1), 0.204

Coefficients are unstandardized coefficients of regression. Adjustment was performed for age, sex, ethnicity, height, weight, heart rate, history of percutaneous coronary intervention and/or coronary artery bypass graft and/or history of chronic obstructive pulmonary disease. Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; and SV, stroke volume.

Table S10. Comparison of 3DE derived LV myocardial indices by image quality in the (experimental) poor technique study).

	Mean±SD		Bias		ICC	
	Good	Sub-optimal	Mean-Δ(95% CI)	Absolute standardized bias (%)*	Good*	Sub-optimal†
LV volumetric indices						
EDV, ml	123.9±19.7	117.7±18.6	-6.2(-8.7, -3.7)	5.0%	0.97	0.91
ESV, ml	53.6±11.0	53.9±9.6	0.2(-1.1, 1.5)	0.4%	0.96	0.89
SV, ml	70.3±9.9	63.9±10.0	-6.4(-8.0, -4.8)	9.1%	0.97	0.90
LV strain indices						
GCS, %	28.1±2.6	25.7±2.1	-2.3(-2.9, -1.8)	8.2%	0.88	0.52
Peak averaged CS, %	28.3±2.6	25.7±2.5	-2.6(-3.2, -2.0)	9.2%	0.86	0.54
Peak averaged LS, %	20.4±2.0	19.7±2.4	-0.7(-1.4, -0.02)	3.4%	0.66	0.39
Peak averaged PTS, %	33.1±2.6	31.4±2.8	-1.6(-2.4, -0.9)	4.8%	0.82	0.36
Peak averaged RS, %	40.9±3.0	38.2±3.0	-2.7(-3.4, -2.0)	6.6%	0.80	0.51
LV rotational indices						
Peak basal rotation, °	8.0±3.9	6.7±5.1	-1.3(-2.7, 0.2)	16.3%	0.54	0.60
Peak apical rotation, °	6.3±2.7	4.6±3.0	-1.6(-2.7, -0.6)	25.4	0.41	0.24
Peak twist, °	13.7±5.8	10.8±7.0	-2.9(-5.2, -0.6)	21.2%	0.40	0.45
Peak torsion, °/cm	1.6±0.7	1.2±0.8	-0.3(-0.6, -0.06)	18.8	0.37	0.40

Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; ESV, end-systolic volume; GCS, global circumferential strain; ICC, intraclass correlation coefficient; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; and SV, stroke volume. *ICC based on un-degraded images. †ICC based on degraded images.

Table S11. The extent of bias proportional to the impairment in image quality of 3DE derived LV myocardial indices in the (experimental) neoprene study.

