Journal of the American Heart Association

ORIGINAL RESEARCH

Vascular Aging Detected by Peripheral Endothelial Dysfunction Is Associated With ECG-Derived Physiological Aging

Takumi Toya, MD; Ali Ahmad, MD; Zachi Attia, MS; Michal Cohen-Shelly, BSc; Ilke Ozcan, MD; Peter A Noseworthy, MD; Francisco Lopez-Jimenez, MD, MSc; Suraj Kapa, MD; Lilach O Lerman, MD, PhD; Paul A Friedman, MD; Amir Lerman, MD

BACKGROUND: An artificial intelligence algorithm that detects age using the 12-lead ECG has been suggested to signal "physiologic age." This study aimed to investigate the association of peripheral microvascular endothelial function (PMEF) as an index of vascular aging, with accelerated physiologic aging gauged by ECG-derived artificial intelligence–estimated age.

METHODS AND RESULTS: This study included 531 patients who underwent ECG and a noninvasive PMEF assessment using reactive hyperemia peripheral arterial tonometry. Abnormal PMEF was defined as reactive hyperemia peripheral arterial tonometry index ≤2.0. Accelerated or delayed physiologic aging was calculated by the Δ age (ECG-derived artificial intelligence–estimated age minus chronological age), and the association between Δ age and PMEF as well as its impact on composite major adverse cardiovascular events were investigated. Δ age was higher in patients with abnormal PMEF than in patients with normal PMEF (2.3±7.8 versus 0.5±7.7 years; P=0.01). Reactive hyperemia peripheral arterial tonometry index was negatively associated with Δ age after adjustment for cardiovascular risk factors (standardized β coefficient, −0.08; P=0.048). The highest quartile of Δ age was associated with an increased risk of major adverse cardiovascular events compared with the first quartile of Δ age in patients with abnormal PMEF, even after adjustment for cardiovascular risk factors (hazard ratio, 4.72; 95% CI, 1.24–17.91; P=0.02).

CONCLUSIONS: Vascular aging detected by endothelial function is associated with accelerated physiologic aging, as assessed by the artificial intelligence–ECG Δ age. Patients with endothelial dysfunction and the highest quartile of accelerated physiologic aging have a marked increase in risk for cardiovascular events.

Key Words: artificial intelligence ■ peripheral microvascular endothelial dysfunction ■ physiological age ■ reactive hyperemia peripheral arterial tonometry index ■ vascular age

Cardiovascular disease is the most common cause of death in older adults, accounting for >40% of deaths among people aged 65 to 74 years and nearly 60% of those aged >85 years.[1] Although aging is inevitable, the rate of physiologic aging may be modifiable with healthy lifestyle, diet, and medical treatments.[2-5] Therefore, understanding and documenting physiological aging may have important implications for management, particularly for patients with cardiovascular risk factors.

The endothelium is a bellwether for the effects of cardiovascular risk factors, which become more prevalent with aging. One such effect is the development of endothelial dysfunction, which is an early manifestation of atherosclerosis.[6,7] Aging may cause phenotypic changes in the vasculature, characterized by impairment of endothelium-dependent vasodilation through age-related reduction of NO bioavailability.[8,9] In this context, endothelial dysfunction may be attributed to

Correspondence to: Amir Lerman, MD, Department of Cardiovascular Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55902. E-mail: Lerman.Amir@mayo.edu

Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.018656

For Sources of Funding and Disclosures, see page 8.

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

CLINICAL PERSPECTIVE

What Is New?

- Patients with abnormal peripheral endothelial dysfunction and the highest degree of Δ age, defined as ECG-derived artificial intelligence–estimated age minus chronological age, appear to be physiologically older than the same chronologically aged cohorts, and have an increased risk for cardiovascular events.

What Are the Clinical Implications?

- A large Δ age can be a biomarker of accelerated physiologic aging.
- Vascular aging may contribute to cardiovascular risk in people with accelerated physiologic aging.

Nonstandard Abbreviations and Acronyms

AI	artificial intelligence
C-age	chronological age
Δ age	ECG-derived artificial intelligence–estimated age minus chronological age
ECG-age	ECG-derived artificial intelligence–estimated age
MACE	major adverse cardiovascular event
PMEF	peripheral microvascular endothelial function
RH-PAT	reactive hyperemia peripheral arterial tonometry

and may be an indicator of vascular aging.[10] Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a noninvasive method to detect peripheral microvascular endothelial dysfunction, which is associated with increased risk of late cardiovascular adverse events even in individuals with minimal cardiovascular risk factors.[11,12]

Recent advances in the application of artificial intelligence (AI) for standard 12-lead ECG enables detection of left ventricular systolic dysfunction and identification of patients with atrial fibrillation during sinus rhythm from single 12-lead ECG.[13,14] More recently, we trained an AI algorithm to estimate age and sex with high accuracy using 12-lead ECG.[15] The difference between ECG-derived AI-estimated age (ECG-age) and chronological age (C-age) was greatest in patients with preexisting comorbidities, such as hypertension, low ejection fraction, coronary artery disease, and atrial fibrillation. Interestingly, patients with a minimal difference between ECG-age and C-age developed fewer cardiovascular events during follow-up than patients in whom the Δ between ECG-age and C-age (Δ age) was greater, indicating that ECG-age better reflects physiological age rather than C-age.[15] A large Δ age may thus be a biomarker of accelerated physiologic aging.

