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Research Article

Measures of Biologic Age in a Community Sample Predict Mortality and Age-Related Disease: The Framingham Offspring Study

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Abstract

Background: We tested the association of biologic age (BA) measures constructed from different types of biomarkers with mortality and disease in a community-based sample.

Methods: In Framingham Offspring participants at Exams 7 (1998–2001, mean age 62 ± 10) and 8 (2005–2008, mean age 67 ± 9), we used the Klemera–Doubal method to estimate clinical BA and inflammatory BA and computed the difference (Δage) between BA and CA. Clinical Δage was computed at Exam 2 (1979–1983, mean age 45 ± 10). At Exam 8, we computed measures of intrinsic and extrinsic epigenetic age. Participants were followed through 2014 for outcomes. Cox proportional hazards models tested the association of each BA estimate with each outcome adjusting for covariates.

Results: Sample sizes ranged from 2532 to 3417 participants. In multivariable models, each 1-year increase in clinical Δage at Exam 2 (hazard ratio [HR] = 1.04–1.06, $p < 2 \times 10^{-16}$) and clinical Δage and inflammatory Δage at Exam 7 significantly increased the hazards of mortality and incident cardiovascular disease (HR = 1.01–1.05, $p < 2 \times 10^{-7}$), whereas inflammatory Δage increased the hazards of cancer (HR = 1.01, $p <$ .05). At Exam 8, increased clinical Δage, inflammatory Δage, and extrinsic epigenetic age all significantly increased the hazard of mortality (HR = 1.03–1.05, all $p <$ .05); clinical Δage and inflammatory Δage increased cardiovascular disease risk (HR = 1.04–1.05, all $p <$ .01); and clinical Δage increased cancer risk (HR = 1.03, $p <$ .01) when all three BA measures were included in the model. Intrinsic epigenetic age was not significantly associated with any outcome.

Conclusions: Our findings suggest BA measures may be complementary in predicting risk for mortality and age-related disease.

Keywords: Inflammation, Epigenetics, Aging, Epidemiology.

The population is aging worldwide not only due to gains in early-life survival but also due to progress with declining late-life mortality (1). Aging in humans is highly variable with wide differences in health at a given chronologic age. Some adults become frail in early old age, whereas others remain fit in their 90s and beyond (2). Understanding the biologic processes of aging and how these processes confer susceptibility to chronic disease may lead to successful interventions that delay aging and improve health span (3).

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Editor's choice

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Given the complexity of the aging process, different measures of biologic aging (BA) and of successful aging have been constructed to better reflect an individual's rate of aging by combining clinical biomarkers representative of different physiologic systems (4–7). Several of the measures have been related to risk for mortality (4,6,7) and physical and cognitive decline (5–7). Despite this body of work, there has not been a consensus on a clinical measure of BA. More recently, genomic data have been used to develop age predictors including transcriptomic and DNA methylation molecular signatures (8–10). DNA methylation age predicts mortality independent of chronologic age and other risk factors (11,12) and is associated with some age-related conditions such as brain aging (13), but not with coronary heart disease (14). It is not clear if each of these different measures of BA captures unique information or adds complementary information over and above chronological age (CA) to predict disease risk and life span.

We had the opportunity to examine a measure of clinical BA over several time points in the adult life course in addition to inflammatory and DNA methylation age constructed in later adulthood in a large community-based sample under continuous surveillance. We hypothesized that different types of BA measures (clinical, inflammatory, and genomic) make unique contributions to age-related disease risk and all-cause mortality. Furthermore, we were able to test the association of the different BA measures in the same cohort accounting for important confounders.

Methods

Study Sample

The Framingham Heart Study (FHS) is a community-based longitudinal cohort study initiated in 1948 to study determinants of cardiovascular disease (CVD) and its risk factors. In 1971, 5,124 children of the original participants and spouses of the children were enrolled into the FHS Offspring cohort (15). Offspring participants have been examined every 4–8 years, have completed nine research examinations, and remain under active surveillance for cardiovascular events, cancer, and death. The Boston University Medical Campus Institution Review Board reviews and approves the protocol for each research examination, and informed consent is obtained at every attended examination. Research examinations consist of a physician administered medical history and resting blood pressure, laboratory assessment, and various noninvasive measures of cardiovascular and lung function.