		Extent of bias relative to the reference			P (trend)
	Reference	**Mild**	**Moderate**	**Severe**	
EDV, ml					
Mean Δ(95% CI)	-	-7.8(-14.5, -1.0)	-11.8(-18.5, -5.0)	-19.5(-26.3, -12.8)	<0.0001
*Absolute standardized bias (%)**	-	5.6%	8.5%	14%	
Mean±SD	139.6±25.5	131.8±20.9	127.8±19.9	120.0±22.3	
ESV, ml					
Mean Δ(95% CI)	-	-1.9(-5.1, 1.3)	-2.5(-5.7, 0.69)	-5.1(-8.3, -1.8)	0.002
*Absolute standardized bias (%)**	-	3.0%	4.0%	8.2%	
Mean± SD	62.5±13.1	60.6±11.6	59.9±10.5	57.4±11.1	
SV, ml					
Mean Δ(95% CI)	-	-5.8(-9.6, -2.1)	-9.3(-13.0, -5.5)	-14.5(-18.3, -10.7)	<0.0001
*Absolute standardized bias (%)**	-	7.5%	12.1%	18.8%	
Mean±SD	77.1±13.1	71.2±10.1	67.8±10.0	62.6±11.6	
GCS, %					
Mean Δ(95% CI)	-	-1.6(-2.4, 0.9)	-1.8(-2.5, -1.0)	-2.6(-3.4, -1.9)	<0.0001
*Absolute standardized bias (%)**	-	6.1%	6.9%	9.9%	
Mean±SD	26.2±2.2	24.6±1.9	24.5±1.3	23.6±2.1	
Peak averaged CS, %					
Mean Δ(95% CI)	-	-1.4(-2.3, -0.5)	-1.6(-2.5, -0.7)	-2.9(-3.8, -2.0)	<0.0001
*Absolute standardized bias (%)**	-	5.4%	6.2%	11.2%	
Mean±SD	26.0±2.0	24.6±2.0	24.5±1.6	23.1±2.5	
Peak averaged LS, %					
Mean Δ(95% CI)	-	-0.6(-1.5, 0.3)	-1.1(-2.0, -0.2)	-2.0(-2.9, -1.1)	<0.0001
*Absolute standardized bias (%)**	-	2.9%	5.4%	9.8%	
Mean±SD	20.5±1.6	19.9±1.5	19.4±1.9	18.5±2.2	
Peak averaged PTS, %					
Mean Δ(95% CI)	-	-1.0(-2.0, 0.0)	-1.4(-2.4, -0.4)	-1.8(-2.8, -0.8)	<0.0001
*Absolute standardized bias (%)**	-	3.1%	4.4%	5.7%	
Mean±SD	-31.8±1.8	30.7±2.0	30.4±1.7	29.9±2.3	
Peak averaged RS, %					
Mean Δ(95% CI)	-	-1.7(-2.6, -0.7)	-2.2(-3.1, -1.3)	-4.0(-5.0, -3.1)	<0.0001
*Absolute standardized bias (%)**	-	4.3%	5.6%	10.2%	
Mean±SD	39.2±2.2	37.5±2.2	36.9±2.0	35.1±2.5	
Peak basal rotation, °					
Mean Δ(95% CI)	-	-0.1(-1.8, 1.5)	-1.9(-3.5, -0.16)	-2.6(-4.3, -0.9)	0.001
*Absolute standardized bias (%)**	-	1.4%	26.8%	36.6%	
Mean±SD	7.1±3.7	6.9±2.8	5.2±2.1	4.5±3.4	
Peak apical rotation, °					
Mean Δ(95% CI)	-	-1.1(-2.8, 0.5)	-2.6(-4.3, -1.0)	-3.0(-4.7, -1.4)	<0.0001
*Absolute standardized bias (%)**	-	16.4%	38.8%	44.8%	
Mean±SD	6.7±5.3	5.5±2.1	4.0±2.3	3.6±2.3	
Peak twist, °					
Mean Δ(95% CI)	-	-1.5(-4.6, 1.6)	-4.7(-7.8, -1.6)	-5.9(-9.1, -2.8)	<0.0001

*Absolute standardized bias (%)**	-	11.1%	34.8%	43.7%	
Mean±SD	13.5±8.5	12.1±4.7	8.8±4.0	7.6±5.4	
Torsion,°/cm					
Mean Δ(95% CI)	-	-0.1(-0.5, 0.2)	-0.5(-0.8, -0.1)	-0.6(-1.0, -0.3)	<0.0001
*Absolute standardized bias (%)**	-	6.7%	33.3%	40%	
Mean±SD	1.5±0.9	1.3±0.5	1.0±0.4	0.8±0.6	

Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; ESV, end-systolic volume; GCS, global circumferential strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume.

Table S12. Bland & Altman Analysis of 3DE derived LV myocardial indices by image quality (Experimental: poor technique study).

	Good 1 vs. Good 2		Good 1	Good 2	Sub-optimal 1 vs. Sub-optimal 2		Sub-optimal 1	Sub-optimal 2
	$Mean_{Diff}$ ± SD	**95% LOA**	**Mean**±SD		**$Mean_{Diff}$ ± SD**	**95% LOA**	**Mean**±SD	
LV volumetric indices								
3D-EF, %	0.7±0.8	-0.8, 2.2	57.3±3.3	56.6±3.2	0.2±1.7	-3.2, 3.5	54.4±2.7	54.2±2.6
EDV, ml	-1.4±4.1	-9.5, 6.7	123.2±19.5	124.6±20.5	-0.5±7.9	-16.0, 15.1	117.5±18.4	118.0±19.2
ESV, ml	-1.5±2.3	-6.0, 3.0	52.9±10.9	54.4±11.4	-0.3±4.3	-8.7, 8.2	53.7±9.9	54.0±9.5
SV, ml	0.1±2.2	-4.2, 4.5	70.4±10.0	70.2±10.2	-0.2±4.5	-9.0, 8.6	63.8±9.5	64.0±10.8
LV strain indices								
3D-GLS, %	-0.4±1.6	-3.5, 2.6	21.7±2.3	21.2±1.5	-1.4± 2.2	-5.9, 3.0	21.0±2.3	19.5±2.5
GCS, %	-0.5±1.1	-2.7, 1.7	28.3±2.9	27.8±2.4	-0.4±2.0	-4.4, 3.7	25.9±2.2	25.5±2.1
Peak averaged CS, %	-0.4±1.2	-2.9, 2.0	28.5±2.8	28.1±2.4	-0.3±2.3	-4.9, 4.3	25.9±2.6	25.6±2.4
Peak averaged LS, %	-0.4±1.5	-3.5, 2.5	20.7±2.3	20.2±1.7	-1.2±2.3	-5.8, 3.4	20.3±2.0	19.1±2.7
Peak averaged PTS, %	-0.8±1.3	-3.4, 1.8	33.5±2.9	32.7±2.3	-1.4±2.8	-7.0, 4.1	32.2±2.9	30.7±2.7
Peak averaged RS, %	0.8±1.7	-2.5, 4.1	41.3±3.2	40.5±2.8	1.3±2.6	-3.9, 6.5	38.9±3.0	37.6±2.9
LV rotational indices								
Peak basal rotation,	0.0±0.2	-7.3, 7.3	8.0±4.0	8.0±3.9	-1.6±4.3	-10.0, 6.8	7.6±5.3	5.9±5.0
Peak apical rotation	0.2±2.9	-5.5, 5.9	6.4±2.5	6.2±2.9	0.47±3.6	-6.7, 7.7	4.9±2.7	4.4±3.3
Peak twist, °	0.1±6.4	-12.6, 12.7	13.7±5.7	13.7±6.1	2.1±7.0	-11.6, 15.9	11.9±6.4	9.7±7.5
Peak torsion, °/cm	0.0±0.8	-1.4, 1.4	1.6±0.6	1.6±0.7	0.3±0.8	-1.3, 1.8	1.4±0.7	1.1±0.8