We hypothesized that endothelial dysfunction, an accepted indicator of vascular aging, is associated with physiological aging, as measured by ECG-age. We aimed to investigate the relationship between peripheral microvascular endothelial dysfunction and the Δ age, and its impact on cardiovascular outcomes.

METHODS

The data that supported the findings of this study are available from the corresponding author on reasonable request.

Study Population

In this cross-sectional and observational cohort study, we enrolled 531 patients who underwent ECG and peripheral microvascular endothelial function (PMEF) testing using the EndoPAT 2000 device (Itamar Medical Inc, Caesarea, Israel) at Mayo Clinic between January 17, 2006, and February 14, 2014, and were followed up until November 5, 2019. The decision to perform ECG and PMEF testing for assessment of chest pain and/or cardiovascular risk was at the clinical discretion of the evaluating physicians. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Mayo Clinic Institutional Review Board approved the study protocol. All patients provided written informed consent for participation in the current study.

Assessment of Peripheral Microvascular Endothelial Function

RH-PAT was used to evaluate PMEF, as previously described.[12,16-18] Briefly, the study protocol included a 5-minute baseline measurement, followed by 5-minute inflation of a blood pressure cuff around the study participant's test arm with a pressure of 60 mm Hg above baseline systolic blood pressure up to 200 mm Hg, followed by a 6-minute period of PAT measurement after deflation of the cuff. Blood pressure cuff occlusion was not applied to the control arm (contralateral arm). RH-PAT ratio was determined as the average pulse wave amplitude for a 1-minute period beginning 1 minute after pressure cuff deflation (test arm=A; control arm=C) divided by the average pulse wave amplitude during the 3.5-minute baseline

period before pressure cuff inflation (test arm=B; control arm=D). The RH-PAT index was computed automatically by normalizing baseline signal and indexing the RH-PAT ratio on the test arm to that of the control arm (RH-PAT index=[A/B]/[C/D]×[baseline correction]). Per clinical protocol, patients were instructed to stop all vasoactive medications, including calcium channel blockers, β blockers, and long-acting nitrates, for at least 24 hours before endothelial function testing. Patients fasted for 4 hours before the study and abstained from coffee and tobacco use on the day of the RH-PAT testing. A calculated RH-PAT index ≤2.0 is a clinically used cutoff value for the diagnosis of abnormal PMEF at Mayo Clinic and was comparable to the median RH-PAT index of study participants (2.07; interquartile range, 1.72-2.52).[19-21]

Assessment of ECG-Age From 12-Lead ECG

A convolutional neural network model using Keras with a Tensorflow (Google, Mountain View, CA) and Python backend was previously developed. Briefly, a total of 774 783 unique subjects with ECG were used to develop the neural network: 399 750 in the training set, 99 977 in the internal validation set, and 275 056 ECGs in the holdout testing set. The training, validation, and test sets were mutually exclusive for patient identification.[15] The convolutional neural network was trained by inputting raw 12-lead ECGs and the patients' C-age at the time of the ECG, during the training process, and the weights of the convolutional filters were adjusted to extract meaningful and relevant features of the inputs in respect to the patients' age. The network had a single output (age) as a continuous number.[15]

We used the previous AI-ECG algorithm with no additional retraining to calculate ECG-age for our study population. ECG-age was calculated using ECGs that were obtained within 1 year from the PMEF testing. When multiple ECGs existed within 1 year, the closest ECG to the PMEF assessment was chosen for analysis.

Clinical Assessment

Clinical history, laboratory data, and current medications were collected from a detailed chart review by an investigator blinded to ECG-age and RH-PAT data. Data were collected on the following parameters: (1) sex, age, and smoking status; (2) dyslipidemia, defined by a documented history of hyperlipidemia, treatment with lipid-lowering therapy, a low-density lipoprotein cholesterol level above the target (<130 mg/dL for low-risk patients, <100 mg/dL for moderatehigh-risk patients, <70 mg/dL for very high-risk patients, and <55 mg/dL for extremely high-risk patients, on the basis of 10-year atherosclerotic cardiovascular disease risk), high-density lipoprotein cholesterol <40 mg/dL in men or <50 mg/dL in women, or triglycerides >150 mg/dL; (3) type 2 diabetes mellitus, defined as a documented history of or treatment for type 2 diabetes mellitus; (4) hypertension, defined as a documented history of or treatment for hypertension; and (5) coronary artery disease, diagnosed by coronary angiography or computed tomography coronary angiography. Significant coronary artery disease was defined as the presence of >50% stenosis in the major epicardial vessels. Patients were followed up from the date of RH-PAT testing for individual major adverse cardiovascular events (MACEs: all-cause death; myocardial infarction; clinically driven coronary revascularization; cerebrovascular disease, such as transient ischemic attack, ischemic stroke, and hemorrhagic stroke; and peripheral artery disease causing claudication). Individual events were ascertained by a combination of public and institutional databases, death certificates, and detailed chart review and were independently adjudicated by 2 investigators. Composite MACE outcome refers to the occurrence of ≥1 individual events over follow-up.

