

# Predictive value of vascular response to cuff inflation–induced pain in the control arm for adverse cardiovascular events <sup>☆</sup>



Tao Sun <sup>a,b</sup>, Yasushi Matsuzawa <sup>a</sup>, Robert J. Widmer <sup>a</sup>, Joerg Hermann <sup>a</sup>, Lilach O. Lerman <sup>c</sup>, Amir Lerman <sup>a,\*</sup>

<sup>a</sup> Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN, USA

<sup>b</sup> Division of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China

<sup>c</sup> Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA

## ARTICLE INFO

### Article history:

Received 22 September 2020

Received in revised form 4 January 2021

Accepted 15 January 2021

### Keywords:

Cardiovascular events

Framingham risk score

Pain-induced peripheral artery tonometry

Reactive hyperemia index

## ABSTRACT

**Background:** The high incidence rate of cardiovascular (CV) events had led to a comprehensive appraisal for identifying patients who are at risk for CV disease. However, CV traditional risk factors, such as Framingham risk score (FRS), failed exhaustively to predict CV events.

**Methods:** 402 participants (mean age, 58 [12] years; 45% male) using fingertip peripheral artery tonometry at Mayo Clinic in Rochester, Minnesota, were recruited in the present study. Measurements included reactive hyperemia index (RHI) and pain-induced peripheral artery tonometry (PIPAT).

**Results:** After a median follow-up of 3.8 (2.7–7.7) years, 95 CV events occurred. Both first minute PIPAT and RHI were independently associated with events (hazard ratio [HR], 0.77 [95% CI, 0.61–0.98];  $P = 0.038$  and HR, 0.75 [95% CI, 0.59–0.96];  $P = 0.019$ , respectively). The C statistic values of FRS, FRS + first minute PIPAT, FRS + RHI, and FRS + RHI + first minute PIPAT were 0.704, 0.722, 0.694, and 0.726, respectively. Furthermore, the addition of first minute PIPAT, RHI, and first minute PIPAT + RHI to FRS results in net reclassification improvement (NRI) in the intermediate-risk group (18.1%,  $P = 0.031$ ; 18.1%,  $P = 0.035$ ; 21%,  $P = 0.013$ ).

**Conclusion:** First minute PIPAT is a risk marker for adverse CV. Addition of first minute PIPAT to FRS increased the discrimination in the receiver operating characteristic analysis. It also increased NRI compared with FRS alone.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

There is a high incidence rate of cardiovascular (CV) events in most industrialized countries, even though individual treatment is based on cardiovascular risk [1–3]. This phenomenon has led to a comprehensive appraisal [2,4] for identifying patients who are at risk for CV disease. However, CV risk assessment using established risk factors, such as Framingham risk score (FRS), failed exhaustively to predict CV events. In our previous study [5], Pain-induced peripheral artery tonometry (PIPAT) in the control arm was first proposed and established to be associated with metabolic risk factors. Using the EndoPAT 2000 device, the ratio of the control arm peripheral artery tonometry signal during five-minute cuff inflation compared with baseline was calculated and

PIPATs were obtained. In the present study, we hypothesize that PIPATs could predict long-term adverse events and, when adding it as an assessment of microvascular function to FRS, could provide substantial CV event prognostic information in selected patients.

## 2. Methods

### 2.1. Study population

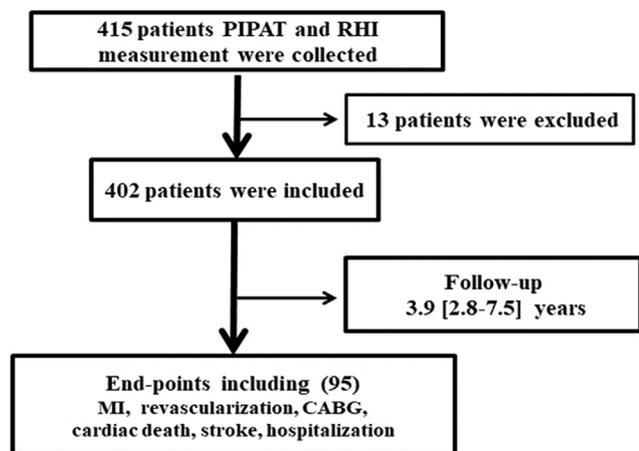
In our study, patients were drawn from a registry for screening of endothelial dysfunction using the EndoPAT2000 device (Itamar Medical Ltd.) at Mayo Clinic in Rochester, Minnesota. As shown in Fig. 1, of the 415 patients identified, we included 402 patients. Exclusion criteria were unstable angina, uncontrolled hypertension, pregnancy, or other severe chronic diseases [6]. This study was approved and reviewed by the Mayo Clinic Institutional Review Board. Written informed consent was obtained from each participant.

Demographics, medical history, and laboratory data for the present study were taken from the examination as close as possible to

<sup>☆</sup> This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

\* Corresponding author at: Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA.