Abbreviations: CS, circumferential strain; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; LOA, limits of agreement; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume.

Table S13. Intra-observer reproducibility based on re-reading the good quality scans (n=10) from the experimental poor technique study.

	Mean±SD		Bias (reading 1 – reading 2)	ICC
	1st reading	2nd reading	Mean Δ (95% CI)	
LV volumetric indices				
3D-EF, %	57.8±3.2	58.1±3.5	-0.3 (-0.1, 0.8)	0.97
EDV, ml	126.2±17.8	125.5±17.9	0.8 (-1.2, -0.3)	0.99
ESV, ml	53.5±9.9	52.8±10.1	0.7 (-1.2, -0.1)	0.99
SV, ml	72.7±9.5	72.7±9.9	-0.1 (-0.7, 0.6)	0.99
LV strain indices				
3D-GLS, %	21.2±2.5	20.5±2.7	0.7 (0.0, 1.3)	0.90
GCS, %	28.5±2.6	27.8±2.4	0.7 (0.1, 1.2)	0.93
Peak averaged CS, %	28.8±2.6	27.8±2.6	1.0 (0.4, 1.6)	0.91
Peak averaged LS, %	20.5±2.3	20.1±2.6	0.3 (-0.4, 1.1)	0.86
Peak averaged PTS, %	33.4±2.8	32.3±2.8	1.1 (0.4, 1.8)	0.91
Peak averaged RS, %	41.3±3.7	40.2±3.4	1.1 (0.5, 1.6)	0.96
LV rotational indices				
Peak basal rotation,	6.9±2.7	5.7±2.8	1.2 (0.3, 1.9)	0.86
Peak apical rotation	5.7±1.9	6.2±1.9	-0.5 (-1.0, -0.0)	0.89
Peak twist, °	11.6±3.9	11.3±4.3	0.3 (-0.7, 1.3)	0.91
Peak torsion,°/cm	1.3±0.4	1.3±0.5	0.1 (-0.1, 0.2)	0.92