Statistical Analysis

Continuous variables distributed normally were expressed as mean±SD, and those with a skewed distribution were expressed as the median with interquartile range. Categorical variables were expressed as frequency (percentage). To compare variables between groups, we performed an unpaired *t*-test for normally distributed continuous variables, a Mann-Whitney *U* test for nonnormally distributed variables, and a χ^2 test (or Fisher exact test) for categorical variables. Linear regression analysis was performed to identify correlations between 2 parameters. The associations between parameters were assessed using the Pearson or Spearman correlation test, as appropriate. Multiple regression analyses were performed to estimate the effects of covariates on the Δ age calculated by ECG-age minus C-age. AI algorism tends to underestimate age in older individual, whereas it tends to overestimate age in younger individual[15]; given prior data, the decision was made to correct Δ age for the C-age in the analyses. There was no predefined cutoff of the Δ age; thus, patients were divided into 4 groups by the quartile of the Δ age. Cox proportional hazards analyses were performed to evaluate the independent prognostic power for composite MACEs, including all-cause death; myocardial infarction; clinically driven coronary revascularization; cerebrovascular disease, such as transient ischemic attack, ischemic stroke, and hemorrhagic

stroke; and peripheral artery disease causing claudication. For patients with multiple events, only the first event was used for the analyses. In multivariate analyses, 3 covariate sets were investigated: multivariate (1) Δ age quartile and C-age; multivariate (2) Δ age quartile, C-age, sex, hypertension, dyslipidemia, diabetes mellitus, and smoking history; and multivariate (3) Δ age quartile, C-age, and abnormal PMEF. The covariates in multivariate analysis were chosen for clinical relevance. For sensitivity analysis, we repeated all the analyses when excluding patients' ECGs used in the training set. Finally, we evaluated the discriminatory power of the Δ age for identifying composite MACEs when adding Δ age to RH-PAT index by calculating net reclassification improvement and integrated discrimination improvement. For all tests, a 2-tailed $P<0.05$ was considered statistically significant. All statistical analyses were performed using JMP Pro software (SAS Institute, Inc, Cary, NC) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). The data were analyzed from December 17, 2019, to March 9, 2020.

RESULTS

Baseline Characteristics

Between January 2006 and February 2014, 687 patients underwent noninvasive endothelial function testing at Mayo Clinic using the EndoPAT device for assessment of chest pain and/or cardiovascular risk. A total of 156 patients were excluded because of the lack of 12-lead ECG within 1 year of PMEF testing, leaving a total of 531 patients in the analyses. Patients (mean C-age, 51.7±13.8 years; mean ECG-age, 53.0±12.2 years; 38% men) were followed up for a maximum of 13.6 years (median, 4.2 years) at Mayo Clinic from the date of the index PMEF testing. A total of 241 patients (45%) had abnormal PMEF (defined as an RH-PAT index ≤2.0), and 290 patients (55%) had normal PMEF (defined as an RH-PAT index >2.0). Table 1 outlines the baseline characteristics of the study sample, categorized on the basis of abnormal versus normal PMEF. A higher proportion of patients with abnormal PMEF were men. Patients with abnormal PMEF were significantly more likely to have traditional cardiovascular risk factors (diabetes mellitus, dyslipidemia, and chronic kidney disease) as well as significant coronary artery disease. Patients with abnormal PMEF were more likely to be treated with aspirin, antihypertensive medications, and antidiabetic medications (Table 1). ECG-age tended to be higher in patients with abnormal PMEF than in patients with normal PMEF (54.1±12.1 versus 52.1±12.2; $P=0.07$). Δ age, defined as ECG-age minus C-age, was significantly higher in patients with abnormal PMEF than in patients with normal PMEF (2.3±7.8 versus 0.5±7.7; $P=0.01$) (Table 1).

Correlation Between ECG-Age, Δ Age, and C-Age

There was a strong positive correlation between ECG-age and C-age (r=0.83; $P<0.0001$) (Figure 1A). In contrast, the Δ age was negatively correlated with C-age (r=0.47; $P<0.0001$) (Figure 1B). To estimate the effects of covariates on the Δ age, we performed multiple regression analyses. C-age and RH-PAT index independently had negative effects on the Δ age (C-age: standardized β coefficient, −0.48 [$P<0.0001$]; RH-PAT index: standardized β coefficient, −0.08 [$P=0.048$]) (Table 2). These associations were consistent after additional adjustment for the presence of significant coronary artery disease (C-age: standardized β coefficient, −0.49 [$P<0.0001$]; RH-PAT index: standardized β coefficient, −0.08 [$P=0.056$]). In 301 patients with available hs-CRP (high-sensitivity C-reactive protein) data, we performed multiple regression analysis with the same covariates and hs-CRP, demonstrating the consistent results; only C-age and RH-PAT index independently had negative effects on the Δ age (C-age: standardized β coefficient, −0.48 [$P<0.0001$]; RH-PAT index: standardized β coefficient, −0.16 [$P=0.003$]; hs-CRP: standardized β coefficient, 0.02 [$P=0.65$]).