E-mail address: [lerman.amir@mayo.edu](mailto:lerman.amir@mayo.edu) (A. Lerman).



**Fig. 1.** Flow Chart. CABG indicates coronary artery bypass graft; MI, myocardial infarction; PIPAT, pain-induced peripheral artery tonometry; RHI, reactive hyperemia index.

the EndoPAT2000 measurements. Current smoking was defined as having smoked a cigarette in the last 30 days. Diabetes mellitus was defined as a fasting glucose  $\geq 126$  mg/dL or the use of hypoglycemic medications. Using antihypertensive and other medications was based on review of prescribed medication containers. Hypertension was defined as systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg, or use of medication prescribed for hypertension. Body mass index was calculated as weight in kilograms divided by height in meters squared ( $\text{kg}/\text{m}^2$ ). Total and high-density lipoprotein cholesterol were measured from blood samples obtained after a 12-hour fast. Low-density lipoprotein cholesterol was estimated by the Fried Ewald equation.

2.2. Measures

2.2.1. PIPAT and reactive hyperemia index measurement

Participants fasted for 4 h before the study and abstained from coffee or tobacco use on the day of the examination. As previously published by Martin et al [7], the study was conducted in the sitting position in a comfortable chair with armrests. A fitted blood pressure cuff was placed on one arm, and the finger cuffs of the EndoPAT2000 device were placed on the middle fingers of each hand.[8] This noninvasive device is US Food and Drug Administration approved and allows continuous recording of the signal; interpretation of the data is operator independent. The ratio of the PAT signal after cuff release compared with baseline was calculated through a computer algorithm automatically normalizing for baseline signal and indexed to the contra lateral arm and endothelial

function was measured via reactive hyperemia index (RHI), as previously described.[8,9] PIPAT in the control arm was obtained using the EndoPAT2000 device, as described previously [5]. Ratios of the 5 each 1-minute control arm peripheral arterial tone signals compared with baseline during test arm cuff inflations were calculated and reflected PIPAT (Fig. 2).

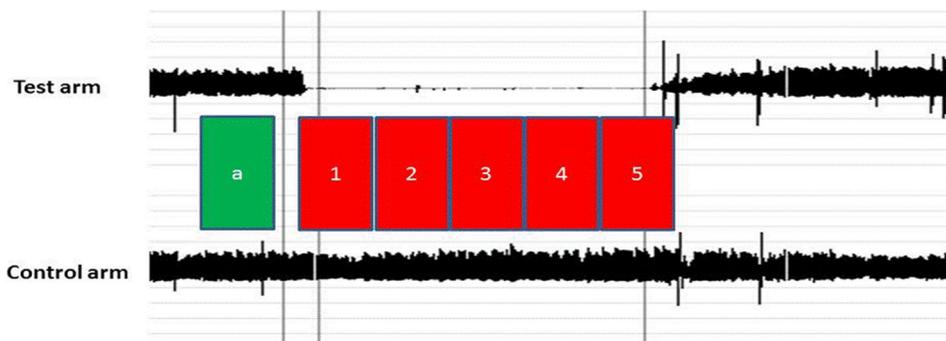
2.2.2. Follow-up and CV end points

Telephone follow-up was obtained for all subjects to detect the occurrence of any of the following outcome events: all cause death, CV death, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, carotid endarterectomy, diagnosed ischemic or hemorrhagic stroke or transient ischemic attack, or hospitalization for any cardiac cause, defined as hospitalization for chest pain, dyspnea, palpitations, or syncope. The combined end point of CV events was defined as the occurrence of CV death, myocardial infarction, stroke, revascularization, or cardiac hospitalization.

2.3. Statistical analysis

Continuous variable distributions were tested for normality using the Kolmogorov-Smirnov test. Continuous variable data with normal distribution were expressed as mean (Standard Deviation, SD), and the data with skewed distributions were expressed as medians (interquartile range). Differences between normally distributed continuous variables were analyzed by an unpaired *t* test. We used the Mann-Whitney *U* test for continuous variables with a skewed distribution. We compared groups using the  $\chi^2$  test. We calculated cumulative CV event incidence with the Kaplan-Meier method and compared CV incidence with the log-rank test. Cox proportional hazards model was used to predict adverse events during follow-up. We used the best cut-off points of PIPAT for predicting CV events, and *P* values were adjusted for the multiple tests performed to identify the cut-off point. We also estimated improvement, using the methods of DeLong et al [10] in discrimination by comparing the area under the receiver operator characteristic curve (AUC) in the models with first minute PIPAT, RHI, and PIPAT + RHI, respectively.