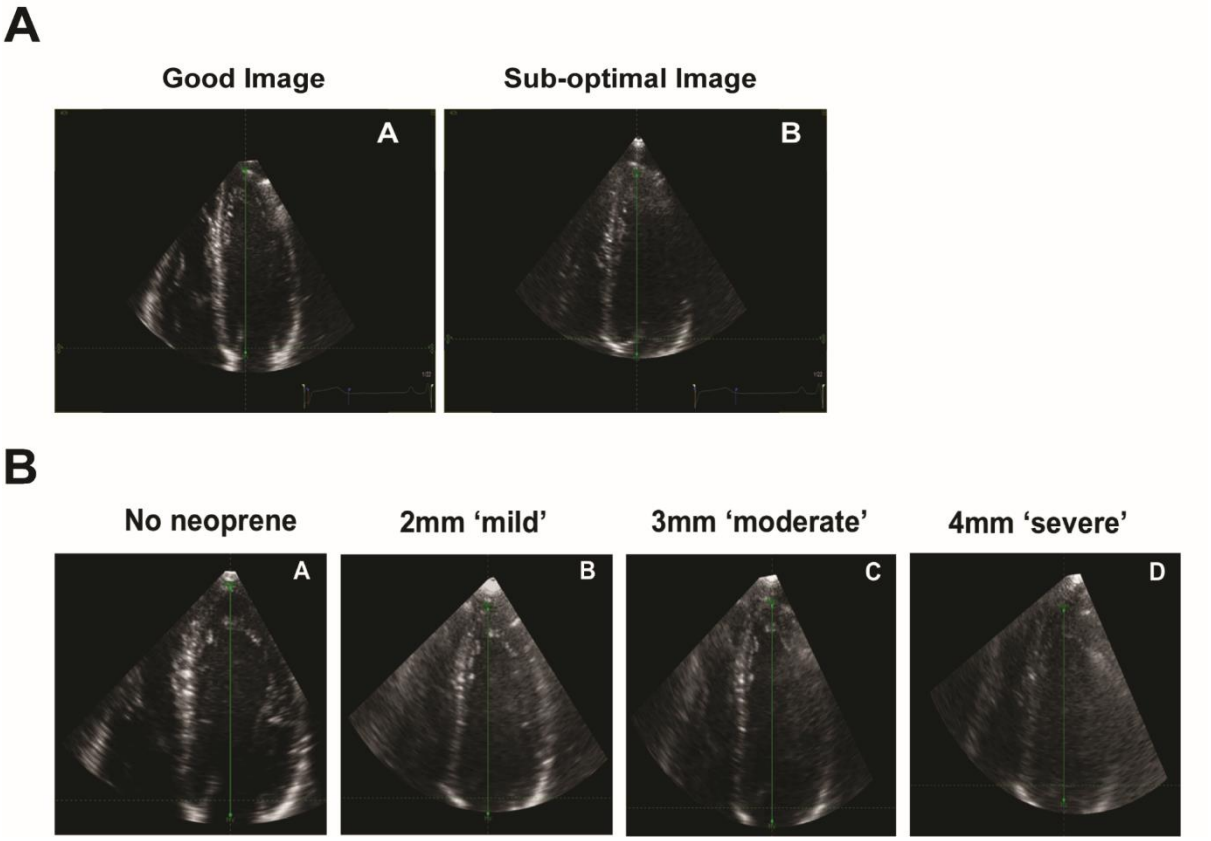
Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume.

Table S14. Inter-observer reproducibility based on re-reading the good quality scans (n=10) from the experimental poor technique study.

	Mean±SD		Bias (reading 1 – reading 2)	ICC
	1st reading	2nd reading	Mean Δ (95% CI)	
LV volumetric indices				
3D-EF, %	57.8±3.2	59.2±4.9	-1.4 (-3.3, 0.5)	0.71
EDV, ml	126.2±17.8	124.0±18.1	2.2 (1.2, 5.6)	0.95
ESV, ml	53.5±9.9	50.9±11.3	2.6 (0.3, 5.5)	0.89
SV, ml	72.7±9.5	73.1±9.9	-0.4 (-3.1, 2.3)	0.89
LV strain indices				
3D-GLS, %	21.2±2.5	21.6±2.4	-0.4 (-1.1, 0.3)	0.87
GCS, %	28.5±2.6	27.4±3.9	1.1 (-0.1, 2.3)	0.80
Peak averaged CS, %	28.8±2.6	28.3±4.0	0.4 (-0.8, 1.7)	0.79
Peak averaged LS, %	20.5±2.3	20.4±2.2	0.0 (-0.7, 0.8)	0.82
Peak averaged PTS, %	33.4±2.8	33.5±3.5	-0.1 (-1.5, 1.3)	0.73
Peak averaged RS, %	41.3±3.7	40.9±4.0	0.4 (0.6, 1.5)	0.89
LV rotational indices				
Peak basal rotation,	6.9±2.7	5.7±3.4	1.2 (-0.3, 2.7)	0.66
Peak apical rotation	5.7±1.9	5.3±2.6	0.4 (-0.7, 1.4)	0.69
Peak twist, °	11.6±3.9	10.2±5.8	1.4 (-1.2, 4.0)	0.59
Peak torsion,°/cm	1.3±0.4	1.1±0.6	0.2 (-0.1, 0.5)	0.60