Clinical Biologic Age

Prior reports demonstrated that a combination of clinical biomarkers used to define biologic age (BA) predicted mortality better than CA (4). By definition, BA varies even in a sample of individuals all of the same CA (5). We used six clinical biomarkers representing diverse physiologic systems that were consistently available over three examinations and were used in prior reports: systolic blood pressure, forced expiratory volume at 1 s (FEV1), total cholesterol, fasting glucose, C-reactive protein, and serum creatinine. We chose examinations to evaluate the change in clinical BA from midlife (Examination 2: 1979–1983, mean age 44 years) to later adulthood (Examination 7: 1998–2001, mean age 62 years) and to examine the relationship of clinical BA to other measures of BA available at later examinations (inflammatory BA available at Exams 7 and 8; DNA methylation BA available at Exam 8: 2005–2008, mean age 67 years). Participants were excluded from the clinical BA sample at a given examination if any of the six biomarkers were missing (16%–28% of attendees). FEV1 was the biomarker resulting in missing values 95%–98% of the time. In additional, at Exam 8, we excluded 13 participants due to missing covariates.

Inflammatory Biologic Age

We chose to create an inflammatory BA phenotype because inflammation plays a central role in aging and development of age-related

Table 1. Characteristics of Clinical and Inflammatory Biologic Age Study Samples at Exam 7

Clinical Biologic Age Sample	*N* = 2,532	Inflammatory Biologic Age Sample	*N* = 3,134
Chronological age (y)	61 (9.3)	Chronological age (y)	62 (9.5)
Sex, female	55%	Sex, female	53%
Clinical BA	61.0 (11.7)	Inflammatory BA	61.5 (12.9)
ΔAge	0.0 (7.0)	ΔAge	−0.1 (8.8)
Clinical Variables[a]		Inflammatory Marker Variables[b]	
Systolic blood pressure (mm Hg)	129 (20)	C-reactive protein (mg/L)	2.2 (1.0, 5.1)
Forced expiratory volume at 1 s (L)	2.7 (0.8)	Monocyte chemoattractant protein-1 (pg/mL)	313 (254, 382)
Total cholesterol (mg/dL)	200 (37)	Osteoprotegerin (pmol/L)	5.4 (4.4, 6.5)
Glucose (mg/dL)	104 (27)	P-selectin (ng/mL)	36 (29, 45)
C-reactive protein (mg/L)	4.2 (7.4)	Intercellular adhesion molecule 1 (ng/mL)	242 (211, 283)
Creatinine (mg/100 mL)	1.1 (0.2)	Interleukin-6 (pg/mL)	2.7 (1.8, 4.3)
		LP-PLA2 mass (ng/mL)	288 (230, 361)
		LP-PLA2 activity (nmol/mL/min)	141 (119, 165)
		Tumor necrosis factor receptor II (pg/mL)	1,977 (1,666, 2,418)
Covariates[a]		Covariates[a]	
Current smoking	12	Current smoking	13
Diabetes	12	Diabetes	13
Hypertension treatment	32	Hypertension treatment	34
Lipid treatment	20	Lipid treatment	21
Prevalent CVD	5	Prevalent CVD	6
Prevalent cancer	8	Prevalent cancer	9

Note: BA = biologic age; CVD = cardiovascular disease; LP-PLA2 = lipoprotein-associated phospholipase A2.

[a]Variables are percentage or mean (*SD*). [b]Variables are median, Q1, Q3; C-reactive protein included in both study samples; intersection of clinical biological age and inflammatory biological age samples: *N* = 2,408.

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disease (16). We selected inflammatory biomarkers measured at Examinations 7 and 8. The markers function across the inflammation process including acute phase reactants, chemokines, cytokines, selectins, and cell adhesion molecules (Table 1) (17). The samples were obtained fasting, and the details of the assays and measurements have been previously reported with intra- and inter-assay coefficients of variation less than 10% (18). Participants were excluded from the inflammatory BA sample at a given examination if they did not provide a fasting morning sample or if any of the biomarkers were missing (9%–11% of exam attendees). At Exam 8, a further 16 participants were excluded do to missing covariates.