To account for the fact that actual follow-up was less than 10 years, we redefined the risk in terms of median 3.8 years when calculating the net reclassification improvements (NRI), using Cox proportional hazards model to reflect sampling from the overall cohort. Reclassifications were developed separately, and all subjects in the reclassification had a minimum of 3.8 years follow-up. We used a Cox proportional hazards model of FRS alone to generate predicted probabilities for the primary outcome among those classified as low, intermediate, and high risk by FRS. The absolute event rate cut points for the primary outcome between the 3 FRS



**Fig. 2.** Representative Pulses Amplitude Tracing in Measuring Pain-Induced Peripheral Artery Tonometry (PIPAT) at Different Intervals After Cuff Inflation in Control Arm. First minute PIPAT was expressed by the ratio 1/a, second minute PIPAT 2/a, third minute PIPAT 3/a, fourth minute PIPAT 4/a, and fifth minute PIPAT 5/a.

**Table 1**  
Baseline Demographics.

Variables	Entire Cohort (n = 402)	Event-free Group (n = 307)	Event Group (n = 95)	P Value
Age, y	57 ± 12.6	56 ± 13	62 ± 10	<0.001
Male sex, n (%)	178 (44)	124 (40)	54 (57)	0.005
Race, n (%)				
White	377 (96)	295 (96)	92 (97)	0.72
Hispanic	2 (0.4)	2 (0.65)	0 (0)	0.29
Black	0 (0)	0 (0)	0 (0)	–
Chinese	4 (0.9)	3 (0.98)	1 (1.04)	0.96
SBP, mmHg	126 ± 17	125 ± 16	131 ± 19	<0.001
Family history, n (%)	165 (41)	135 (44)	30 (32)	0.025
FRS (%) median [M <sub>1</sub> , M <sub>3</sub> ]	4 [1,8]	3 [1,6]	7 [13,14]	<0.001
Diabetes mellitus, n (%)	44 (11)	30 (9.8)	14 (14.7)	0.19
PAD, n (%)	14 (3.5)	9 (2.9)	5 (5.3)	0.41
HNT, n (%)	184 (46)	130 (42)	54 (56)	0.017
Current smoker, n (%)	53 (13)	36 (11.7)	17 (17.8)	0.14
BMI, kg/m <sup>2</sup>	29 ± 6	29 ± 5.8	30.8 ± 6.4	0.013
Heart rate, bpm	70 ± 13	70 ± 14	71 ± 11	0.66
TC, mg/dL	188 ± 39	189 ± 40	184 ± 37	0.38
HDL, mg/dL	53 ± 15	55 ± 16	48 ± 13	<0.001
TG, mg/dL				
Median [M <sub>1</sub> , M <sub>3</sub> ]	106 [73,154]	107 [73,155]	106 [75,147]	0.053
LDL, mg/dL	105 ± 36	104 ± 38	106 ± 32	0.81
1st minute PIPAT	0.95 ± 0.18	0.96 ± 0.18	0.91 ± 0.15	0.026
2nd minute PIPAT	1.02 ± 0.20	1.03 ± 0.20	0.99 ± 0.19	0.18
3rd minute PIPAT	1.05 ± 0.24	1.06 ± 0.25	1.04 ± 0.21	0.50
4th minute PIPAT	1.07 ± 0.26	1.08 ± 0.27	1.04 ± 0.23	0.26
5th minute PIPAT	1.11 ± 0.66	1.12 ± 0.74	1.06 ± 0.25	0.40
RHI	1.94 [1.58, 2.39]	1.98 [1.60, 1.98]	1.78 [1.54, 2.22]	0.043
Ln RHI	0.66 ± 0.28	0.68 ± 0.29	0.61 ± 0.27	0.044
Aspirin use, n (%)	180 (45)	141 (46)	39 (41)	0.40
β-blocker use, n (%)	115 (29)	93 (30)	22 (23)	0.17
ACE inhibitor use, n (%)	73 (18)	56 (18)	17 (18)	0.94
Calcium channel blocker, n (%)	90 (22)	69 (22.5)	21 (22)	0.94
HMG-CoA use, n (%)	129 (32)	103 (33.5)	26 (27)	0.25
hs-CRP, mg/L	0.87 [0.30,3.0]	0.84 [0.30,3.0]	0.94 [0.30,3.0]	0.70
Homocysteine, mg/L	8.10 ± 2.90	7.93 ± 2.89	8.85 ± 2.82	0.12

Values expressed as mean ± SEM. ACE indicates angiotensin-converting enzyme; BMI, body mass index; FRS, Framingham risk score; HDL, high-density lipoprotein; HNT, hypertension; LDL, low-density lipoprotein; PIPAT, pain-induced peripheral artery tonometry; RHI, reactive hyperemia index; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride. PAD, peripheral arterial disease.

risk categories were as follows: low, ≤2.6%; intermediate, 2.6%–6.5%; high, ≥6.5%. We used similar model to generate predicted probability for primary outcome using FRS + first minute PIPAT, FRS + Ln RHI, and FRS + first minute PIPAT + Ln RHI, respectively. We evaluated the classification of risk using NRIs. NRI = (probability [being correctly reclassified to a higher-risk category/event] – probability [being incorrectly reclassified to a lower-risk category/event]) + (probability [being correctly reclassified to a lower-risk category/nonevent] – probability [being incorrectly reclassified to a higher-risk category/nonevent]) [11]. In the present study, we evaluated NRI in those subjects at intermediate risk.  $P < 0.05$  was set a priori and considered statistically significant. All statistical analyses were performed with the Statistical Package for Social Sciences version 11.5 for Windows (SPSS Inc.)