Impact of Δ Age and Abnormal PMEF on Cardiovascular Outcomes

A total of 33 patients (14%) with abnormal PMEF and 20 patients (7%) with normal PMEF developed MACEs during follow-up ($P=0.01$). Next, to assess the impact of the Δ age on cardiovascular outcomes, Cox proportional hazard analyses were performed. When dividing patients into 4 groups by the quartile of the Δ age, the highest quartile of the Δ age was significantly associated with an increased risk of MACEs after adjustment for C-age (multivariate [1]: C-age adjusted hazard ratio [HR], 2.64; 95% CI, 1.02–6.78; $P=0.04$) (Table 3). The highest quartile of the Δ age was significantly associated with an increased risk of MACEs, even after adjustment for other cardiovascular risk factors (multivariate [2]: C-age, sex, hypertension, dyslipidemia, diabetes mellitus, and smoking history; adjusted HR, 2.78; 95% CI, 1.06–7.29; $P = 0.04$); however, after adjustment for C-age and abnormal PMEF, the highest quartile of the Δ age was not significantly associated with an increased risk of MACEs (multivariate [3]: adjusted HR, 2.22; 95% CI, 0.85–5.79; $P=0.10$) (Table 4). Therefore, we performed multivariate analysis 2 with further categorization based on abnormal versus normal PMEF. The highest quartile of the Δ age was significantly associated with an increased risk of MACE only in patients with abnormal PMEF (adjusted

Table 1. Baseline Characteristics Comparing Patients With Normal Versus Abnormal PMEF

Characteristics		RH-PAT Index		*P* Value
		≤2.0 (N=241)	>2.0 (N=290)	
Clinical characteristics				
C-age, mean (SD), y	51.7 (13.8)	51.8 (13.6)	51.7 (14.0)	0.92
ECG-age, mean (SD), y	53.0 (12.2)	54.1 (12.1)	52.1 (12.2)	0.07
Δ age, mean (SD), y	1.3 (7.8)	2.3 (7.8)	0.5 (7.7)	0.01
ECG-age>C-age, N (%)	306 (58)	148 (61)	158 (55)	0.11
Men, N (%)	203 (38)	109 (45)	94 (32)	0.003
Hypertension, N (%)	230 (43)	108 (45)	122 (42)	0.53
Diabetes mellitus, N (%)	53 (10)	35 (15)	18 (6)	0.002
Dyslipidemia, N (%)	377 (71)	182 (76)	195 (67)	0.04
Smoking history, N (%)	190 (36)	95 (39)	95 (33)	0.11
CAD, N (%)				
None	315 (59)	131 (55)	184 (64)	0.02
<50%	93 (18)	40 (17)	53 (18)	
≥50%	122 (23)	69 (29)	53 (18)	
Body mass index, median (IQR), kg/m^2	27.0 (23.9–31.3)	28.4 (25.4–33.1)	25.7 (23.1–29.6)	<0.0001
Systolic BP, mean (SD), mm Hg	122 (17)	121 (17)	122 (17)	0.67
Diastolic BP, mean (SD), mm Hg	75 (11)	74 (10)	75 (10)	0.04
Laboratory data				
LDL, median (IQR), mg/dL	103 (80–129)	103 (77–125)	104 (83–130)	0.30
HDL, median (IQR), mg/dL	53 (44–66)	50 (41–63)	58 (46–70)	<0.0001
Triglyceride, median (IQR), mg/dL	110 (77–159)	120 (79–184)	107 (74–147)	0.04
Glucose, median (IQR), mg/dL	96 (90–104)	97 (91–106)	95 (89–103)	0.01
HbA1c, median (IQR), %	5.4 (5.1–5.9)	5.5 (5.1–6.0)	5.4 (5.2–5.9)	0.52
Creatinine, median (IQR), mg/dL	0.9 (0.8–1.1)	0.9 (0.8–1.1)	0.9 (0.8–1.0)	0.23
eGFR, mean (SD), mL/min per 1.73 m^2	75.8 (18.5)	77.9 (19.6)	74.1 (17.4)	0.02
RH-PAT index, median (IQR)	2.07 (1.72–2.52)	1.70 (1.48–1.84)	2.49 (2.22–2.89)	<0.0001
Ln RH-PAT index, mean (SD)	0.74 (0.28)	0.50 (0.15)	0.94 (0.18)	< 0.0001
Medications, N (%)				
Aspirin	264 (50)	132 (55)	132 (46)	0.03
Statins	217 (41)	106 (44)	111 (38)	0.18
ACEi/ARB	138 (26)	74 (31)	64 (22)	0.02
β Blocker	169 (32)	84 (35)	85 (29)	0.17
CCB	121 (23)	68 (28)	53 (18)	0.01
Diuretics	88 (17)	44 (18)	44 (15)	0.35
Antihypertensive agents	272 (51)	136 (56)	136 (47)	0.03
Antidiabetic agents	43 (8)	29 (12)	14 (5)	0.002

ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BP, blood pressure; CAD, coronary artery disease; C-age, chronological age; CCB, calcium channel blocker; Δ age, ECG-age–C-age; ECG-age, ECG-derived artificial intelligence–estimated age; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; PMEF, peripheral microvascular endothelial function; and RH-PAT, reactive hyperemia peripheral arterial tonometry.