Methylation Age

Epigenetic changes are a key hallmark of aging (19), and DNA methylation-based biomarkers often referred to as the "epigenetic clock" have been shown to be robust measures of biologic age (9,10). Recent work incorporating blood cell metrics into the epigenetic measures demonstrate significant associations with mortality (12). Therefore, we examined two epigenetic measures we reported previously (12): (a) intrinsic epigenetic age (IEAA) is the residual obtained from a multivariate regression of the Horvath epigenetic age estimate (353 CpGs) on CA and measures of blood cell counts (9) and is constructed to be independent of blood cell count changes that occur with age and (b) extrinsic epigenetic age (EEAA) defined using the Hannum epigenetic age estimate (71 CpGs) (10) and creating a weighted average of the estimate taking into account imputed blood cell types using the Klemera–Doubal approach (20). EEAA is strongly correlated with blood cell counts (12).

Blood samples collected at Examination 8 were used to extract genomic DNA and the Illumina Infinium Human Methylation 450K BeadChip (Illumina, San Diego, CA, USA) was used for DNA methylation measurement as previously reported (11,12).

Incident Events

Participants are under continuous surveillance for cardiovascular events and death. An end point committee of three senior investigators reviews all available information including hospital records, death certificates, and next-of-kin interviews to determine the date and cause of death. Cardiovascular events (coronary heart disease: including coronary insufficiency, myocardial infarction, coronary heart disease death; stroke, heart failure, and coronary or CVD death) are adjudicated by the committee using standardized criteria previously reported (21). A study neurologist adjudicates cerebrovascular outcomes (atherothrombotic infarction, cerebral embolism, intracerebral hemorrhage, subarachnoid hemorrhage, death due to stroke). Cancer cases are identified at routine research examinations and by medical history updates. The vast majority of cancers were validated with pathology reports with less than 5% of cases based on clinical diagnosis or death certificate. Non-melanoma skin cancers were excluded.

Cognitive and Physical Function at Examination 8

At Exam 8, trained technicians administered the mini-mental state examination (MMSE), a 30-point questionnaire used to measure cognitive function including the domains of orientation, attention, recall, and ability to follow simple commands. Hand grip strength was measured in kilograms using a Jamar dynamometer (Sammons Preston, Bolingbrook, IL, USA) obtaining three trials in each hand. Gait speed was measured over a 4-m course to the nearest 0.01 second, and the faster of two normal paced walks was used for analyses.

Covariates

At each research exam, participants were asked about smoking habits. Current smoking was defined as smoking one or more cigarettes per day in the year preceding the exam. Participants were asked about medication use, and lipid-lowering medications were recorded. Diabetes was defined as fasting glucose of 126 mg/dL or higher or use of oral hypoglycemic agents or insulin. Hypertension was defined as blood pressure ≥ 140/90 or use of anti-hypertensive medications.

Statistical Methods

Biologic age estimates

We used the Klemera and Doubal method (20) to compute clinical BA estimate and the inflammatory BA estimate. Compared with other methods for computing BA, the Klemera–Doubal algorithm with CA as one of the biomarkers, showed the best performances in precision of estimation (20) and predictive ability (4). The key idea of the Klemera–Doubal method is to minimize distance between BA and biomarkers in an m-dimensional space (m is number of biomarkers) and also to minimize the variability of BA estimates. This is achieved by running simple linear regressions on CA using biomarkers as outcomes. The BA variable is constructed based on parameter estimates and residuals from these simple linear regressions (20). We defined Δage for each BA measure as the BA minus CA. Thus, individuals with Δage > 0 have greater BA than their CA, whereas individuals with Δage < 0 have younger BA than their CA. Clinical and inflammatory BA were significantly correlated with CA at all exams (all $p < 2.2 \times 10^{-16}$); however, clinical and inflammatory Δage measures as well as IEAA and EEAA were not correlated with CA (all $p > .12$).

