JACC: CLINICAL ELECTROPHYSIOLOGY
VOL. 10, NO. 4, 2024

STATE-OF-THE-ART REVIEW

JACC FAMILY SERIES

Assessing Biological Age

The Potential of ECG Evaluation Using Artificial Intelligence: *JACC* Family Series

Francisco Lopez-Jimenez, MD, MSc, MBA,[a] Suraj Kapa, MD,[a] Paul A. Friedman, MD,[a] Nathan K. LeBrasseur, PhD,[b] Eric Klavetter, JD,[a] Kathryn E. Mangold, PhD,[a] Zachi I. Attia, PhD[a]

ABSTRACT

Biological age may be a more valuable predictor of morbidity and mortality than a person's chronological age. Mathematical models have been used for decades to predict biological age, but recent developments in artificial intelligence (AI) have led to new capabilities in age estimation. Using deep learning methods to train AI models on hundreds of thousands of electrocardiograms (ECGs) to predict age results in a good, but imperfect, age prediction. The error predicting age using ECG, or the difference between AI-ECG–derived age and chronological age (delta age), may be a surrogate measurement of biological age, as the delta age relates to survival, even after adjusting for chronological age and other covariates associated with total and cardiovascular mortality. The relative affordability, noninvasiveness, and ubiquity of ECGs, combined with ease of access and potential to be integrated with smartphone or wearable technology, presents a potential paradigm shift in assessment of biological age. (J Am Coll Cardiol EP 2024;10:775–789) © 2024 Published by Elsevier on behalf of the American College of Cardiology Foundation.

Aging is defined as ongoing functional decline: a progressive reduction in physiological and cellular function over time.[1] With decreased physiological response to stresses, and a failure of complex molecular mechanisms, aging is associated with a reduction in repair and regeneration in the tissues and organs.[2] Advancing age is commonly accompanied by increased morbidity and a higher risk of mortality, and is particularly associated with the development of chronic conditions.[1,3,4]

THE RATE OF AGING

The rate of aging, beyond the passing of years, is influenced by the hallmarks of aging, which include genetic factors, telomere attrition, cellular senescence, epigenetic changes, loss of proteostasis, and lifestyle and environmental influences.[1,4] The stability of DNA is continuously challenged by extrinsic physical, chemical, and biological agents, and by intrinsic events including DNA replication errors, spontaneous hydrolytic reactions, and reactive oxygen species.[5] Genomic instability interferes with mechanisms responsible for maintaining the length and functionality of telomeres and for ensuring the integrity of mitochondrial DNA (mtDNA), both of which are also hallmarks of the aging process.[6,7] Somatic variations accumulate within cells with advancing age, and copy number variations, chromosomal aneuploidies, and increased clonal mosaicism for large chromosomal anomalies have been associated with aging.[1,8-10] The oxidative microenvironment of the mitochondria, lack of protective histones, and limited repair mechanism efficiency mean that aging-associated somatic mutations occur at a higher rate in the mtDNA than in chromosomal DNA.[11] However, the role of mtDNA

From the [a]Department of Cardiovascular Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota, USA; and the [b]Robert and Arlene Kogod Center on Aging, Mayo Clinic College of Medicine, Rochester, Minnesota, USA.
The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received October 20, 2023; revised manuscript received February 8, 2024, accepted February 11, 2024.

ISSN 2405-500X/$36.00
https://doi.org/10.1016/j.jacep.2024.02.011

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 10, NO. 4, 2024
APRIL 2024:775–789

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

AI = artificial intelligence

CAC = coronary artery calcification

CNN = convolutional neural network

CVD = cardiovascular disease

ECG = electrocardiogram

MLR = multiple linear regression

PCA = principal component analysis

δage = delta age

mutation in aging has been controversial because of the heteroplasmic nature mitochondrial genomes (coexistence of mutant and wild-type genomes within the same cell).[1]

Telomeres are repetitive DNA sequences found at the ends of linear chromosomes; telomeres shorten with each cell division, eventually leading to replicative senescence or apoptosis.[6] As telomeres reach a critical length, they become unable to bind sufficient telomere-capping proteins and are sensed as exposed DNA ends, which activates a DNA damage response, which enforces a permanent proliferative arrest and contributes to cellular senescence.[12]

Cellular senescence is a stable state of growth arrest in which cells are unable to proliferate despite optimal growth conditions and mitogenic stimuli, and typically arises from chromatic remodeling, processes activated by telomere dysfunction, oxidative stress caused by accumulation of dysfunctional mitochondria, and oncogene expression.[13-15]

There are 3 key mechanisms of epigenetic aging: DNA methylation, histone modification, and chromatin remodeling.[1,16,17] DNA methylation is the most studied epigenetic mechanism. The process engages the proteins required in gene silencing or impeding the interaction between DNA and transcription factors, regulating gene expression.[16] DNA methylation occurs at the cytosines in CpG dinucleotides to form 5-mC, and 60% to 90% of CpG sites in the mammalian genome are methylated. The genome is generally hypomethylated during aging, although many CpGs are subject to age-related hypermethylation.[16,17] The change in level of methylation at CpG sites with age has been used as a biomarker to predict chronological age—the epigenetic clock.[18-21]

Post-translational modification of histones, often by methylation or acetylation at the lysine residues, can activate or silence gene expression and regulate the aging process. Global changes to H3K9me3, H4K20me3, H3K27me3, and H3K9ac levels have been observed during aging.[16,17] A reduction in expression the H3K9me3 methyltransferase SUV39H1 is observed with aging and is associated with perturbed heterochromatin function; this loss of heterochromatic integrity is a common feature of aging.[22]

Chromatin remodeling refers to genome-wide changes in the nuclear architecture that can be observed at specific chromosomes or chromosome domains.[17] Age-related dysregulation of chromatin organization can lead to cellular malfunctions and exacerbate the aging process. A loss of trimethylation of H3K9me3 with increasing age is associated with impaired formation of heterochromatin, leading to the de-repression of silenced genes and aberrant gene expression patterns.[23]

The proteostatic pathways control the synthesis, folding, and degradation of proteins. Proteotoxic stress increases with age and can result in misfolded proteins and then protein aggregates. Protein aggregation has been linked to Alzheimer's and Parkinson's disease.[24]

Environmental and lifestyle factors including diet, exercise levels, metabolic dysfunction/obesity, exposure to UV radiation, and toxins (including smoking) can accelerate aging by inducing DNA damage, oxidative stress, and other cellular deteriorations.[3,25,26]

HIGHLIGHTS

- Chronological age only partially predicts morbidity and mortality.
- Measures of biological age can provide additional insight into a person's physiological and health status.
- AI can derive age from ECGs; delta age is the difference between AI-ECG age and chronological age.
- Large positive delta age is associated with increased risks of all-cause and cardiovascular mortality, suggesting that AI-ECG is a valid surrogate measure of biological aging.

DISEASE AND AGING

It is understood that aging, at both the cellular and organismal levels, contributes to the development and progression of chronic diseases. However, less is known about the inverse relationship: the contribution of chronic diseases and their treatments to the progression of aging.[27]

Several specific diseases have been linked to accelerated aging through several different mechanisms. Both type 1 and type 2 diabetes drive metabolic changes that affect various organs and systems in the body. If untreated, disturbances in insulin signaling and carbohydrate homeostasis may contribute to the development of other age-related complications.[27,28] Chronic kidney disease, hypertension, atherosclerosis, and the presence of certain cancers can lead to increased oxidative stress and inflammation.[27,29-31] Chronic inflammation is associated with both normal and pathological aging. Murine

model data suggest that that systemic chronic inflammation can accelerate aging via reactive oxygen species-mediated exacerbation of telomere dysfunction and cell senescence in the absence of any other genetic or environmental factor.[32]

Alzheimer's disease and Parkinson's disease have been associated with increased oxidative stress and mitochondrial dysfunction, leading to elevated cellular senescence.[33] Human immunodeficiency virus (HIV) infection has been linked to changes in T cell telomere length, CDKN2A expression, accumulation of $CD28^{+}CD8^{-}$ T cells, reduced naive T cell generation, and persistent chronic immune activation, leading to accelerated immune senescence.[34] SARS-CoV-2 (COVID-19) infection has been associated with epigenetic aging, and an accumulation of epigenetic alterations during infection may contribute to post-COVID-19 syndrome (long COVID).[35]

Stress is associated with rapid increases in biological age; however, these increases can be short-lived, and have been shown to reverse on resolution of the stress episode.[36] Stress can be associated with other conditions and is common in patients with cancer or HIV (in which the stress may be a longer-term consequence), and recently in people with COVID-19.[27,36]

In terms of how disease treatment accelerates aging, there is a relatively small pool of research. Highly active antiretroviral therapy in HIV appears to accelerate telomere attrition.[27] Several cancer treatments increase genomic instability, cellular senescence, and stem cell exhaustion.[1,27] Chemotherapy has been associated with increased markers of aging,[27,31] and could be thus considered a progeronic intervention.