HR, 4.72; 95% CI, 1.24–17.91; *P*=0.02), whereas the highest quartile of the Δ age was not associated with MACEs in patients with normal PMEF (adjusted HR, 0.87; 95% CI, 0.09–8.28; *P*=0.90) (Table 5). In addition, we performed multivariate analysis 2 with further categorization based on sex in patients with abnormal PMEF. Seventeen female patients (13%) and 16 male patients (15%) with abnormal PMEF developed MACEs during follow-up (*P*=0.69). The highest quartile of the Δ age was significantly associated with an increased risk of MACEs only in women (adjusted HR, 11.04; 95% CI, 1.43–85.38; *P*=0.02), whereas the highest quartile of the Δ age was not associated with MACEs in men (adjusted HR, 3.60; 95% CI, 0.36–35.58; *P*=0.27) (*P* for

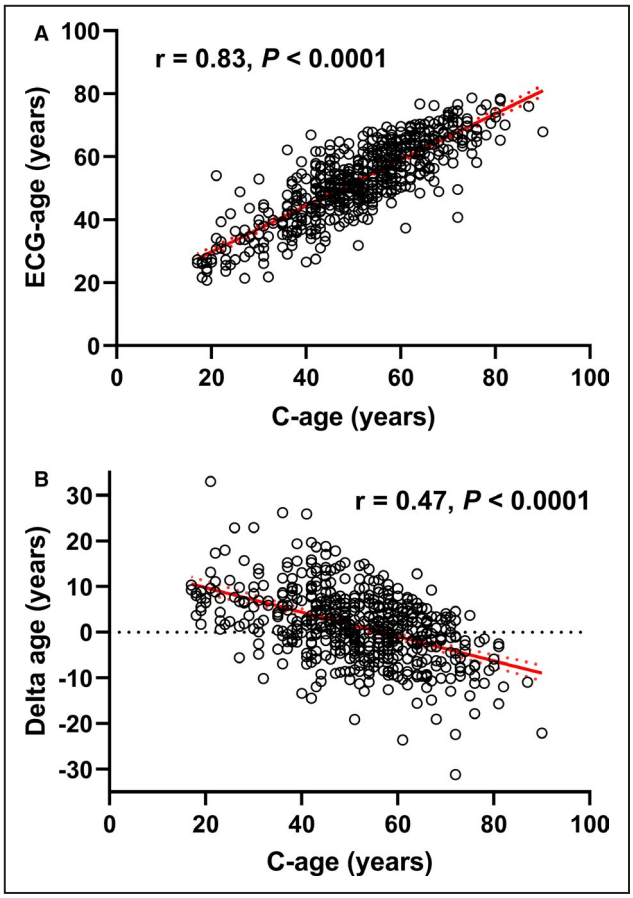

Figure 1. Correlation between ECG-age, Δ age, and chronological age (C-age).
(**A**) There was a strong correlation between ECG-age and C-age (r=0.83; *P*<.0001). (**B**) Δ age was negatively correlated with C-age (r=0.47; *P*<.0001).

interaction=0.04). Next, we assessed the discriminatory power of Δ age for MACEs when adding Δ age to RH-PAT index by calculating net reclassification improvement and integrated discrimination improvement. The discriminatory accuracy for MACEs did not significantly improve after adding Δ age to RH-PAT index in this population (integrated discrimination improvement, 0.002 [95% CI, −0.002 to 0.014] [*P*=0.16]; net reclassification improvement, 0.32 [95% CI, 0.04–0.60] [*P*=0.03]).

Sensitivity Analysis

For sensitivity analysis, we repeated the analysis with the population, excluding patients whose ECGs were used in the training set of the AI algorithm. A total of 513 patients were ultimately included in the sensitivity analysis. The baseline characteristics of the population for sensitivity analysis were similar to the whole population (mean C-age, 51.7±13.9 years; mean ECG-age, 53.0±12.3 years; 38.6% men) (Table S1). There was a borderline association between the highest quartile of the Δ age and an increased risk of MACEs after adjustment for C-age (C-age adjusted HR, 2.33; 95% CI, 0.91–5.93; *P*=0.08) (Table S2). The highest quartile of the Δ age was significantly associated with an increased risk of MACEs after adjustment for other cardiovascular risk factors in patients with abnormal PMEF (adjusted HR, 4.72; 95% CI, 1.24–17.96; *P*=0.02) (Tables S3 and S4).

DISCUSSION

In this study, we demonstrated that the vascular aging detected by abnormal peripheral endothelial function was associated with an increased difference between AI determined age and chronologic age (Δ age), potentially suggesting a mechanism of accelerated physiological aging. Furthermore, the highest quartile of the Δ age was significantly associated with an increased risk of MACEs in the presence of peripheral endothelial dysfunction, indicating that vascular aging may contribute to cardiovascular risk in people with accelerated physiologic aging.