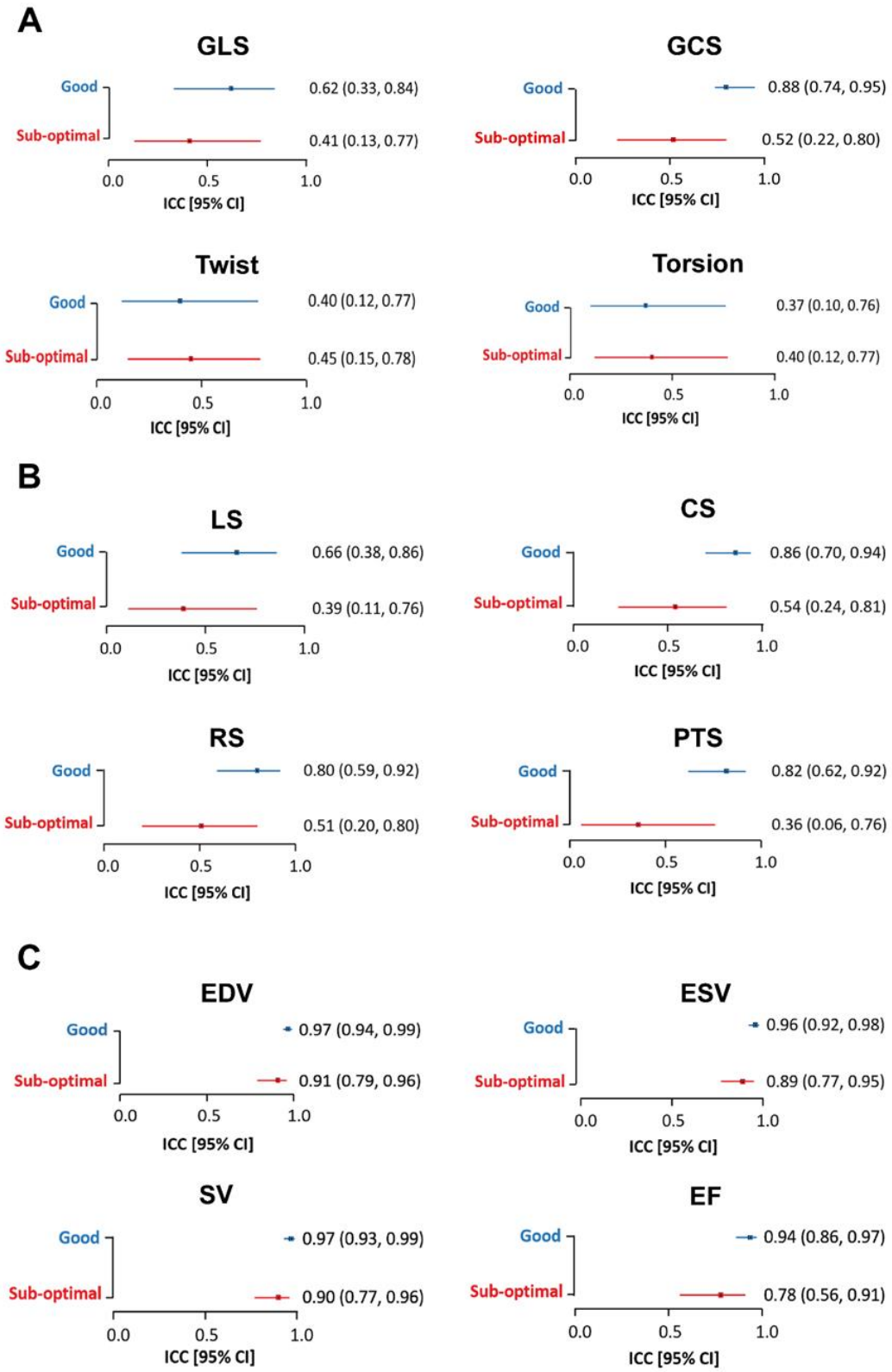
Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; LS, longitudinal strain; LV, left ventricular; PTS, principle tangential strain; RS, radial strain; SD, standard deviation; and SV, stroke volume.

Figure S1. Examples of impaired 3D echocardiographic (3DE) image quality.