SOCIODEMOGRAPHIC FACTORS AND PREMATURE AGING

Sociodemographic factors influence lifestyle choices like diet, exercise, and smoking, all of which are known contributors to premature physiological aging,[3,25,26,37] and socioeconomic-associated health disparities can lead to chronic stress and premature aging.[38] Individuals with lower income and education levels often have limited access to health care, which may limit the management of chronic conditions.[39,40] Minority populations often experience premature aging, driven by relatively poor access to care.[41] Gender differences in biological, psychological, and social roles can also influence aging. In general, women live longer than men with relatively lower biological ages; however, women have worse health at the end of life, while men perform better in physical function examinations.[42,43]

Living in neighborhoods with high pollution, violence, or lack of access to health care can lead to synergistic forms of stress, contributing to premature aging.[44] A lack of social support and feelings of isolation can lead to chronic stress, and loneliness has been identified as a risk factor for increased morbidity and mortality and accelerated physiological aging.[45]

INTRODUCING BIOLOGICAL AGE

Biological age refers to an individual's internal physiological state, as opposed to their chronological age, and captures how well or poorly the body is functioning relative to an average state of health for individuals of a specific chronological age. Biological age is driven by epigenetic, cellular, genomic, disease, and treatment states, and environmental modifiers (**Figure 1**), and is expected to provide insight beyond chronological age into risk for morbidities and mortality.[46]

MEASURING BIOLOGICAL AGE

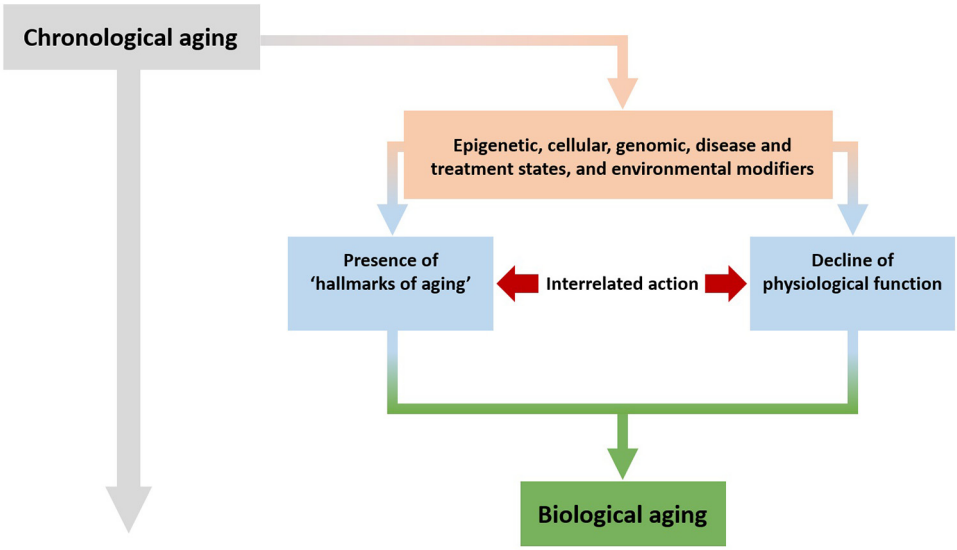
The search for reliable and accessible indicators of biological age has seen meaningful advances in recent years.[47] Although biomarker studies have commonly found cross-sectional associations, determining predictive associations has been challenging.

Senescence-associated beta-galactosidase staining was one of the first biomarkers for senescence and is still a widely used measure.[13,48] Senescent cells demonstrate an increased expression of cell cycle-inhibitory proteins, and the cyclin-dependent kinase inhibitor $p16^{INK4A}$ is a robust marker of senescence.[13]

There are several epigenetic clocks that analyze DNA methylation levels at sets of CpG sites;[19,49] these clocks are recognized as accurate measures of age and predictors of mortality. Although the clocks work on the same principle, they analyze methylation in different ways: the Horvath clock is based on methylation levels of 353 CpG sites,[19] and the Hannum clock uses 71 CpG sites.[49] Developments in this field continue, including DNAm PhenoAge and GrimAge.[50-52] AgeAccelGrim added quantification of the difference between GrimAge and a patient's chronological age, and was found to be broadly predictive of time to congestive heart disease, coronary heart disease, hypertension, and type 2 diabetes.[51] A recent meta-analysis found that each 5-year increase in DNA methylation age was associated an 8% to 15% increased risk of mortality and that there was "some, although inconsistent, evidence for an association between increased DNA methylation age and risk of disease," suggesting that further research is needed

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 10, NO. 4, 2024
APRIL 2024:775–789

FIGURE 1 Biological Aging Is a Multifactorial Process



Biological aging is different from chronological aging, and is accelerated by genetic, disease, and lifestyle factors.

in order to use DNA methylation as a clinical biomarker.[53]

Telomeres can be measured using several methods, the most common being quantitative polymerase chain reaction, terminal restriction fragment analysis, a variety of quantitative fluorescence in situ hybridization methods, single telomere length analysis, and telomere shortest length assay.[54,55] The rate of telomere shortening may be more predictive of life span and mortality than structure length alone.[38]

Several omics-based biomarkers of aging have been investigated. Transcriptomics studies the output of the transcriptome, which encompasses the full range of RNA and messenger RNA expressed at a given time, is highly dynamic, and is responsive to environmental changes. Transcriptomic age correlates well with epigenetic age but less so with chronologic age. However, the combination of transcriptional markers and other and epigenetic markers can outperform either marker alone in age prediction.[56] Proteomics provides a quantitative description of the sum of protein expression in an organism and demonstrates influence by perturbation.[56] Metabolomics studies the metabolic responses of living systems to manipulation and was incorporated into a Metabolic Age Score.[57] However, this assessment cannot yet estimate the metabonomic changes of aging at the individual level.[56]

A study of a panel of blood biomarkers found that various biomarker signatures exist with significant associations with physical function, morbidity, and mortality. However, a signature of dysregulation of a single biomarker can change with dysregulation of other biomarkers, and age-related changes of individual blood biomarkers alone do not necessarily indicate disease or functional decline.[46]

Several mathematical models are commonly used to measure biological age. In use for over 50 years, the multiple linear regression (MLR) approach is a basic and preliminary method of estimation. MLR uses clinical biomarkers of aging, including renal, cardiovascular (cholesterol), hepatic, hematocrit, and immunoglobin measurements, that are determined based on their correlation with chronological age.[58,59] The key limitation of the MLR estimate is that because biological age is constructed linearly with chronological age, it cannot be determined whether chronological age is performing as a biomarker or a selection criterion. MLR also distorts biological age at the regression edge and does not account for the discontinuity of the aging rate over the individual's lifetime.[58]

Principal component analysis (PCA) uses parameters closely related to chronological age, and is performed using uncorrelated parameters to identify biomarkers that can explain the majority of biological age variance—the principal component.[58,60] The

biomarkers of aging, which include clinical measures of renal function, blood pressure, hemoglobin levels, height, and waist and thigh circumference, are weighted and combined according to the PCA to give a biological age score.[59] The biological age score is not expressed as an age in years, and this age score is difficult to compare directly with chronological age.[58] Recently, research groups have modified the PCA model to account for this by adding a chronological age input.[58]

Klemera and Doubal's method is a graphing method that uses a reverse regression technique for biological age estimation. Several presumptions are made about the relationship between chronological age, biological age, and biomarkers, including that the speed of aging is different between individuals of the same species, and that measurable indicators that change with chronological age should be defined as aging biomarkers.[58] In this model, chronological age is input as a standard biomarker and not an outcome to be predicted, and estimates are based on minimizing the distance between regression lines and biomarker points, within a dimensional space of all biomarkers.[58,59,61] This results in a biological age that is equal to the chronological age, plus the effect of any variables.[61] Although Klemera and Doubal's method is generally accepted as the optimal estimate of biological age, including in young adults, it still has significant limitations.[58] Comparisons between the presumptions are unavailable for the evaluation of lifespan and longevity. Biomarkers to predict mortality and those of longevity may not necessarily be reciprocal and may not be the same biomarker or hallmark.[58]

CARDIAC AGE IN PRACTICE

Cardiac, or vascular, age is used to evaluate the risk of cardiovascular disease (CVD) and is calculated based on variables such as blood pressure, cholesterol levels, family history of heart disease, and lifestyle factors like smoking status and exercise levels.[62]

Developed in 2008, the HEART score combines patient history, electrocardiogram (ECG) findings, chronological age, established cardiovascular risk factors, and troponin levels to give a score (0-10) that stratifies 3 levels of risk.[63,64]

Whereas cardiac age focuses specifically on the vascular system, biological age assessment provides a comprehensive view of an individual's aging status, incorporating multiple biological systems.[47]