Peripheral Microvascular Endothelial Dysfunction as an Indicator of Vascular Aging

In this study, we used peripheral microvascular endothelial dysfunction as an indicator of vascular

Table 2. Multiple Linear Regression Analysis for Predictors of Δ Age

Variable	Unstandardized Coefficient		Standardized Coefficient	*t* Value	*P* Value
	β	SE	β		
Chronological age	−0.27	0.02	−0.48	−11.6	<0.0001
Male sex	−0.13	0.31	−0.02	−0.41	0.69
Hypertension	−0.35	0.33	−0.04	−1.04	0.30
Dyslipidemia	0.40	0.35	0.05	1.13	0.26
Diabetes mellitus	−0.43	0.52	−0.03	−0.83	0.41
Smoking history	0.03	0.31	0.004	0.11	0.91
RH-PAT index	−0.97	0.49	−0.08	−1.98	0.048

Δ Age indicates ECG-derived artificial intelligence–estimated age minus chronological age; and RH-PAT, reactive hyperemia peripheral arterial tonometry.

Table 3. Association Between Δ Age and Age-Adjusted HR of MACEs

Δ Age Quartile	Age-Adjusted HR	95% CI	*P* Value
1	1.00		
2	1.50	0.73–3.07	0.28
3	1.61	0.74–3.53	0.23
4	2.64	1.02–6.78	0.04

Δ Age indicates ECG-derived artificial intelligence–estimated age minus chronological age; HR, hazard ratio; and MACE, major adverse cardiovascular event.

aging. Growing evidence suggests that senescent endothelial cells can acquire a particular senescent phenotype through multifaceted pathways, such as reduced NO availability, oxidative stress–induced DNA damage, mitochondrial dysfunction, impaired angiogenesis, and senescent endothelial progenitor cell–associated imbalance between endothelial damage and repair.[1,22-29] Furthermore, increased oxidative stress in aging may lead to form highly reactive oxidant, peroxynitrite, via the reaction with NO and cause cardiovascular diseases in aging.[30-33] These phenotypic changes are thought to be the main drivers of endothelial senescence and vascular aging, both of which are associated with endothelial dysfunction.[34-36]

Peripheral Microvascular Endothelial Dysfunction and Physiological Aging

In this study, we showed the association between abnormal peripheral endothelial function and Δ age and the impact of Δ age on the adverse cardiovascular outcomes in the presence of peripheral endothelial dysfunction, even after adjustment for other cardiovascular risk factors, indicating the close link between vascular aging and physiological aging. Possibly, ECG-age might be reflecting endothelial dysfunction of the coronary vasculature; therefore, the association between peripheral endothelial dysfunction and Δ age may reflect the systemic nature of endothelial dysfunction leading to end organ damage, predominantly in the heart, brain, and kidney, and aging-related functional decline.[11,37-39]

Lifestyle intervention may reverse some of the processes involved in vascular aging. For example, caloric restriction exhibits significant antioxidant and anti-inflammatory vascular effects, mediated by SIRT (Sirtuin) 1 (SIRT1)-dependent pathway.[40,41] Furthermore, routine physical activity was shown to improve age-related endothelial dysfunction, which is hypothetically attributed to a cardiovascular risk reduction with exercise through reduction of oxidative stress, mitochondrial protection, and anti-inflammatory effects.[42-44] Also, pharmacological inhibition of renin-angiotensin system was shown to exert anti-vascular aging effect by mitigating angiotensin-2–induced chronic low-grade vascular inflammation and oxidative stress.[45] However, whether the improvement of endothelial function and the reduction of the Δ age with lifestyle and/or pharmacological intervention translates into reduction of cardiovascular diseases remains to be determined in future studies.

Limitations

This study has several limitations. First, because of its retrospective observational cohort design, it is challenging to derive causal associations from the current study. The evaluation of RH-PAT index and 12-lead ECG was performed at the discretion of the evaluating physicians. Thus, a certain element of selection bias cannot be excluded. Second, after excluding the patient ECGs that had been used in the training set, the statistical significance showing the association between Δ age and MACEs was weakened because

Table 4. Multivariate Cox Regression Analysis for MACEs

Variable	All Patients			All Patients		
	Adjusted Hazard Ratio	95% CI	*P* Value	Adjusted Hazard Ratio	95% CI	*P* Value
Δ age fourth vs first quartile	2.78	1.06–7.29	0.04	2.22	0.85–5.79	0.10
Chronological age	1.07	1.04–1.11	<0.0001	1.09	1.06–1.12	<0.0001
Male sex	1.30	0.75–2.27	0.35			
Hypertension	3.34	1.57–7.10	0.002			
Dyslipidemia	1.40	0.58–3.40	0.45			
Diabetes mellitus	2.73	1.42–5.24	0.003			
Smoking history	0.58	0.32–1.07	0.08			
Abnormal PMEF				1.82	1.03–3.21	0.04

Δ age indicates ECG-derived artificial intelligence–estimated age minus chronological age; MACE, major adverse cardiovascular event; and PMEF, peripheral microvascular endothelial function.