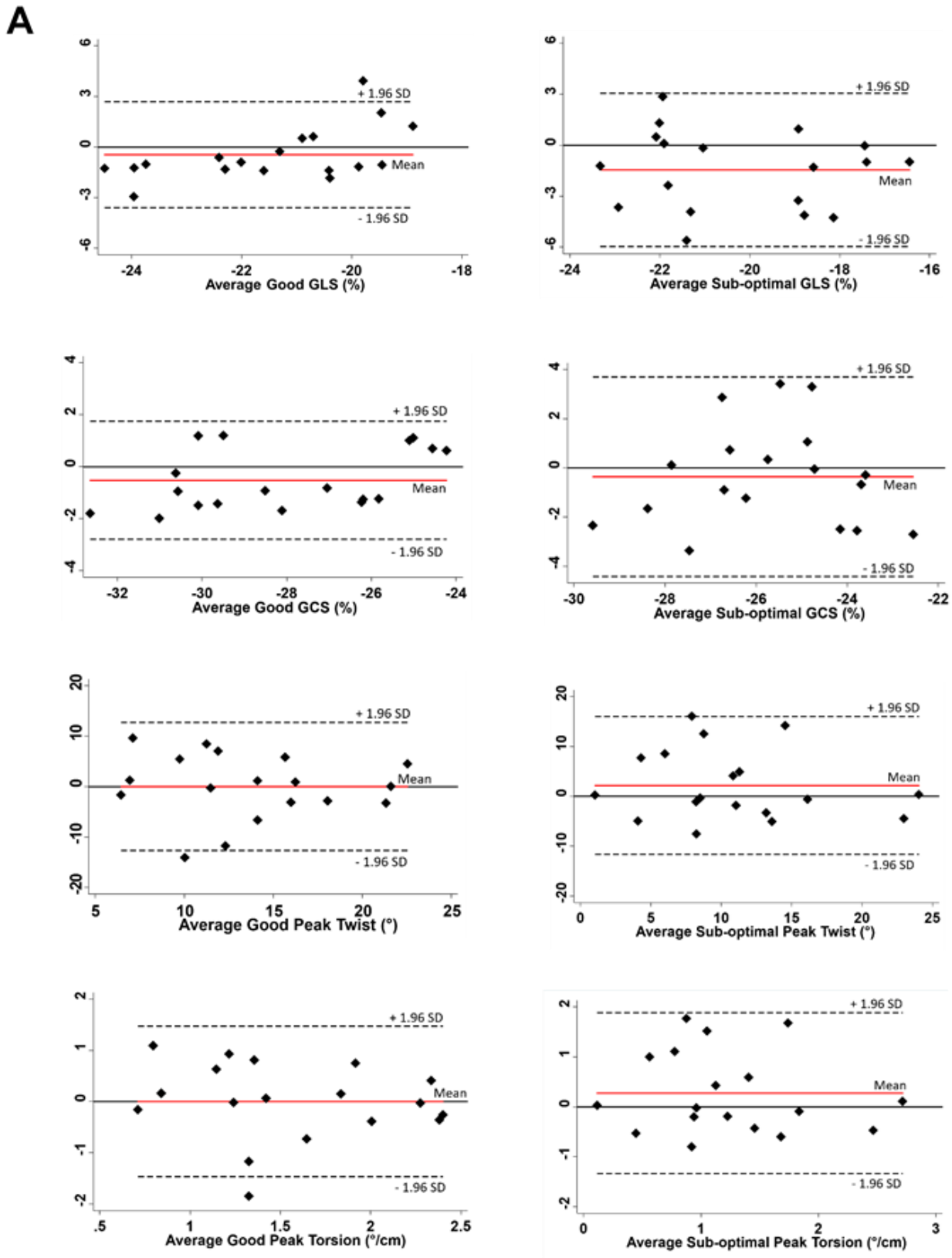
An example of a good and suboptimal 3DE image quality obtained from the same participant in the poor technique study**(A)**. An example of a 3DE with an optimal quality reference (no neoprene), mild (2mm neoprene), moderate (3mm neoprene), and severe (4mm neoprene) impairment of 3DE image quality obtained from the same participant in the neoprene study **(B)**.

Figure S2. Test-retest (scan re-scan) reliability.

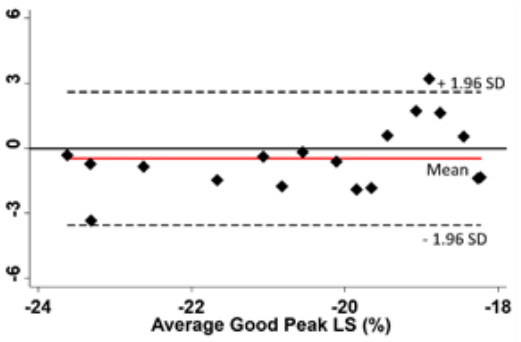


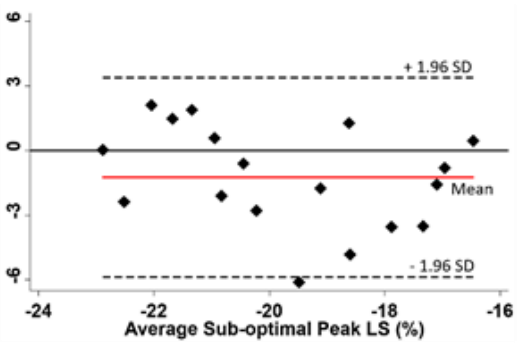
Intraclass correlation coefficient (ICC) of left ventricular (LV) global strain and rotational indices (A); peak averaged segmental LV strain indices (B); and volumetric indices (C). Good ICC represents the analysis of un-distorted quality images and sub-optimal ICC represents the analysis of distorted quality images. Abbreviations: CS, circumferential strain; CI, confidence interval; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GCS, global circumferential strain; GLS, global longitudinal strain; LS, longitudinal strain; PTS, principle tangential strain; RS, radial strain; and SV, stroke volume.