USING ECG TO ASSESS BIOLOGICAL AGE

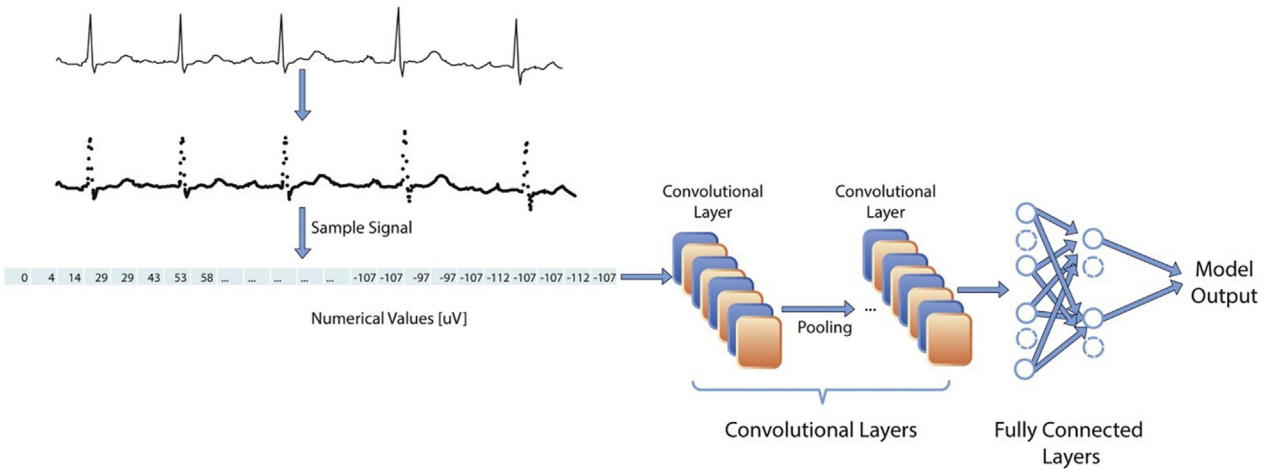
Invented in 1901 by Willem Einthoven, the ECG has become a cornerstone of cardiovascular risk stratification and disease and management.[65] With recent rapid developments in machine learning, neural networks, and artificial intelligence (AI), the boundaries of the "classic" ECG have been pushed, resulting in positive findings for application in serum potassium level estimation and hyperkalemia screening,[66,67] screening for asymptomatic left ventricular systolic dysfunction,[68,69] identification of patients with atrial fibrillation (AF) during sinus rhythm,[70] and detecting the use of cocaine.[69]

Following preliminary results from the experiments trying to predict left ventricular systolic dysfunction with ECGs through convolutional neural networks (CNNs), the group at Mayo Clinic attempted to optimize the ECG-only model by incorporating age and sex, variables known to be associated with systolic dysfunction. The incorporation of those variables had no effect on the receiver-operating characteristic area under the curve, implying collinearity. The authors hypothesized that the CNN had already incorporated age and sex prediction, or in other words, that the model already knew the age and sex of the individuals.[65] This led to the seminal analysis and subsequent publication in 2019 by Attia et al[65] confirming such a hypothesis (The CNN schematic is shown in **Figure 2**).[71] In this study, the group trained using deep learning on a set of 10-second 12-lead ECG data from 499,727 patients; 399,750 unique ECGs were used in the training set and 99,977 unique ECGs were in the internal validation set.[65] This CNN was then tested on a separate cohort of 275,056 patient ECGs. A total of 100 patients, for whom several ECGs were available throughout the course of their lives, were selected to test intrapatient accuracy of CNN age estimation. Hereafter in this review, this methodology is referred to as the Mayo Clinic ECG-age CNN.

For prediction of sex, the Mayo Clinic ECG-age CNN model was 90.4% accurate. Age was estimated as a continuous variable with an average error of 6.9 ± 5.6 years. For a multigroup classification to the age groups of 18 to 25, 25 to 50, 50 to 75, and 75 years and older, the overall accuracy of the CNN was 71.6% (**Figure 3B**).

There was a difference between chronological age and CNN-predicted age of <7 years in 51% of the 100 participants with multiple ECGs.[65] Rather than assuming this suboptimal prediction of chronological age as an error, the group hypothesized that the difference between predicted age and actual

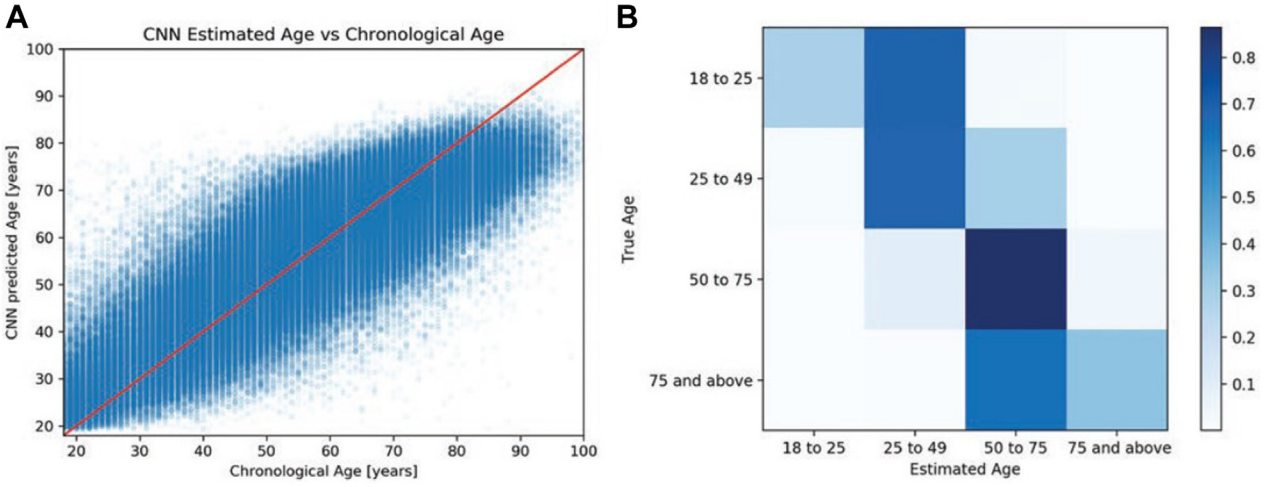
FIGURE 2 Schematic of the Development of a Convolutional Neural Network



The physical signal (the electrocardiogram) is converted to a digital signal (sampled) to obtain a list of numerical values. The digital values are then convolved with the network weights at each level to yield the layer output, each layer is continuously fed to the next, and the last layer represents the final model output. Network weights are set during training by feeding the network signals with known outputs and adjusting them until the optimal matching between the real labels and the network output is achieved. Reprinted from Lopez-Jimenez, et al. Artificial Intelligence in Cardiology: Present and Future. *Mayo Clinic Proceedings*. 2021;95(5):1015-39.[71]

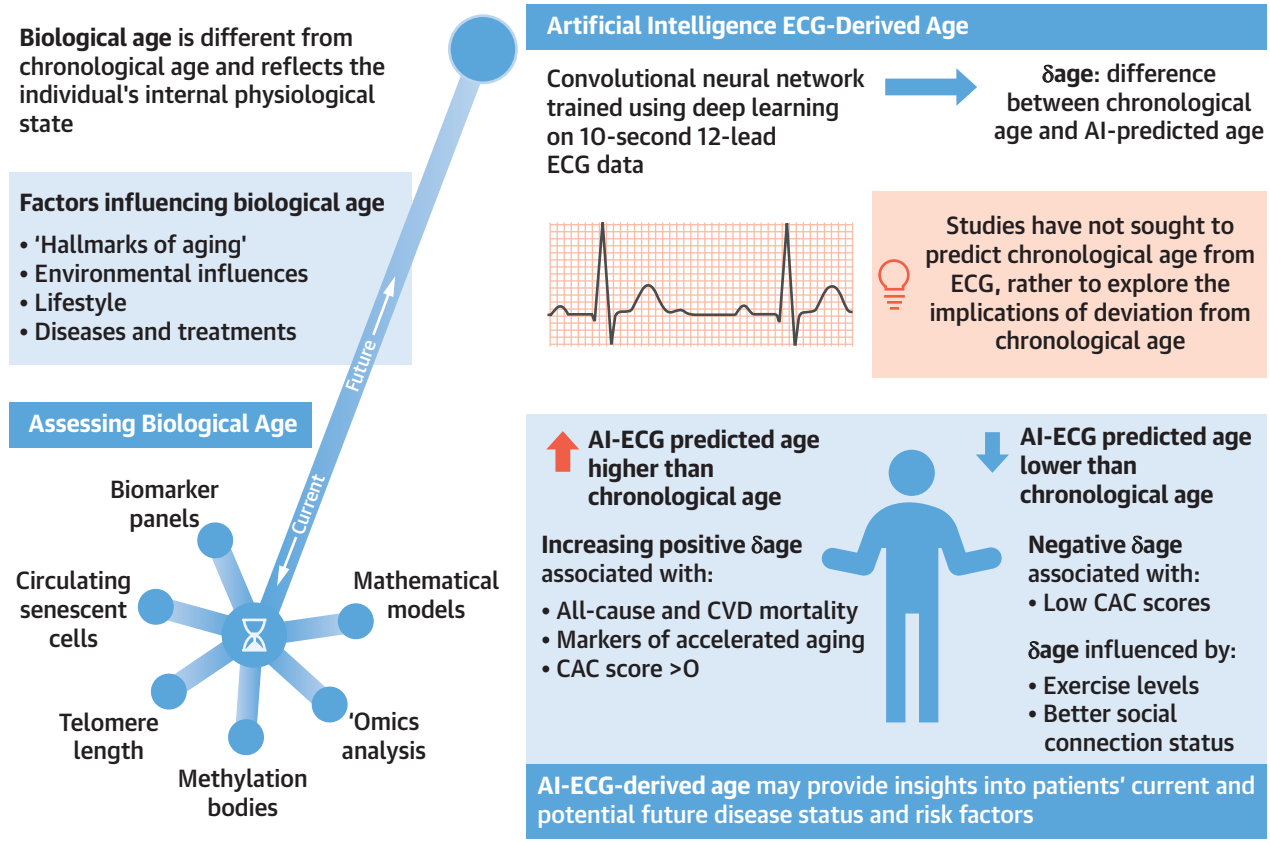
chronological age represented disease states or even advanced aging. The results suggested the age gap between predicted and actual age represented indeed something more than random error. In those with a difference between chronological age and AI-ECG-predicted age of >7 years, there was a significant increase in major cardiovascular risk factors: low ejection fraction, hypertension, and coronary artery