Table 5. Multivariate COX Regression Analysis for MACE With Further Categorization Based on Normal Versus Abnormal PMEF

	Abnormal PMEF			Normal PMEF		
	Adjusted Hazard Ratio	95% Confidence Interval	*P* Value	Adjusted Hazard Ratio	95% Confidence Interval	*P* Value
Delta age 4th vs 1st quartile	4.72	[1.24–17.91]	0.02	0.87	[0.09–8.28]	0.90
Chronological age	1.09	[1.04–1.13]	0.0003	1.06	[1.02–1.12]	0.01
Male sex	0.88	[0.41–1.87]	0.74	2.29	[0.87–6.02]	0.09
Hypertension	1.80	[0.69–4.74]	0.23	7.58	[1.72–33.50]	0.01
Dyslipidemia	1.19	[0.34–4.19]	0.78	1.81	[0.51–6.44]	0.36
Diabetes mellitus	3.56	[1.53–8.31]	0.003	2.31	[0.47–11.30]	0.30
Smoking history	0.47	[0.21–1.05]	0.06	1.03	[0.34–2.89]	0.95

Delta age, artificial intelligence-estimated age—chronological age. PMEF indicates peripheral microvascular endothelial function.

of a decreased number of samples. However, there remained a strong trend demonstrating the same results, and the association between the highest quartile of the Δ age and MACEs in patients with abnormal PMEF remained statistically significant. In addition, although we calculated the predictive value of the Δ age using a multivariate analysis, we could not adjust for all the variables because of the small numbers of events in our sample. Finally, although our prior work suggests that the ECG-age is a valuable marker of physiologic age and that the difference between ECG-age and C-age may be a marker of accelerated physiologic aging, there is no "gold standard" test for physiologic age, so the nature of the relationships between physiologic age and physiologic signals remains speculative.

CONCLUSIONS

Abnormal peripheral endothelial dysfunction is likely associated with an increased ECG-age. Patients with abnormal peripheral endothelial dysfunction and the highest degree of accelerated physiologic aging appear to be physiologically older than the same C-aged cohorts, and have an increased risk for cardiovascular events. This observation suggests that peripheral endothelial function is associated with physiologic aging and that it is a marker for risk of cardiovascular events, although further validation is necessary in different cohorts. Future studies need to evaluate the utility of these indexes of physiological aging for management of patients at high cardiovascular risk.

ARTICLE INFORMATION

Received October 21, 2020; accepted November 16, 2020.

Affiliations

From the Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN (T.T., Z.A., M.C.-S., I.O., P.A.N., F.L.-J., S.K., P.A.F., A.L.); Division of Cardiology, National Defense Medical College, Tokorozawa, Saitama, Japan (T.T.); and Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN (L.O.L.).

Sources of Funding

This study was partly supported by the National Institute of Health (grants DK120292 and DK 122734) and the Mayo Foundation.

Disclosures

Dr Lerman declared consulting for Itamar Medical. Dr Friedman, Z. Attia, Dr Lopez-Jimenez, and Dr Kapa have filed intellectual property related to the artificial intellegence algorithm used herein to detect age from the ECG. The remaining authors have no disclosures to report.

Supplementary Material

Tables S1–S4

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Supplemental Material

Table S1. Baseline characteristics comparing patients with normal vs abnormal PMEF (Removing patients whose ECGs were used in the training set).

	All Patients N = 513	RH-PAT index		*P* value
		≤ 2.0 N = 233	> 2.0 N = 280	
Clinical characteristics				
C-age, mean (SD), years	51.7 (13.9)	51.9 (13.7)	51.6 (14.1)	0.84
ECG-age, mean (SD), years	53.0 (12.3)	54.1 (12.2)	52.1 (12.3)	0.07
Delta age, mean (SD), years	1.3 (7.8)	2.3 (7.9)	0.5 (7.7)	0.01
ECG-age > C-age, N(%)	296 (58)	141 (61)	155 (55)	0.24
Male, N (%)	198 (39)	107 (46)	91 (33)	0.002
Hypertension, N (%)	222 (43)	103 (44)	119 (43)	0.70
Diabetes mellitus, N (%)	51 (10)	34 (15)	17 (6)	0.001
Dyslipidemia, N (%)	364 (71)	177 (76)	187 (67)	0.02
Smoking history, N (%)	181 (35)	91 (39)	90 (32)	0.10
CAD, N (%)				
None	305 (60)	127 (55)	178 (64)	
< 50%	90 (18)	38 (16)	52 (19)	0.01
≥ 50%	117 (23)	67 (29)	50 (18)	
Body mass index, median (IQR), kg/m^2	27.0 (23.7-31.4)	28.3 (25.4-33.1)	25.8 (23.0-29.6)	< 0.0001
Systolic BP, mean (SD), mmHg	122 (17)	121 (17)	122 (17)	0.57
Diastolic BP, mean (SD), mmHg	75 (10)	74 (10)	75 (11)	0.04
Laboratory data				
LDL, median (IQR), mg/dL	103 (80-128)	103 (75-125)	103 (83-130)	0.35
HDL, median (IQR), mg/dL	53 (44-67)	50 (41-63)	58 (46-70)	< 0.0001
TG, median (IQR), mg/dL	109 (76-158)	120 (78-183)	107 (74-146)	0.05
Glucose, median (IQR), mg/dL	96 (90-104)	97 (91-105)	95 (89-103)	0.03