### 3. Results

#### 3.1. Baseline characteristics

The overall study cohort (n = 402) consisted of mainly middle-aged subjects (57 [12.6] years) with a low FRS of 4 [1,8]. Of the participants, 44% were male, 87% was non-smokers, 46% had hypertension, 11% had diabetes mellitus and 3.5% had peripheral arterial disease. The patients were taking the following medications during their hospitalization: β-blockers (n = 115, 29%), angiotensin converting enzyme inhibitors or angiotensin receptor blockers (n = 73, 18%), aspirin (n = 180, 45%), or statins (n = 129, 32%) (Table 1).

#### 3.2. CV events

There were 95 CV events during 3.8 (2.7–7.7) years follow-up. Events included 10 deaths from a definite CV cause, 7 myocardial infarctions, and 18 revascularizations (2 coronary artery bypass grafts and 16 percutaneous coronary interventions), 6 S, 3 undergoing carotid endarterectomy, 6 heart failures, and 45 cardiac hospitalizations.

#### 3.3. Predictors of CV events

Individual predictors of CV events are shown in Table 2. Age (per 10 years) (1.36 [1.14–1.63];  $P < 0.001$ , male sex (2.07 [1.38–3.15];  $P < 0.001$ ), systolic blood pressure (per SD) (1.24 [1.03–1.50];  $P = 0.023$ ), body mass index (per SD) (1.26 [1.05–1.50];  $P = 0.012$ ), and high-density lipoprotein (per SD) (0.58 [0.43–0.76];  $P < 0.001$ ) were significant predictors of CV events. Both first minute PIPAT (0.79 [0.64–0.97];  $P = 0.026$ ) and Ln RHI (0.77 [0.62–0.96];  $P = 0.017$ ) were inversely associated with CV events in the univariate model. In the multivariable Cox proportional hazards model, age (per 10 years) (1.32 [1.10–1.60];  $P = 0.0025$ ), male sex (2.81 [1.68–4.81];  $P < 0.0001$ ), systolic blood pressure (per SD) (1.23 [1.002–1.51];  $P = 0.048$ ), first minute PIPAT (per SD) (0.77 [0.61–0.98];  $P = 0.038$ ), and Ln RHI (0.75 [0.59–0.96];  $P = 0.019$ ) remained significant predictors after adjustment for age, sex, systolic blood pressure, body mass index, high-density lipoprotein, and Ln RHI. In addition, there were no interactions between Ln RHI and first minute PIPAT in the multivariable model ( $P = 0.257$ ). First minute PIPAT remained a predictor of CV events

**Table 2**  
Univariate (Age- and Sex-Adjusted) and Multivariable HRs for Cardiovascular Events.

Variable	Univariate HR (95%CI)	P Value	Multivariable HR (95% CI)	P Value
Age per 10 yrs.	1.36 (1.14–1.63)	0.0005	1.32 (1.10–1.60)	0.0025
Sex, male	2.07 (1.38–3.15)	0.0005	2.81 (1.68–4.81)	<0.0001
Race, white	0.82 (0.31–3.36)	0.75	---	---
SBP per SD	1.24 (1.03–1.50)	0.023	1.23 (1.002–1.51)	0.048
Family history, yes	0.70 (0.45–1.07)	0.097	---	---
DM, yes	1.33 (0.72–2.27)	0.34	---	---
Current smoking, yes	1.59 (0.90–2.62)	0.10	---	---
BMI, kg/m <sup>2</sup> per SD	1.26 (1.05–1.50)	0.012	1.04 (0.81–1.32)	0.75
Heart rate, per SD	0.99 (0.80–1.20)	0.96	---	---
TC, mg/dL per SD	0.85 (0.68–1.07)	0.18	---	---
HDL, mg/dL per SD	0.58 (0.43–0.76)	<0.001	0.77 (0.55–1.07)	0.13
TG, mg/dL per SD	1.10 (0.93–1.23)	0.21	---	---
LDL, mg/dL per SD	1.11 (0.83–1.46)	0.45	---	---
Ln RHI per SD	0.77 (0.62–0.96)	0.017	0.75 (0.59–0.96)	0.019
1st minute PIPAT per SD	0.79 (0.64–0.97)	0.026	0.77 (0.61–0.98)	0.038
2nd minute PIPAT per SD	0.84 (0.68–1.03)	0.10	---	---
3rd minute PIPAT per SD	0.89 (0.71–1.11)	0.32	---	---
4th minute PIPAT per SD	0.89 (0.70–1.10)	0.28	---	---
5th minute PIPAT per SD	0.92 (0.74–1.14)	0.46	---	---