We examined the distribution of clinical Δage at Exam 2 by attendance at Exam 7 to determine whether greater clinical Δage at Exam 2 was associated with attendance at the later exam and with ability to construct clinical and inflammatory BA phenotypes at Exams 7 and 8. Next, we evaluated the correlation between clinical Δage at Exam 2 and clinical Δage at Exam 7, nearly 20 years later. Finally, we examined the correlation between pairs of Δage phenotypes based on the clinical and inflammatory biomarkers both across exams and at the same exam.

We constructed separate Cox proportional hazards models to examine the association of each Δage estimate at each time point (clinical BA Δage at Exams 2, 7, 8; inflammatory BA Δage at Exams 7 and 8; IEAA and EEAA at Exam 8) with mortality, CVD events, and cancer. Participants were followed through 2014. Multivariable-adjusted models included CA, sex, current smoking, diabetes, hypertension treatment, lipid treatment, and for models investigating mortality, prevalent CVD and prevalent cancer. In the models examining CVD or cancer, participants with prevalent disease at the exam at which the BA was measured were excluded. Finally, to investigate whether each measure of BA contributed to event risk independently of the others, we included all BA measures in the same model.

We examined the cross-sectional association of clinical Δage, inflammatory Δage, IEAA, and EEAA at Exam 8 with gait speed and grip strength after adjusting for CA, sex, height, and body mass index and with MMSE score after adjusting for CA, sex, and education.

All analyses were performed in R version 3.2.3 using R packages Himsc, psych, pROC.

Results

Characteristics of the individuals included for each BA measure are shown in Table 1 for Exam 7. Sample sizes for each BA measure differ due to availability of the biomarkers used to compute the BA. Sample

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characteristics for Exams 2 and 8 are presented in Supplementary Tables 1 and 2. Between 51% and 55% of the individuals included were female across all exams. The mean CA of the clinical BA and inflammatory BA subsamples were similar: 61 (9.3) and 62 (9.5) years, respectively. The clinical BA sample was smaller than the inflammatory BA sample mainly due to missing data for the FEV1 component of clinical BA; the two samples were largely overlapping (n = 2,408 in both samples). ΔAge was centered at zero with a wide distribution –20 to 30 (Supplementary Figure 1). CA of the clinical BA sample was 45.0 (10.1) years at Exams 2 and 67 (8.9) years at Exam 8 for all BA samples. The distribution of the individual components of clinical BA differ over the exams in accordance with the increasing CA (eg, mean FEV1 declines and mean systolic blood pressure increases).

Clinical Δage at Exam 2 was more favorable in participants who returned to Exams 7 and 8, whereas the distribution was shifted to larger Δages indicative of advanced aging for participants missing at the later exams (Supplementary Figures 1 and 2). Clinical Δage at Exam 2 was correlated with clinical Δage (r = .5) and inflammatory Δage at Exam 7 (r = .2); similar correlations were observed for Δage measures at Exam 8 (Supplementary Figures 1 and 2). The correlation between clinical Δage at Exams 7 and 8 (r = .7) and inflammatory Δage at Exam 7 and 8 (r = .6) is higher (Supplementary Figure 3). The correlation between clinical and inflammatory Δage within exam was lower (r = .37 and r = .35 at Exams 7 and 8, respectively) than the correlation of clinical or inflammatory Δage with its corresponding measure across the two exams. IEAA and EEAA at Exam 8 were not correlated with clinical Δage or inflammatory Δage at Exam 8.

There were large numbers of outcomes for all BA measures at all exams (Figure 1, Table 2, and Supplementary Table 3). In multivariable-adjusted models, each 1-year increase in clinical Δage and inflammatory Δage at Exam 7 (older adulthood), significantly increased the hazards of all-cause mortality and incident CVD, whereas inflammatory Δage also increased the hazard of cancer (Table 2; hazard ratios [HR] = 1.01–1.05, $p < 2 \times 10^{-7}$ except cancer $p < .05$). Similar increased hazards for increased clinical Δage in models constructed in midlife (Exam 2, mean age 45 years, median follow-up >32 years) were also observed (Supplementary Table 3; HR = 1.04–1.06, $p < 2 \times 10^{-16}$).