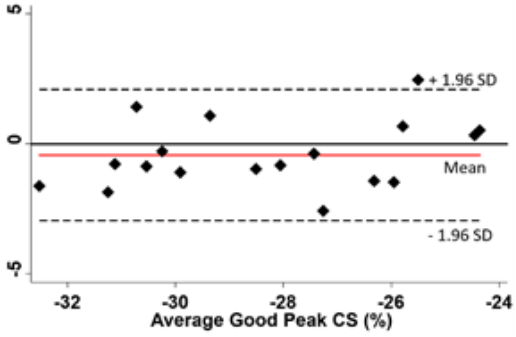
Figure S3. Bland & Altman Graphs.

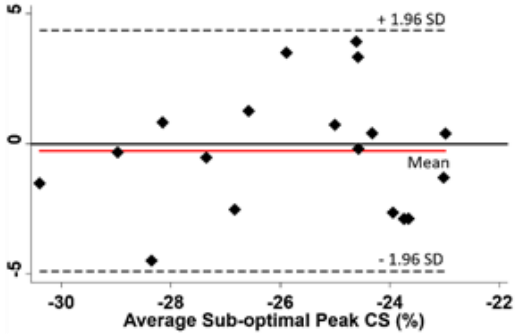


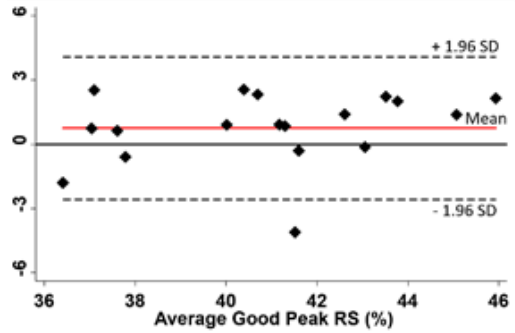
B

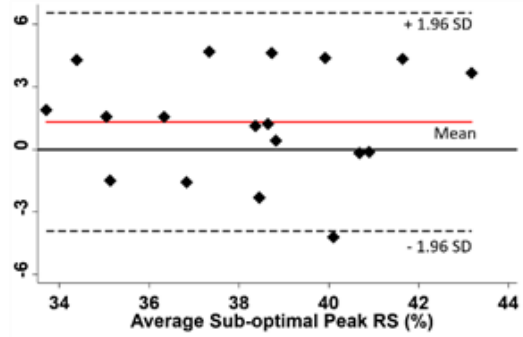

+ 1.96 SD
Mean
- 1.96 SD
Average Good Peak LS (%)

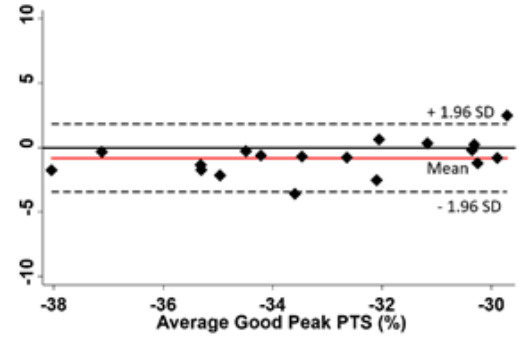

+ 1.96 SD
Mean
- 1.96 SD
Average Sub-optimal Peak LS (%)

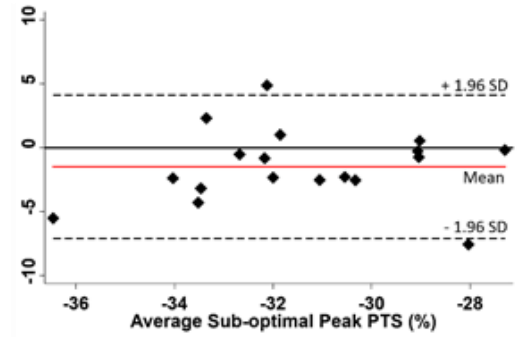

+ 1.96 SD
Mean
- 1.96 SD
Average Good Peak CS (%)