BMI indicates body mass index; DM, diabetes mellitus; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; PIPAT, pain-induced peripheral artery tonometry; RHI, reactive hyperemia index; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

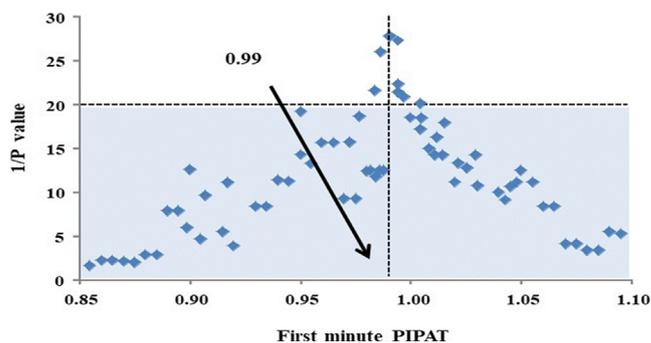
(hazard ratio [HR], 0.74 [95% CI, 0.60–0.92]; *P* = 0.006) in the FRS-adjusted model (Fig. 3).

Comparison of the significance of this cut point versus others to differentiate patients by risk of CV event is shown as the relation between (inverted) *P* values and first minute PIPAT (Fig. 4). Adverse event rates differed according to first minute PIPAT results, as patients with lower first minute PIPAT had higher event rates during the follow-up period, and the most useful cut-off point was 0.99. Survival curves showed separate curves during the follow-up period (Fig. 5). Further Cox proportional hazards model analysis showed that first minute PIPAT < 0.99 was independently associated with increased CV event rate (HR, 1.57 [95% CI, 1.03–2.45]; *P* = 0.034) during follow-up and remained an independent predictor of CV events when included in an age-adjusted model (per 10 years) (HR, 1.50, [95% CI, 1.01–2.34]; *P* = 0.048) and FRS-adjusted model (per SD) (HR, 1.55, [95% CI, 1.02–2.43]; *P* = 0.030) (Table 3).

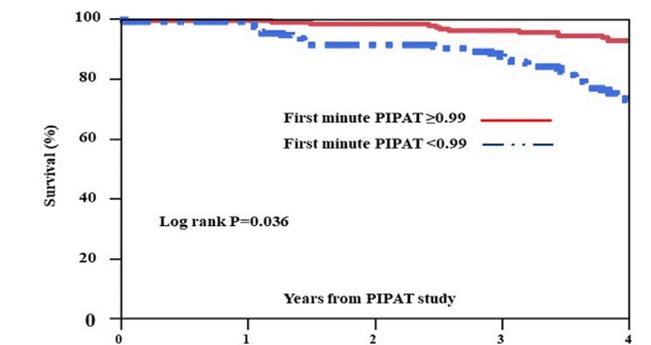
In the receiver operating characteristic analyses, the C statistic (AUC) for FRS was 0.704. In the univariate model, Ln RHI and first minute PIPAT were 0.568 and 0.562, respectively. When microvascular parameters were added to FRS, Ln RHI did not increase the C index (0.694); however, there was an increase in the C index with first minute PIPAT (0.722) and the combination of Ln RHI and first

minute PIPAT (0.726), but the increase did not achieve statistical significance (Table 4). The smallest Akaike and Bayes information criteria were also observed when Ln RHI and first minute PIPAT were added to FRS.

As illustrated in Table 5, the 3.8-year NRI of intermediate-risk patients was 18.1% for Ln RHI (*P* = 0.031), 18.1% for first minute

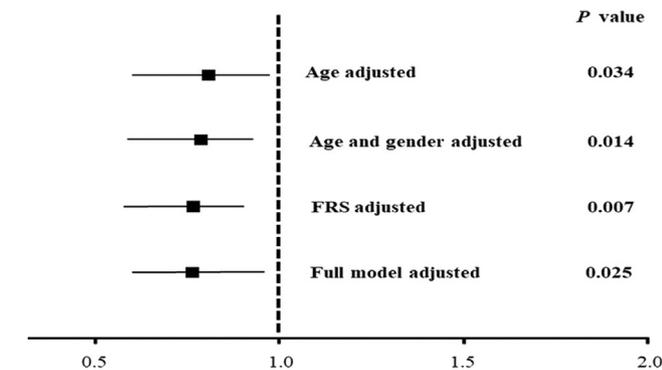


**Fig. 4.** Plot of the *P* Value vs Pain-Induced Peripheral Artery Tonometry (PIPAT) Cut Points in Stratifying Patients for Adverse Events.



No. at risk	0	1	2	3	4
<0.99	238	229	198	159	102
≥0.99	164	153	138	120	50

**Fig. 5.** Survival Stratified by Cut-off Value of the enrolled patients.



**Fig. 3.** First Minute Pain-Induced Peripheral Artery Tonometry (PIPAT) Adjusted in Different Cox Proportional Hazards Models. First minute PIPAT denotes PIPAT per SD. Full model was adjusted for age, sex, systolic blood pressure, body mass index, high-density lipoprotein, and Ln reactive hyperemia index. FRS indicates Framingham risk score.