FIGURE 3 The Mayo Clinic Electrocardiographic Age CNN Model: Predicted Age vs Reported Age



(A) Estimated convolutional neural network (CNN)-predicted electrocardiographic age (blue) vs the reported chronological age (in years; red line), (B) multigroup classification to the age groups of 18 to 25, 25 to 50, 50 to 75, and 75 years and accuracy of CNN-predicted age (x-axis, estimated age) vs the actual age (y-axis) in terms of the percentage of patients with a specific actual age who had a specific corresponding CNN-predicted age within a similar range. Used with permission from Attia, et al. Age and Sex Estimation Using Artificial Intelligence From Standard 12-Lead ECGs. *Circulation: Arrhythmia and Electrophysiology*. 2019;12(9):e007284.[65]

CENTRAL ILLUSTRATION Assessment of Biological Age



Lopez-Jimenez F, et al. J Am Coll Cardiol EP. 2024;10(4):775-789.

The potential of AI-ECG-derived age differences between chronological and biological age in predicting clinical risk and outcomes. AI-ECG = artificial intelligence electrocardiogram; CAC = coronary artery calcification; CVD = cardiovascular disease.

disease. In the 27% of patients in which correlation was >0.8 between AI-ECG-predicted age and chronologic age, no incident events occurred over follow-up (33.0 ± 12 years). This suggested that any "error" in AI-ECG prediction of chronological age was an insight into a person's biological age, and that the age difference could be predictive of cardiovascular risk.

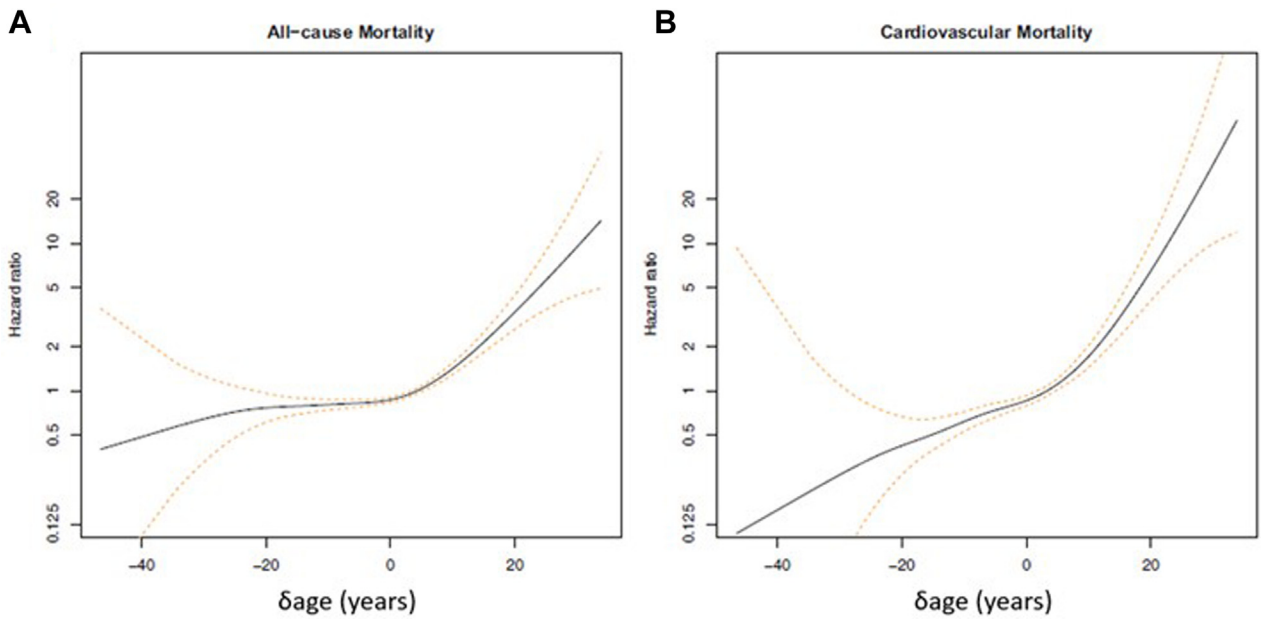
DELTA AGE

The difference between AI-ECG-predicted age and chronological age may give pause to the medical professional and patient to further explore undetected disease or simply accelerated aging in the absence of specific underlying disease mechanisms. Therefore, the AI-ECG-derived delta age (δage) is a potential biomarker for biological aging (Central Illustration).

INVESTIGATING THE VALUE OF AI-ECG–DERIVED δAGE

AI-ECG-derived δage has been investigated in 2 studies as a biomarker of biological age and accelerated aging. The Mayo Clinic ECG-age CNN was used on a cohort of 25,144 participants ≥30 years of age who had primary care outpatient visits from 1997 to 2003, who had been followed for 12.4 ± 5.3 years. Patients with an existing diagnosis of coronary artery disease, stroke, and AF were excluded from the analysis.[72] In this study, the mean chronological age was 53.7 years, the AI-ECG-derived age was 54.6 years, and the mean δage was 0.88 ± 7.4 years. Patients with δage ≥1SD higher than their chronological age had higher all-cause and CVD mortality than patients with a δage within 1 SD (Figure 4). Patients whose δage was ≤1 SD lower

FIGURE 4 Relationship Between Artificial Intelligence-Electrocardiogram-Derived δage and Mortality Outcomes



Spline curves showing the relationship between delta age (δage) and (A) all-cause mortality and (B) cardiovascular mortality. Used with permission from Ladejobi, et al. The 12-lead electrocardiogram as a biomarker of biological age. *European Heart Journal - Digital Health.* 2021;2(3):379-89.[72] CV = cardiovascular.

than their chronological age had lower all-cause and CVD mortality. Results were unchanged after adjusting for CVD risk factors and other factors influencing survival.

These findings demonstrated that AI-ECG-derived δage was associated with both cardiovascular and all-cause mortality. Because the association was evident even when adjusting for comorbidities known to predict survival, this suggests that δage is predictive to a greater extent than would be expected using chronological age alone. The study did not seek to predict chronological age from the ECG, but rather to examine the implications of deviation from the chronological age, indicating that AI-ECG-derived δage could act as a marker of either advanced or slower biological aging.

In a separate study, the Mayo Clinic ECG-age CNN was used to predict the age of 4,542 participants in the Know Your Heart study conducted in 2 cities in Russia (2015-2018). The association of δage with known CVD risk factors and markers of cardiac abnormalities was assessed using linear regression models.[73] The mean chronologic age was 54.6 years, the mean AI-ECG-derived age was 59.8 years, and the mean δage was 5.32 years. When adjusted for sex and chronological age, δage was significantly associated with systolic and diastolic blood pressure, mean arterial pressure, body mass index, low-density lipoprotein over high-density lipoprotein ratio, and smoking status. AI-ECG-derived δage was also significantly associated with 2 key markers of cardiac pathology: N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin T. Mean δage was higher in younger adults (35-60 years of age), and the strength of the association with both risk factors and markers of cardiac abnormalities increased further when only including this younger cohort in the model; AI-ECG-derived δage may help identify working-age people at risk of developing CVD.

The association between AI-ECG-derived δage and coronary artery calcification (CAC) was investigated using the Mayo Clinic ECG-age CNN in 41,202 consecutive patients that underwent clinically indicated CAC and ECG testing within 1 year (1997-2020).[74] The mean chronological age was 55.2 years, the mean AI-ECG-derived age was 56.0 years, and the mean δage was -0.78 ± 7.0 years. After adjustment for age and sex, those with a positive δage were more likely to have CAC score >0, with increasing odds with a larger δage. Those who were estimated as younger than their chronological by AI-ECG (negative δage) were less likely to have any CAC. These findings are meaningful because they suggest that AI-ECG-derived δage can identify

people who are more likely to have subclinical coronary atherosclerosis.