+ 1.96 SD
Mean
- 1.96 SD
Average Sub-optimal Peak CS (%)

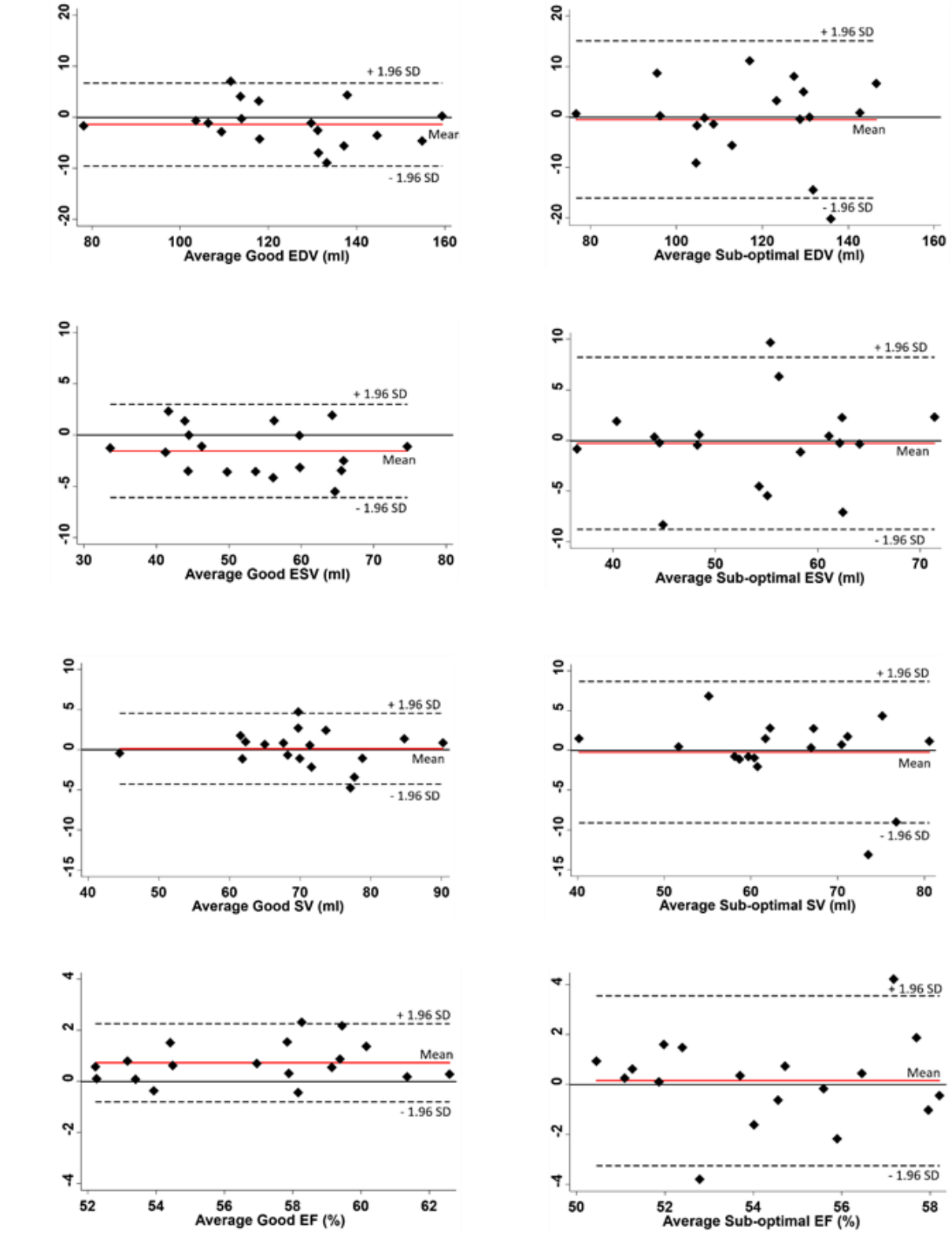

+ 1.96 SD
Mean
- 1.96 SD
Average Good Peak RS (%)


+ 1.96 SD
Mean
- 1.96 SD
Average Sub-optimal Peak RS (%)


+ 1.96 SD
Mean
- 1.96 SD
Average Good Peak PTS (%)


+ 1.96 SD
Mean
- 1.96 SD
Average Sub-optimal Peak PTS (%)

C



For these plots, actual strain not absolute strain values have been plotted of left ventricular (LV) global strain and rotational indices (A); peak averaged segmental LV strain indices (B); and volumetric indices (C).