HbA1c, median (IQR), %	5.4 (5.1-5.9)	5.6 (5.1-6.0)	5.4 (5.1-5.8)	0.40
Creatinine, median (IQR), mg/dl	0.9 (0.8-1.1)	0.9 (0.8-1.1)	0.9 (0.8-1.0)	0.16
eGFR, mean (SD), ml/min/1.73 m^2	75.6 (18.6)	77.7 (19.7)	74.0 (17.5)	0.03
RH-PAT index, median (IQR),	2.07 (1.72-2.52)	1.69 (1.47-1.84)	2.49 (2.22-2.90)	< 0.0001
Ln RH-PAT index, mean (SD)	0.74 (0.28)	0.49 (0.14)	0.94 (0.18)	< 0.0001
Medications				
Aspirin, N (%)	255 (50)	127 (55)	128 (46)	0.05
Statins, N (%)	211 (41)	102 (44)	109 (39)	0.27
ACEi /ARB, N (%)	134 (26)	71 (31)	63 (23)	0.04
β blocker, N (%)	163 (32)	79 (34)	84 (30)	0.34
CCB, N (%)	119 (23)	67 (29)	52 (19)	0.01
Diuretics, N (%)	85 (17)	43 (19)	42 (15)	0.30
Anti-hypertensive, N (%)	265 (52)	131 (56)	134 (48)	0.06
Anti-diabetics, N (%)	42 (82)	28 (12)	14 (5)	0.004

ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BP, blood pressure; CAD, coronary artery disease; C-age, chronological age; CCB, calcium channel blockers; Delta age, ECG-age – C-age; ECG-age, electrocardiogram-derived artificial intelligence-estimated age; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HbA1c, haemoglobin A1c; IQR, interquartile range; LDL, low-density lipoprotein; RH-PAT, reactive hyperemia peripheral arterial tonometry; SD, standard deviation.

Table S2. Association between delta age and age adjusted hazard ratio of major cardiovascular events (Removing patients whose ECGs were used in the training set).

Delta age quartile	Age adjusted hazard ratio	95% confidence interval	*P* value
1	1.00		
2	1.39	[0.68-2.85]	0.37
3	1.29	[0.59-2.83]	0.23
4	2.33	[0.91-5.93]	0.08

Delta age, artificial intelligence-estimated age – chronological age

Table S3. Multivariate COX regression analysis for MACE (Removing patients whose ECGs were used in the training set).

	All patients			All patients		
	Adjusted hazard ratio	**95% confidence interval**	***P* value**	**Adjusted hazard ratio**	**95% confidence interval**	***P* value**
Delta age 4th vs 1st quartile	2.39	[0.91-6.30]	0.08	1.99	[0.77-5.15]	0.15
Chronological age	1.07	[1.04-1.10]	< 0.0001	1.09	[1.06-1.12]	< 0.0001
Male sex	1.37	[0.78-2.41]	0.27			
Hypertension	3.64	[1.61-8.20]	0.002			
Dyslipidemia	1.35	[0.56-3.28]	0.50			
Diabetes mellitus	2.82	[1.44-5.51]	0.003			
Smoking history	0.50	[0.27-0.94]	0.03			
Abnormal PMEF				1.73	[0.98-3.07]	0.06

Delta age, artificial intelligence-estimated age – chronological age; PMEF, peripheral microvascular endothelial function.

Table S4. Multivariate COX regression analysis for MACE with further categorization based on normal vs abnormal PMEF (Removing patients whose ECGs were used in the training set).

	Abnormal PMEF			Normal PMEF		
	Adjusted hazard ratio	**95% confidence interval**	***P* value**	**Adjusted hazard ratio**	**95% confidence interval**	***P* value**
Delta age 4th vs 1st quartile	4.72	[1.24-17.96]	0.02	0.56	[0.06-5.40]	0.61
Chronological age	1.08	[1.04-1.14]	0.0004	1.06	[1.01-1.11]	0.01
Male sex	0.99	[0.47-2.12]	0.99	2.53	[0.97-6.59]	0.06
Hypertension	2.35	[0.85-6.53]	0.10	8.55	[1.83-40.09]	0.01
Dyslipidemia	1.03	[0.29-3.67]	0.96	2.42	[0.62-9.40]	0.20
Diabetes mellitus	3.39	[1.45-7.91]	0.01	1.98	[0.39-10.02]	0.41
Smoking history	0.37	[0.16-0.86]	0.02	0.97	[0.34-2.80]	0.96

Delta age, artificial intelligence-estimated age – chronological age; PMEF, peripheral microvascular endothelial function.