**Table 3**  
First minute PIPAT Hazard Ratios for Events in Cox Proportional Hazards Model.

Variable	Estimate	HR (95%CI)	P Value
Model 1			
PIPAT < 0.99	0.45	1.57 (1.03–2.45)	0.034
Model 2: adjusted for age			
PIPAT < 0.99	0.42	1.50 (1.01–2.34)	0.048
Age per 10 years	0.30	1.34 (1.13–1.62)	0.0007
Model 3: adjusted for FRS			
PIPAT < 0.99	0.45	1.55 (1.02–2.43)	0.030
FRS (per SD)	0.37	1.43 (1.23–1.67)	<0.001

FRS indicates Framingham risk score; HR, hazard ratio; PIPAT, pain-induced peripheral artery tonometry.

**Table 4**  
C statistic to predict cardiovascular events during follow-up.

Risk Factors and Vascular Parameters	C Index	AIC	BIC
FRS	0.704	412	420
FRS + 1st PIPAT	0.722	406	419
FRS + Ln RHI	0.694	411	423
FRS + Ln RHI + 1st PIPAT	0.726	403	418

\*There were no statistical differences between the C index of FRS and the C index of the other covariates. FRS, Framingham risk score; PIPAT, pain-induced peripheral artery tonometry; RHI, reactive hyperemia index.

PIPAT ( $P = 0.035$ ), and 21% for Ln RHI + first minute PIPAT ( $P = 0.013$ ).

#### 4. Discussion

In the present study, we investigated the potential value of novel microvascular function measures for CV event prediction in

apparently healthy subjects. We demonstrated first minute PIPAT has an independent association with CV events, and low first minute PIPAT was predictive of higher incidence of events during the follow-up period. We also established the additional value for the use of first minute PIPAT as a tool for refining CV risk prediction over FRS in intermediate-risk subjects. To our knowledge, this is the first study to describe the improvement in risk prediction provided by novel physical stress-induced microvascular function.

Numerous noninvasive techniques to measure vascular function have been developed [12–18]. We first described PIPAT in apical ballooning syndrome and demonstrated its significant relationship with vascular response to mental stress [5]. In our present study, we individually entered the 5 1-minute PIPAT cuff inflations into the univariate Cox proportional hazards model; first minute PIPAT remained an independent predictor of CV events in the FRS-adjusted model and multivariable model. Therefore, we used the first minute PIPAT as the parameter measuring the vascular response when the cuff inflated on the control arm. Moreover, both first minute PIPAT and RHI were significantly entered the multivariable model, and hazard ratios decreased, implying that measuring different aspects of vascular biology provide incremental predictions regarding the clinical events.

We also established that the addition of first minute PIPAT to FRS increased the C statistic for CV events; when combined with RHI, the C index increased to 0.726. However, the addition of RHI failed to increase the discrimination in the receiver operating characteristic analysis. Previous studies [15–18] showed that novel risk markers improve the classification of risk over FRS. Anderson et al [15] further investigated if hyperemia velocity, not flow-mediated dilation, was a novel marker of microvascular function and could provide a net clinical improvement of 28.68% in lower-risk healthy men. Yeboah et al [19] established that the addition of flow-mediated dilation resulted in an overall NRI of 2.4% in an intermediate-risk cohort. We took these previous studies into

**Table 5**  
Net reclassification improvements for cardiovascular events with additional individual and combined vascular responses to FRS.

Variables	FRS Risk Category			Net Correct Reclassification, %	P Value
	Low	Intermediate	High		
FRS + Ln RHI with events					
Low	1	0	0		
Intermediate	12	3	0		
High	9	14	9		
FRS + Ln RHI with no events					
Low	9	2	0		
Intermediate	78	7	0		
High	30	19	8		
Intermediate risk only, %				18.1	0.031
FRS + PIPAT with events					
Low	0	0	0		
Intermediate	16	2	0		
High	6	15	9		
FRS + PIPAT with no events					
Low	7	0	0		
Intermediate	81	8	1		
High	29	20	7		
Intermediate risk only, %				18.1	0.035
FRS + Ln RHI and PIPAT with events					
Low	1	0	0		
Intermediate	12	2	0		
High	9	15	9		
FRS + Ln RHI and PIPAT with no events					
Low	18	2	0		
Intermediate	60	9	1		
High	39	17	7		
Intermediate risk only, %				21	0.012

FRS indicates Framingham risk score; PIPAT, pain-induced peripheral artery tonometry; RHI, reactive hyperemia index.

account and found first minute PIPAT provided an NRI of 18.1% ( $P = 0.035$ ); the combination with RHI created a higher NRI of 21% and lessened the degree of statistical significance ( $P = 0.013$ ).