Both exercise and social isolation have been discussed here as factors that can influence the rate of biological aging. The Mayo Clinic ECG-age CNN was applied to 268,002 participants (mean age 59.7 ± 16.4 years) to assess the effect of moderate-to-strenuous exercise on biological age.[75] Participants performed a median 3 days per week of exercise, and the average time of exercising was 31.3 ± 30 minutes per day; a quarter (25.7%) of the cohort were not engaged in any exercise. AI-ECG–derived δage diminished progressively with increased duration of moderate-to-strenuous exercise, and linear regression analysis identified exercise as an independent predictor of δage.

A cohort of 280,323 adults seen at Mayo Clinic (2019-2022) who completed the social determinants of health questionnaire and had a 12-lead ECG within 1 year of completing the questionnaire was studied to examine the effect of social interaction on aging.[76] In this study, the mean chronological age was 59.7 years, and mean AI-ECG–derived age was 59.5 years. When adjusted for chronological age and sex, a better social connection status significantly correlated with lower δage. Participants reporting the least social connection were an average of 2 years older than their chronological age; those with the most social connection were 2 years younger than their chronological age, both as derived from the AI-ECG age.

Other studies are ongoing using the Mayo Clinic ECG-age CNN, including examining how obesity, depression, and adverse childhood experiences can influence AI-ECG–derived δage.

AI-ECG–DERIVED AGE AND GENETIC MARKERS OF CVD AND AGING

Following the initial proof-of-concept studies that associated AI-ECG–derived δage with risk of CVD and mortality, a genome-wide association study into the genetic underpinning of δage was performed.[77] The Mayo Clinic ECG-age CNN was used in conjunction with the UK Biobank to test association with approximately 6.4 million autosomal variants from 34,432 individuals.[65,77] Overall, 15 loci were found to be associated with δage with at least suggestive significance.

The gene *SIPA1L1* on chromosome 14 had the strongest association with δage; this gene has been associated with kidney function and blood flow control. Variation at this locus may also affect *RGS6* expression, which is demonstrably linked to systolic blood pressure, heart rate, and heart rate variability. Other associated genes have roles in: 1) hypertension and immune system (*DEFB136*, *DEFB135*, *DEFB134*, and *CTSB*); 2) cardiac sodium regulation/cvd/hf (*CAMK2D*); and 3) BP, AF, BMI, waist circumference (*VGLL2*). The genome-wide association study catalog mapped a variety of cardiovascular phenotypes and ECG traits to *TTN*, on chromosome 2, ranging from AF to the PR interval and left ventricular ejection fraction. In this analysis, genes associated with other forms of biological aging (eg, telomere length) were mostly absent from the loci identified as associated with AI-ECG–derived δage.

Mutations in the lamin A/C (*LMNA*) gene are linked to a spectrum of rare genetic disorders (laminopathies) that manifest across a range of phenotypes including arrhythmogenic cardiomyopathy and muscular dystrophies and atrophies. More than 90% of patients with Hutchinson-Gilford progeria syndrome, which is characterized by early aging and premature death due to myocardial infarction and stroke, carry a heterozygous de novo mutation, *LMNA* (c.1824C>T, p.Gly608Gly) in exon 11. A study by Shelly et al[78] retrospectively reviewed medical records from patients with *LMNA* mutations who underwent at least a 12-lead ECG at the Mayo Clinic (2003-2019) and looked at association with AI-ECG–derived age. Thirty-one patients (271 ECGs) were included. Patients with *LMNA* mutations had markers of senescence and accelerated aging, either in the presence or absence of cardiac abnormalities, and a higher AI-ECG–derived age than chronological age. This study demonstrated that raw ECG, interpreted by the CNN and AI, can identify differential aging rates caused by mutations in *LMNA*, which is known to be involved in aging.

AI-ECG age might be influenced by the presence/absence/extent of hallmarks of aging (DNA damage, epigenetic changes, senescence, inflammation, mitochondrial dysfunction) in the cardiovascular system or elsewhere and be manifested in the ECG in ways yet imperceptible to the human eye.

A WIDER LOOK AT AI-ECG INVESTIGATIONS

Research into the development, validation, and application of AI-driven interpretation of ECGs has been rapid and extensive in recent years.[79-82]

Following the original report from the Mayo Clinic using CNN to predict age using ECG, a couple of years later Lima et al[83] published findings from a deep neural network that assess 3 cohorts in a Brazilian population (218,169 patients from a subset of the CODE-15 [Clinical Outcomes in Digital Electrocardiography Study] cohort, 14,263 from the ELSA-Brasil

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 10, NO. 4, 2024
APRIL 2024:775–789

[Brazilian Longitudinal Study of Adult Health] study, and 1631 from the SaMi-Trop [São Paulo-Minas Gerais Tropical Medicine Research Center] study). The study found that patients with an ECG age 8 years greater than their chronological age had a significantly higher mortality rate, and those with an ECG age 8 years lower than their chronological age had a significantly lower mortality rate,[83] replicating the results by Attia et al.[65]

The relevance of a difference between AI-ECG age and chronological age was also explored by Chang et al,[84] with a deep learning model applied to 71,741 ECGs, then tested on 32,707 cases and tuned on another 8,295 ECGs. Patients who had >7 years difference between predicted and actual age had higher risk on all-cause mortality and cardiovascular-cause mortality and worse outcomes in terms of heart failure, diabetes, chronic kidney disease, myocardial infarction, stroke, coronary artery disease, AF, and hypertension. In the external validation sets (SaMi-Trop and CODE-15), an ECG age 7 years greater than chronological age was associated with greater risk of all-cause mortality.[84]

A deep learning-based algorithm, trained on 425,051 12-lead ECGs by Baek et al,[85] was used to estimate the AI-ECG heart age and assess whether any difference from chronological age would be predictive of mortality or cardiovascular outcomes. The model was tested on a separate set of 97,058 ECGs. After adjusting for relevant comorbidity factors, the patients with an AI-ECG heart age of 6 years older than their chronological age had greater all-cause mortality and more major adverse cardiovascular events; conversely, those with an AI-ECG heart age 6 years younger had lower all-cause mortality and fewer major adverse cardiovascular events.[85]

In 2022, Lindow et al[86] published the heart age by Bayesian interpretation method, which uses statistical analysis of a 10-second 12-lead ECG to determine the age of the heart. This estimate of age, and the resultant heart age gap (estimated heart age vs chronological age) increased in line with cardiovascular risk factors and the presence of CVD.[86]

These studies confirm and validate the original findings that the age gap is a manifestation of aging rate beyond what would be expected by the presence of CVD or risk factors for CVD, opening the door to numerous potential uses in medicine.

POTENTIAL UTILITY OF AI-ECG–DERIVED AGE

AI-ECG–derived age estimation represents a paradigm shift by using deep neural networks that can consider linear and nonlinear dynamics in a manner that other methods typically have not fully accounted for. Allowing AI to explore the raw ECG on its own potentially allows for the avoidance of investigator biases and for AI to see ECG features that may not be readily identified by cardiologists,[72] providing an opportunity to recognize subclinical markers of disease risk.

Using AI-ECG–derived age, physicians can take a single biological age measurement that provides insight into a patients current and potential future disease status and risk factors; this can empower immediate intervention to prevent or manage disease progression, compared with a "wait-and-see" approach informed by assessment along a chronological timeline. AI interpretation of ECGs is likely to provide benefit in the risk assessment and management of AF, sudden cardiac death (including risk stratification in patients with hypertrophic cardiomyopathy or inherited arrhythmia syndromes), congestive heart failure, and angina.[87]

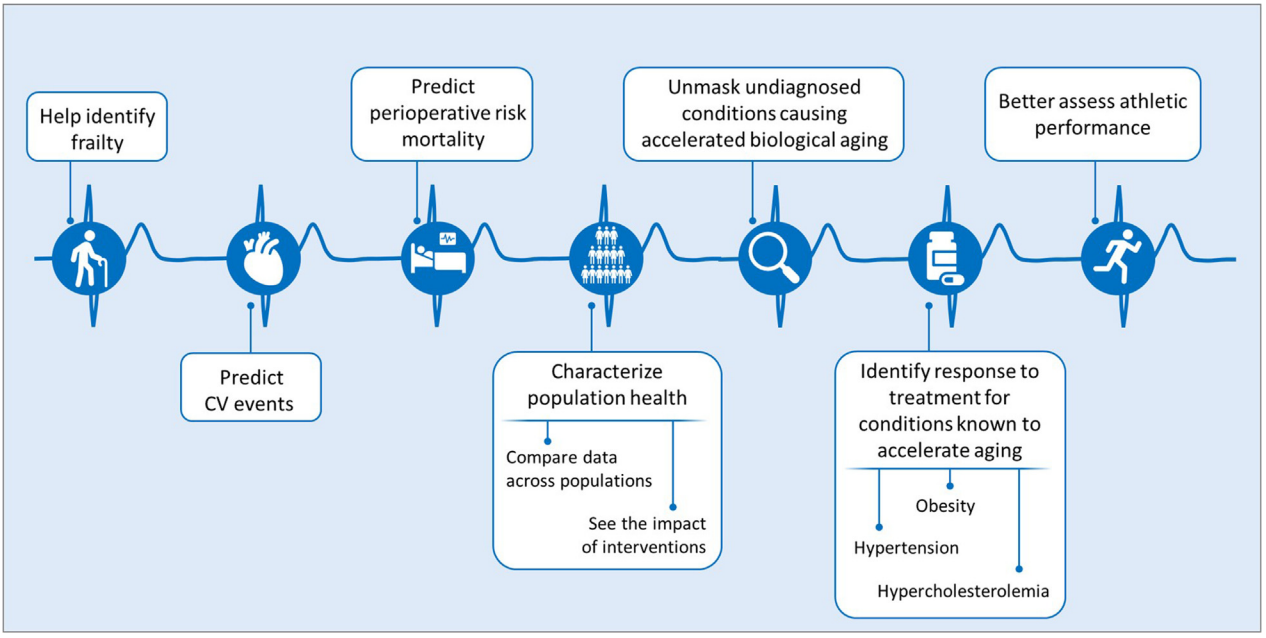
Changes in AI-ECG–derived age could be supportive of data interpretation across cardiovascular, physical, cognitive, metabolic, or other health outcomes, and could serve as an informative surrogate endpoint in future clinical trials that investigate interventions targeting the biology of aging.