It is worth noting that there are several differences between the present study and the above mentioned studies: 1) the present study evaluated the noninvasive microvascular function instead of conduit vessel function using EndoPAT2000; 2) in previous studies, [15,18] 5-year time point for the reclassification was selected due to the nature of the database, and some censored observations were omitted before 5-year follow-up. A median 7.5-year risk was redefined to calculate the NRI. [19] In the present study, a median of 3.8-year risks was redefined; the baseline FRS differed in median (interquartile range), 8.8 (6.5–12.2), [19] 7.9 (4.7–15.6). [15] In the present study, FRS of 4.0 (1.0, 8.0) represented a relatively low heart attack risk in the next 10 years.

PIPATs were supposed to involve the actions of corticotrophin-releasing hormone, cortisol, enhanced sympathetic nerve activation [20–24] and pro-inflammatory cytokines [23,24], immediately ensuing the cuff inflation in the test arm. Notably, among the 5 each 1-minute PIPATs, we observed that only 1st minute PIPAT showed less than 1, which illustrated the vascular response only increased in the 1st minute cuff inflation compared with baseline vascular tone. Interestingly, we assessed how much variation is explained by the cardiovascular risk in 5 each 1-minute PIPATs in multivariate regression models, the 1st minute PIPAT overall model  $R^2$  was maximized (not shown). Therefore, we proposed that 1st minute PIPAT was the most appropriate measure of digital vascular function in the control arm. We then hypothesized that 1st minute PIPAT represented exacerbated the myocardial oxygen tension, and was expected to be a surrogate risk marker for future events. In the present study, we could obtain the amplitude of PIPAT and endothelial functions with peripheral arterial tonometry in a “one-stop” model. Due to its relatively simple, less time-consuming nature, PIPAT could be utilized in different clinical conditions. In addition, the combination of PIPAT and RHI might provide a more efficient risk predictor for CV events. Further studies should be employed to address the clinical utility of this novel microvascular measure.

Our study has limitations related to the relatively small sample size, and the retrospective nature of the design. The patients in the current study represents a population with low cardiovascular risk and thus we elected to use re hospitalization as an endpoint. Another limitation secondary to the nature of the study is the lack of complete adjudication of the symptoms the required the hospitalization. In addition, even though the exact mechanisms need to be further explored, peripheral artery tonometry signal on the control arm finger microvasculature can also be influenced by variable nonendothelial and endothelial factors. Finally, we created the separate reclassification table over a maximum of 3.8-year follow-up, and the analysis failed to include all censored observations.

## 5. Conclusions

First minute PIPAT was associated with adverse CV events. Addition of first minute PIPAT to FRS increased discrimination in the receiver operating characteristic analysis. When combined with RHI, it provided incremental discrimination about CV event risk.

## Funding sources

National Institutes of Health (NIH Grant HL-92954 and AG-31750 to A.L.)

## Declaration of Competing Interest

None.

## Acknowledgements

The authors wish to thank Diana P. Albers for her assistance with these studies.

## References

- [1] R.A. Hayward, H.M. Krumholz, D.M. Zulman, J.W. Timbie, S. Vijan, Optimizing statin treatment for primary prevention of coronary artery disease, *Ann. Intern. Med.* 152 (2010) 69–77.
- [2] G.S. Collins, D.G. Altman, An independent external validation and evaluation of qrisk cardiovascular risk prediction: A prospective open cohort study, *BMJ*. 339 (2009) b2584.
- [3] Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *Jama*. 2001; 285: 2486–2497.
- [4] Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *Jama*. 2001; 285: 2486–2497.
- [5] T. Sun, R.J. Widmer, Y. Matsuzawa, R.J. Lennon, K.H. Park, L.O. Lerman, A. Lerman, Pain-induced peripheral artery tonometry scores in the control arm are impaired in patients with apical ballooning syndrome, *Medicine* 98 (2019) e13841.
- [6] J. Li, A.J. Flammer, R.J. Lennon, R.E. Nelson, R. Gulati, P.A. Friedman, R.J. Thomas, N.P. Sandhu, Q. Hua, L.O. Lerman, A. Lerman, Comparison of the effect of the metabolic syndrome and multiple traditional cardiovascular risk factors on vascular function, *Mayo Clin. Proc.* 87 (2012) 968–975.
- [7] E.A. Martin, A. Prasad, C.S. Rihal, L.O. Lerman, A. Lerman, Endothelial function and vascular response to mental stress are impaired in patients with apical ballooning syndrome, *J. Am. Coll. Cardiol.* 56 (2010) 1840–1846.
- [8] E.A. Martin, S.L. Tan, L.R. MacBride, S. Lavi, L.O. Lerman, A. Lerman, Sex differences in vascular and endothelial responses to acute mental stress, *Clin. Auton. Res.* 18 (2008) 339–345.
- [9] D.A. Goor, J. Sheffy, R.P. Schnall, A. Arditto, A. Caspi, E.E. Bragdon, D.S. Sheps, Peripheral arterial tonometry: A diagnostic method for detection of myocardial ischemia induced during mental stress tests: A pilot study, *Clin. Cardiol.* 27 (2004) 137–141.
- [10] E.R. DeLong, D.M. DeLong, D.L. Clarke-Pearson, Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach, *Biometrics*. 44 (1988) 837–845.
- [11] F.G. Fowkes, G.D. Murray, I. Butcher, C.L. Heald, R.J. Lee, L.E. Chambless, A.R. Folsom, A.T. Hirsch, M. Dramaix, G. deBacker, J.C. Wautrecht, M. Kornitzer, A.B. Newman, M. Cushman, K. Sutton-Tyrrell, A.J. Lee, J.F. Price, R.B. d'Agostino, J.M. Murabito, P.E. Norman, K. Jamrozik, J.D. Curb, K.H. Masaki, B.L. Rodriguez, J.M. Dekker, L.M. Bouter, R.J. Heine, G. Nijpels, C.D. Stehouwer, L. Ferrucci, M.M. McDermott, H.E. Stoffers, J.D. Hooi, J.A. Knottnerus, M. Ogren, B. Hedblad, J.C. Witteman, M.M. Breteler, M.G. Hunink, A. Hofman, M.H. Criqui, R.D. Langer, A. Fronek, W.R. Hiatt, R. Hamman, H.E. Resnick, J. Guralnik, Ankle brachial index combined with framingham risk score to predict cardiovascular events and mortality: A meta-analysis, *JAMA* 300 (2008) 197–208.
- [12] J.T. Kuvin, A.R. Patel, K.A. Sliney, N.G. Pandian, J. Sheffy, R.P. Schnall, R.H. Karas, J.E. Udelson, Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude, *Am. Heart J.* 146 (2003) 168–174.
- [13] L. Linder, W. Kiowski, F.R. Buhler, T.F. Luscher, Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension, *Circulation* 81 (1990) 1762–1767.
- [14] D.S. Celermajer, K.E. Sorensen, V.M. Gooch, D.J. Spiegelhalter, O.I. Miller, I.D. Sullivan, J.K. Lloyd, J.E. Deanfield, Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, *Lancet* 340 (1992) 1111–1115.
- [15] T.J. Anderson, F. Charbonneau, L.M. Title, J. Buithieu, M.S. Rose, H. Conradson, K. Hildebrand, M. Fung, S. Verma, E.M. Lonn, Microvascular function predicts cardiovascular events in primary prevention: Long-term results from the firefighters and their endothelium (fate) study, *Circulation* 123 (2011) 163–169.
- [16] J. Yeboah, J.R. Crouse, F.C. Hsu, G.L. Burke, D.M. Herrington, Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: The cardiovascular health study, *Circulation* 115 (2007) 2390–2397.
- [17] J. Yeboah, A.R. Folsom, G.L. Burke, C. Johnson, J.F. Polak, W. Post, J.A. Lima, J.R. Crouse, D.M. Herrington, Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: The multi-ethnic study of atherosclerosis, *Circulation* 120 (2009) 502–509.
- [18] P.M. Ridker, N.P. Paynter, N. Rifai, J.M. Gaziano, N.R. Cook, C-reactive protein and parental history improve global cardiovascular risk prediction: The reynolds risk score for men, *Circulation* 118 (2008) 2243–2251, 2244p following 2251.

- [19] J. Yeboah, R.L. McClelland, T.S. Polonsky, G.L. Burke, C.T. Sibley, D. O'Leary, J.J. Carr, D.C. Goff, P. Greenland, D.M. Herrington, Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals, *JAMA*. 308 (2012) 788–795.
- [20] A.J. Broadley, A. Korszun, E. Abdelaal, V. Moskvina, C.J. Jones, G.B. Nash, C. Ray, J. Deanfield, M.P. Frenneaux, Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment, *J. Am. Coll. Cardiol.* 46 (2005) 344–350.
- [21] J.S. Gottdiener, W.J. Kop, E. Hausner, M.K. McCeney, D. Herrington, D.S. Krantz, Effects of mental stress on flow-mediated brachial arterial dilation and influence of behavioral factors and hypercholesterolemia in subjects without cardiovascular disease, *Am. J. Cardiol.* 92 (2003) 687–691.
- [22] L. Lind, K. Johansson, J. Hall, The effects of mental stress and the cold pressure test on flow-mediated vasodilation, *Blood Press.* 11 (2002) 22–27.
- [23] C.J. Huang, J.K. Stewart, R.L. Franco, R.K. Evans, Z.P. Lee, T.D. Cruz, H.E. Webb, E. O. Acevedo, Lps-stimulated tumor necrosis factor-alpha and interleukin-6 mrna and cytokine responses following acute psychological stress, *Psychoneuroendocrinology* 36 (2011) 1553–1561.
- [24] K. Aschbacher, E. Epel, O.M. Wolkowitz, A.A. Prather, E. Puterman, F.S. Dhabhar, Maintenance of a positive outlook during acute stress protects against pro-inflammatory reactivity and future depressive symptoms, *Brain Behav Immun.* 26 (2012) 346–352.