Currently, with the technology in its early stages, any observed differences between AI-ECG–derived biological age and chronological age might not be fully actionable; however, there is great promise in the future of integrating AI-ECG–derived age into practice.

AI-ECG–derived age has the potential to offer physicians and healthcare policy makers practical support across a range of diseases (**Figure 5**). AI has the capacity to increase the efficiency and value of nuclear cardiology, electrophysiology, and coronary angiography.[71] With further application in prevention, diagnosis, management, and monitoring of heart failure,[71] AI is poised to transform cardiovascular medicine and be a driver of improving outcomes at both the patient and population levels.[87] In routine practice, AI-ECG–derived age could help predict the likelihood of future CV events, and support both pharmaceutical and lifestyle interventions to reduce this risk. In the surgical setting, AI-ECG–derived age may become essential in accurate and accessible prediction of risks of adverse events or surgery-associated mortality, and of anticipated speed of recovery.

Beyond cardiovascular medicine, changes in AI-ECG–derived age could provide insight into population-level characterization of health. This

FIGURE 5 **The Potential Use of Artificial Intelligence-Electrocardiogram-Derived Age in Clinical Practice in the Future**



Artificial intelligence-electrocardiogram-derived age could provide support in clinical decision making, with roles in diagnosis, risk stratification, and population-level medicine, and in evaluating response to treatment. CV = cardiovascular.

could include both comparative data interpretation across cardiovascular, physical and athletic, cognitive, metabolic, or immune health and the assessment of treatment outcomes in large datasets. A recent study has identified that AI-ECG-derived age varied between geographical regions, and that the differences in AI-ECG-derived age were associated closely with the gaps in life expectancy among those populations, suggesting that the algorithm is sensitive to different risk factors for mortality across at the population level.[88] Understanding the impact of treatment on a large scale can inform disease management policy, as well as practice-based decision making and patient education. Serial assessment AI-ECG-derived age in routine practice may be of value in encouraging adoption of a healthy lifestyle.[72] This insight could prove particularly valuable in understanding the management of, and response to treatment in, diseases that are known to accelerate biological aging: hypertension, obesity, and hypercholesterolemia, among many others.

Frailty is understood to relate to biological age.[72] AI-ECG-derived age could help in early identification of patients at risk of becoming frail, aid in the selection of treatment and shaping of care plans, and help track the effectiveness of interventions used in the management of frailty.

Finally, the awareness that a patient has an unexpectedly high biological age, assessed by AI-ECG-derived delta age, could drive clinical investigation and discovery of undiagnosed illness.[71,72,87]

CURRENT CHALLENGES

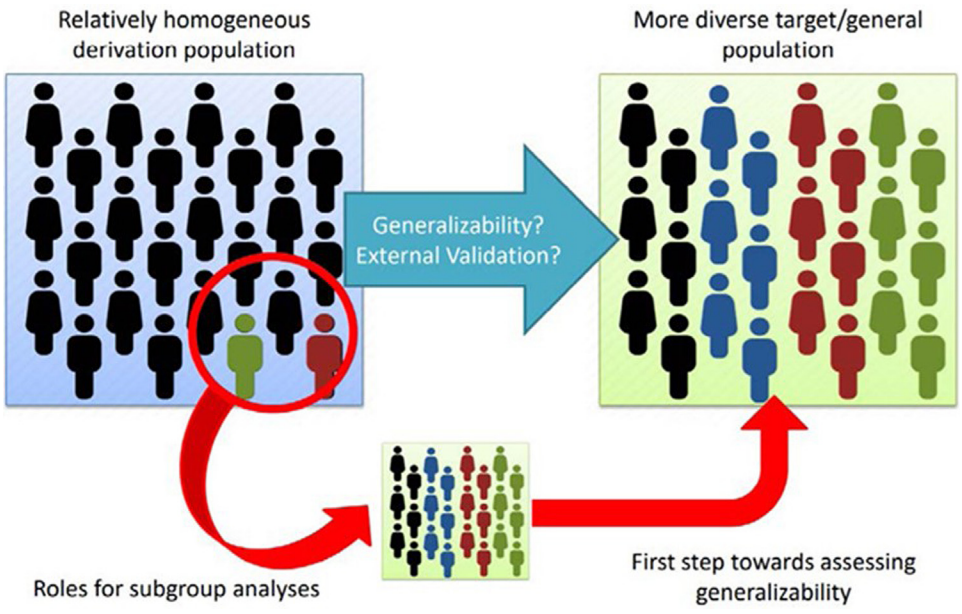
A lack of trust, by both patients and physicians, in AI is a key barrier to overcome. This lack of comfort stems from both a low tolerance for computer failure, compared with the allowance for human error, and the fear of a "black box" system.[89] The black box calculations give rise to several concerns, including whether a physician can explain the process to a patient or assure the accuracy of the AI-derived conclusions.[90-92]

Commentators disagree on whether AI is suited to the "last resort" patient, in which the physician has used all conventional tools at their disposal, or to the triaging and management of routine and uncomplicated cases.[90,93,94]

AI has potential race and sex bias when an algorithm has been trained on a nondiverse population. This has been reported in facial recognition software and some medical applications.[95] In the case of the Mayo Clinic ECG-age CNN, the original AI algorithm was trained and tested using a predominantly

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 10, NO. 4, 2024
APRIL 2024:775–789

FIGURE 6 Subgroup Analysis of a Relatively Homogeneous Population Can Help Increase the Generalizability of a Convolutional Neural Network



The generalizability of a population to smaller, more diverse populations in the application of artificial intelligence reading of datasets is unknown. Therefore, employing and validating subgroup analyses, especially of relatively homogeneous datasets, is important for the responsible use of artificial intelligence in medicine. Used with permission from Noseworthy, et al. Assessing and Mitigating Bias in Medical Artificial Intelligence. *Circulation: Arrhythmia and Electrophysiology*. 2020;13(3):e007988.[95]

Caucasian population.[65,95] Promisingly, subsequent subgroup analysis demonstrated that the algorithm performed well across racial groups, with the interaction between δage and racial subgroups being nonsignificant in the detection of left ventricular ejection fraction.[95] However, while this investigation was not affected by race, other parameters of ECG features may not be race-invariant. It is therefore important to maintain diverse datasets, with consistent subgroup reporting and with external validation (**Figure 6**).

Data sharing and privacy concerns also exist. Because few institutions will have sufficient data to train a neural network, multiple sources will be needed, which raises legal and ethical barriers to the sharing of health information. A solution to this lies in federated learning, which uses a decentralized model to train locally, before the outputs, which are not personal or interpretable, are collated in a central node that does not have access to the raw data. Another method adds noise to the signal, blending the individual data into the mass, and with only small amounts of data being taken from any individual.[96]

EXPLAINABLE AND UNEXPLAINABLE AI

The issue of trust around a "black box" algorithm revolves around the concepts of explainable AI and unexplainable AI. It has been suggested that, by enabling users to uncover the underlying rules that a model finds during training, explainable AI might be trusted to a greater degree.[92,96,97]

However, there is increasing suggestion that reliance on explainable AI may defeat the goals that the user is trying to achieve, and that explainable AI may be more of an explanation of a process, rather than an insight-generating tool.[98] It could be considered that medicine itself is practiced with a degree of inexplicability in both diagnosis and drug action. Some medical decision making relies on validation in which explanation is absent, and it might be argued that this validation-led approach is suited to selecting AI models for use in medicine.[98,99]

By not attempting to be understandable, AI may be able to provide greater insight into a dataset because it will not be working to a preconceived ruleset.

FUTURE DIRECTIONS

Further research is needed to assess whether AI-ECG-derived age will associate with long-term outcomes in nonpatient and more ethnically diverse populations.[72] Other areas for further research include comparing AI-ECG-derived age with other estimators of heart age and determining if AI-ECG will provide incremental prognostic information to CVD risk calculators or to well-accepted comorbidity indices (eg, Charlson comorbidity index, multimorbidity index).[72]

Beyond the work done with the *LMNA* gene and its association with advanced aging,[78] it will be valuable to determine whether neural network predicted age correlates with other known markers of accelerated aging.[72] Recently, the Mayo Clinic machine learning-derived algorithm to assess AI-ECG age was closely associated with brain age. Moreover, ECG-derived δage has been associated with the results of cognitive function tests, in a manner that perhaps offered more insight than that obtained with magnetic resonance imaging-derived brain δage, by capturing vascular risk factors that might not be seen on imaging results.[100] This study concluded that that the standardized, low-cost age difference information could be valuable, alongside imaging, in the diagnosis and management of dementia.[100]

With the development of mobile form factors that allow acquisition of medical-grade ECGs from smartphones and smart wearables, the use of AI-ECG model may enable massive scalability to democratize health care.[96]

Finally, it is still unknown whether changes over time to AI-ECG-derived age reflect changes in aging rate as a biomarker of improvements on the biological aging process in response to therapies or lifestyle modification, or whether they demonstrate measurement error. Longitudinal studies with serial ECGs before and after therapies may provide additional insights to answer this question.

SUMMARY

Measures of biological age may provide additional insights into risk for chronic disease, disability, and adverse health outcomes beyond chronological age. The recent addition of AI to a standard ECG, which benefits from being inexpensive, noninvasive, and readily accessible, allows meaningful insight into the difference between biological age and chronological age. Where these ages differ meaningfully, there is a clear association with risk of disease development and/or worsening, and this is particularly apparent in CVD at present. AI-ECG-derived age offers a point-of-care, potentially scalable tool that represents a paradigm shift in screening and risk stratification.

ACKNOWLEDGEMENTS Medical writing support was provided by Colin Griffin, PhD (Griffin Scientific Ltd, United Kingdom), and this service was funded by the Mayo Clinic.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr LeBrasseur was supported by grant number R01 AG55529. Drs Lopez-Jimenez, Kapa, Friedman, and Attia are co-inventors of the algorithm to determine age using AI-ECG, which algorithm has been licensed to Anumana. The Mayo Clinic and these authors may benefit financially in the future from this licensing. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Francisco Lopez-Jimenez, Division of Preventive Cardiology, Department of Cardiovascular Medicine, Mayo Clinic, 200 First Street Southwest, Rochester, Minnesota 55905, USA. E-mail: lopez@mayo.edu.

REFERENCES

1. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153:1194–1217.

2. Khan SS, Singer BD, Vaughan DE. Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell*. 2017;16:624–633.

3. Kennedy BK, Berger SL, Brunet A, et al. Geroscience: linking aging to chronic disease. *Cell*. 2014;159:709–713.

4. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell*. 2023;186:243–278.

5. Barter JD, Foster TC. Aging in the brain: new roles of epigenetics in cognitive decline. *Neuroscientist*. 2018;24:516–525.

6. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med*. 2006;12:1133–1138.

7. Kazak L, Reyes A, Holt IJ. Minimizing the damage: repair pathways keep mitochondrial DNA intact. *Nat Rev Mol Cell Biol*. 2012;13:659–671.

8. Laurie CC, Laurie CA, Rice K, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat Genet*. 2012;44:642–650.

9. Faggioli F, Wang T, Vijg J, Montagna C. Chromosome-specific accumulation of aneuploidy in the aging mouse brain. *Hum Mol Genet*. 2012;21:5246–5253.

10. Forsberg LA, Rasi C, Razzaghian HR, et al. Age-related somatic structural changes in the nuclear genome of human blood cells. *Am J Hum Genet*. 2012;90:217–228.

11. Linnane AW, Marzuki S, Ozawa T, Tanaka M. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet*. 1989;1:642–645.

12. Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and

age-related diseases. *Nat Cell Biol*. 2022;24:135–147.

13. Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol*. 2021;22:75–95.

14. Ovadya Y, Landsberger T, Leins H, et al. Impaired immune surveillance accelerates accumulation of senescent cells and aging. *Nat Commun*. 2018;9:5435.

15. Rodrigues LP, Teixeira VR, Alencar-Silva T, et al. Hallmarks of aging and immunosenescence: Connecting the dots. *Cytokine Growth Factor Rev*. 2021;59:9–21.

16. la Torre A, Lo Vecchio F, Greco A. Epigenetic mechanisms of aging and aging-associated diseases. *Cells*. 2023;12:1163.

17. Wang K, Liu H, Hu Q, et al. Epigenetic regulation of aging: implications for interventions of aging and diseases. *Signal Transduct Target Ther*. 2022;7:374.

18. Bocklandt S, Lin W, Sehl ME, et al. Epigenetic predictor of age. *PLoS One*. 2011;6:e14821.

19. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14:R115.

20. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19:371–384.

21. McCrory C, Fiorito G, Hernandez B, et al. GrimAge outperforms other epigenetic clocks in the prediction of age-related clinical phenotypes and all-cause mortality. *J Gerontol A Biol Sci Med Sci*. 2021;76:741–749.

22. Djeghloul D, Kuranda K, Kuzniak I, et al. Age-associated decrease of the histone methyltransferase SUV39H1 in HSC perturbs heterochromatin and B lymphoid differentiation. *Stem Cell Rep*. 2016;6:970–984.

23. Lee JH, Kim EW, Croteau DL, Bohr VA. Heterochromatin: an epigenetic point of view in aging. *Exp Mol Med*. 2020;52:1466–1474.

24. Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. *Nature*. 2021;592:695–703.

25. Yi SJ, Kim K. New insights into the role of histone changes in aging. *Int J Mol Sci*. 2020;21:8241.

26. Pagiatakis C, Musolino E, Gornati R, Bernardini G, Papait R. Epigenetics of aging and disease: a brief overview. *Aging Clin Exp Res*. 2021;33:737–745.

27. Hodes RJ, Sierra F, Austad SN, et al. Disease drivers of aging. *Ann N Y Acad Sci*. 2016;1386:45–68.

28. Pal S, Tyler JK. Epigenetics and aging. *Sci Adv*. 2016;2:e1600584.

29. Wang JC, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res*. 2012;111:245–259.

30. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. *Am J Kidney Dis*. 2013;62:339–351.

31. Sanoff HK, Deal AM, Krishnamurthy J, et al. Effect of cytotoxic chemotherapy on markers of molecular age in patients with breast cancer. *J Natl Cancer Inst*. 2014;106:dju057.

32. Jurk D, Wilson C, Passos JF, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat Commun*. 2014;2:4172.

33. Chinta SJ, Woods G, Demaria M, et al. Cellular senescence is induced by the environmental neurotoxin paraquat and contributes to neuropathology linked to Parkinson's disease. *Cell Rep*. 2018;22:930–940.

34. Pathai S, Bajillan H, Landay AL, High KP. Is HIV a model of accelerated or accentuated aging? *J Gerontol A Biol Sci Med Sci*. 2014;69:833–842.

35. Cao X, Li W, Wang T, et al. Accelerated biological aging in COVID-19 patients. *Nat Commun*. 2022;13:2135.

36. Poganik JR, Zhang B, Baht GS, et al. Biological age is increased by stress and restored upon recovery. *Cell Metab*. 2023;35:807–820.e5.

37. Stringhini S, Sabia S, Shipley M, et al. Association of socioeconomic position with health behaviors and mortality. *JAMA*. 2010;303:1159–1166.

38. Adler NE, Rehkopf DHUS. disparities in health: descriptions, causes, and mechanisms. *Annu Rev Public Health*. 2008;29:235–252.

39. Starfield B, Shi L, Macinko J. Contribution of primary care to health systems and health. *Milbank Q*. 2005;83:457–502.

40. Steptoe A, Zaninotto P. Lower socioeconomic status and the acceleration of aging: an outcome-wide analysis. *Proc Natl Acad Sci U S A*. 2020;117:14911–14917.

41. Geronimus AT, Hicken M, Keene D, Bound J. "Weathering" and age patterns of allostatic load scores among blacks and whites in the United States. *Am J Public Health*. 2006;96:826–833.

42. Hagg S, Jylhava J. Sex differences in biological aging with a focus on human studies. *Elife*. 2021;10:e63425.

43. Regitz-Zagrosek V. Sex and gender differences in health. Science & Society Series on Sex and Science. *EMBO Rep*. 2012;13:596–603.

44. Diez Roux AV, Mair C. Neighborhoods and health. *Ann N Y Acad Sci*. 2010;1186:125–145.

45. Hawkley LC, Cacioppo JT. Loneliness matters: a theoretical and empirical review of consequences and mechanisms. *Ann Behav Med*. 2010;40:218–227.

46. Sebastiani P, Thyagarajan B, Sun F, et al. Biomarker signatures of aging. *Aging Cell*. 2017;16:329–338.

47. Jylhava J, Pedersen NL, Hagg S. Biological age predictors. *EBioMedicine*. 2017;21:29–36.

48. Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc*. 2009;4:1798–1806.

49. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. 2013;49:359–367.

50. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)*. 2018;10:573–591.

51. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY)*. 2019;11:303–327.

52. Lohman T, Bains G, Berk L, Lohman E. Predictors of biological age: the implications for wellness and aging research. *Gerontol Geriatr Med*. 2021;7:23337214211046419.

53. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. *Clin Epigenetics*. 2019;11:62.

54. Lai TP, Wright WE, Shay JW. Comparison of telomere length measurement methods. *Philos Trans R Soc Lond B Biol Sci*. 2018;373:20160451.

55. Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. *Nurs Res*. 2014;63:289–299.

56. Diebel LWM, Rockwood K. Determination of biological age: geriatric assessment vs biological biomarkers. *Curr Oncol Rep*. 2021;23:104.

57. Hertel J, Friedrich N, Wittfeld K, et al. Measuring biological age via metabonomics: the metabolic age score. *J Proteome Res*. 2016;15:400–410.

58. Jia L, Zhang W, Chen X. Common methods of biological age estimation. *Clin Interv Aging*. 2017;12:759–772.

59. Bafei SEC, Shen C. Biomarkers selection and mathematical modeling in biological age estimation. *NPJ Aging*. 2023;9:13.

60. Park J, Cho B, Kwon H, Lee C. Developing a biological age assessment equation using principal component analysis and clinical biomarkers of aging in Korean men. *Arch Gerontol Geriatr*. 2009;49:7–12.

61. Klemera P, Doubal S. A new approach to the concept and computation of biological age. *Mech Ageing Dev*. 2006;127:240–248.

62. Vlachopoulos C, Xaplanteris P, Aboyans V, et al. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. *Atherosclerosis*. 2015;241:507–532.

63. Six AJ, Backus BE, Kelder JC. Chest pain in the emergency room: value of the HEART score. *Neth Heart J*. 2008;16:191–196.

64. Green SM, Schriger DL. A methodological appraisal of the HEART score and its variants. *Ann Emerg Med*. 2021;78:253–266.

65. Attia ZI, Friedman PA, Noseworthy PA, et al. Age and sex estimation using artificial intelligence from standard 12-lead ECGs. *Circ Arrhythm Electrophysiol*. 2019;12:e007284.

66. Attia ZI, DeSimone CV, Dillon JJ, et al. Novel bloodless potassium determination using a signal-

processed single-lead ECG. *J Am Heart Assoc*. 2016;5:e002746.

67. Galloway CD, Valys AV, Shreibati JB, et al. Development and validation of a deep-learning model to screen for hyperkalemia from the electrocardiogram. *JAMA Cardiol*. 2019;4:428–436.

68. Attia ZI, Kapa S, Lopez-Jimenez F, et al. Screening for cardiac contractile dysfunction using an artificial intelligence-enabled electrocardiogram. *Nat Med*. 2019;25:70–74.

69. Hossain SM, Ali AA, Rahman M, et al. Identifying drug (Cocaine) intake events from acute physiological response in the presence of free-living physical activity. *IPSN*. 2014;2014:71–82.

70. Attia ZI, Noseworthy PA, Lopez-Jimenez F, et al. An artificial intelligence-enabled ECG algorithm for the identification of patients with atrial fibrillation during sinus rhythm: a retrospective analysis of outcome prediction. *Lancet*. 2019;394:861–867.

71. Lopez-Jimenez F, Attia Z, Arruda-Olson AM, et al. Artificial intelligence in cardiology: present and future. *Mayo Clin Proc*. 2020;95:1015–1039.

72. Ladejobi AO, Medina-Inojosa JR, Shelly Cohen M, et al. The 12-lead electrocardiogram as a biomarker of biological age. *Eur Heart J Digit Health*. 2021;2:379–389.

73. Benavente ED, Lopez-Jimenez F, Attia ZI, et al. Studying accelerated cardiovascular ageing in Russian adults through a novel deep-learning ECG biomarker. *Wellcome Open Res*. 2021;6:12.

74. Sheffeh MA, Medina-Inojosa JR, Medina-Inojosa B, et al. Abstract 15612: association between ECG-derived age with coronary artery calcium. *Circulation*. 2022;146:A15612.

75. Rajai N, Medina-Inojosa B, Sheffeh MA, et al. Abstract 13374: effect of moderate to strenuous exercise on biological aging as determined by artificial-enabled electrocardiography. *Circulation*. 2022;146:A13374.

76. Rajai N, Medina-Inojosa JR, Sheffeh MA, et al. Abstract 13378: association between social connection and biological age as determined by artificial intelligence-enabled electrocardiography. *Circulation*. 2022;146:A13378.

77. Libiseller-Egger J, Phelan JE, Attia ZI, et al. Deep learning-derived cardiovascular age shares a genetic basis with other cardiac phenotypes. *Sci Rep*. 2022;12:22625.

78. Shelly S, Lopez-Jimenez F, Chacin-Suarez A, et al. Accelerated aging in LMNA mutations detected by artificial intelligence ECG-derived age. *Mayo Clin Proc*. 2023;98:522–532.

79. Kwon JM, Lee SY, Jeon KH, et al. Deep learning-based algorithm for detecting aortic stenosis using electrocardiography. *J Am Heart Assoc*. 2020;9:e014717.

80. Khurshid S, Friedman S, Pirruccello JP, et al. Deep learning to predict cardiac magnetic resonance-derived left ventricular mass and hypertrophy from 12-lead ECGs. *Circ Cardiovasc Imaging*. 2021;14:e012281.

81. Khurshid S, Friedman S, Reeder C, et al. ECG-based deep learning and clinical risk factors to predict atrial fibrillation. *Circulation*. 2022;145:122–133.

82. Choi SH, Lee HG, Park SD, et al. Electrocardiogram-based deep learning algorithm for the screening of obstructive coronary artery disease. *BMC Cardiovasc Disord*. 2023;23:287.

83. Lima EM, Ribeiro AH, Paixao GMM, et al. Deep neural network-estimated electrocardiographic age as a mortality predictor. *Nat Commun*. 2021;12:5117.

84. Chang CH, Lin CS, Luo YS, Lee YT, Lin C. Electrocardiogram-based heart age estimation by a deep learning model provides more information on the incidence of cardiovascular disorders. *Front Cardiovasc Med*. 2022;9:754909.

85. Baek YS, Lee DH, Jo Y, Lee SC, Choi W, Kim DH. Artificial intelligence-estimated biological heart age using a 12-lead electrocardiogram predicts mortality and cardiovascular outcomes. *Front Cardiovasc Med*. 2023;10:1137892.

86. Lindow T, Palencia-Lamela I, Schlegel TT, Ugander M. Heart age estimated using explainable advanced electrocardiography. *Sci Rep*. 2022;12:9840.

87. Siontis KC, Noseworthy PA, Attia ZI, Friedman PA. Artificial intelligence-enhanced electrocardiography in cardiovascular disease management. *Nat Rev Cardiol*. 2021;18:465–478.

88. Benavente ED, Lopez-Jimenez F, Iakunchykova O, et al. Capturing population differences in rates of vascular aging using a deep learning electrocardiogram algorithm: a cross-sectional study. Preprint. *medRxiv*. Posted September 14, 2021. https://www.medrxiv.org/content/10.1101/2021.09.09.21263337v1

89. Poon AIF, Sung JJY. Opening the black box of AI-medicine. *J Gastroenterol Hepatol*. 2021;36:581–584.

90. Chan B. Black-box assisted medical decisions: AI power vs. ethical physician care. *Med Health Care Philos*. 2023;26:285–292.

91. Quinn TP, Jacobs S, Senadeera M, Le V, Coghlan S. The three ghosts of medical AI: can the black-box present deliver? *Artif Intell Med*. 2022;124:102158.

92. Reddy S. Explainability and artificial intelligence in medicine. *Lancet Digit Health*. 2022;4:e214–e215.

93. Ornes S. Peering inside the black box of AI. *Proc Natl Acad Sci U S A*. 2023;120:e2307432120.

94. Gordijn B, Ten Have H. What's wrong with medical black box AI? *Med Health Care Philos*. 2023;26:283–284.

95. Noseworthy PA, Attia ZI, Brewer LC, et al. Assessing and mitigating bias in medical artificial intelligence: the effects of race and ethnicity on a deep learning model for ECG analysis. *Circ Arrhythm Electrophysiol*. 2020;13:e007988.

96. Attia ZI, Harmon DM, Behr ER, Friedman PA. Application of artificial intelligence to the electrocardiogram. *Eur Heart J*. 2021;42:4717–4730.

97. Saranya A, Subhashini R. A systematic review of Explainable Artificial Intelligence models and applications: recent developments and future trends. *Decis Analyt J*. 2023;7:100230.

98. Ghassemi M, Oakden-Rayner L, Beam AL. The false hope of current approaches to explainable artificial intelligence in health care. *Lancet Digit Health*. 2021;3:e745–e750.

99. Cutillo CM, Sharma KR, Foschini L, et al. Machine intelligence in healthcare-perspectives on trustworthiness, explainability, usability, and transparency. *NPJ Digit Med*. 2020;3:47.

100. Iakunchykova O, Schirmer H, Vangberg T, et al. Machine-learning-derived heart and brain age are independently associated with cognition. *Eur J Neurol*. 2023;30:2611–2619.

KEY WORDS artificial intelligence, AI, biological age, delta age, ECG, mortality, risk