In fully adjusted models, IEAA constructed at Exam 8 was not significantly associated with any outcome (Figure 1, all $p > .1$). EEAA was significantly associated with mortality ($p = 6.1 \times 10^{-8}$) and incident CVD ($p = .04$) but not cancer (Figure 1). To determine whether each Δage measure contributed to the hazard of the outcomes under investigation, all three measures were included in a single model. Increased clinical Δage, inflammatory Δage, and EEAA all significantly increased the hazard of mortality (HR = 1.03–1.05, all $p <$ .05); clinical Δage and inflammatory Δage increased risk for CVD (HR = 1.04–1.05, all $p < .01$) and clinical Δage alone increased risk for cancer (HR = 1.03, $p < .01$) in the multivariable model with all three measures in the model (Figure 2).

We examined the cross-sectional associations of BA measures and functions defined with gait speed, hand grip strength, and mini-mental state examination score at Exam 8. Older clinical and inflammatory Δage and EEAA was associated with slower gait speed and weaker hand grip strength (all $p < .05$, Supplementary Table 4). Inflammatory Δage was also associated with lower MMSE score ($p < .001$). IEAA was not associated with the functional measures.

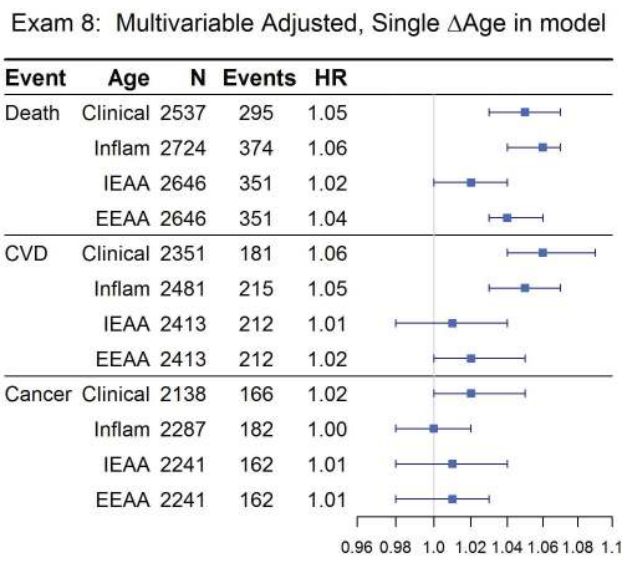

Figure 1. Incident events according to measures of Δage and epigenetic age: Framingham Offspring Study, Exam 8. Each outcome and each Δage measure is a separate model. Median follow-up time for all outcomes was ≈8 years. Models are multivariable adjusted with covariates: age, sex, current smoking, diabetes, hypertension treatment, lipid treatment, mortality only additionally adjusted for prevalent CVD and prevalent cancer.

Discussion

In this large well-characterized community-based cohort followed for over 30 years, our findings of several estimates of BA in the same individuals constructed from clinical, inflammatory and methylation data are threefold. First, midlife clinical BA is correlated with measures of BA in older adult life with the exception of DNA methylation age. Accelerated aging in midlife is associated with lower attendance at later exams, a metric often associated with poorer health. Second, increased clinical and inflammatory aging, corresponding to older BA than CA, results in greater hazard of death and incident CVD across exams even after accounting for CA and important potential confounders. Third, in models that included all three BA measures, increased aging from all three remained significantly predictive of increased mortality, whereas clinical and inflammatory Δage estimates increased risk for disease. Therefore, our findings suggest the three BA measures may be complementary in predicting risk for mortality and age-related disease. Finally, in cross-sectional analyses, all three BA measures were associated with functional measures of gait speed and grip strength.

Other clinical biomarker measures used to reflect the heterogeneity in human health span including frailty indices with large numbers of clinical and laboratory items have been developed in older adults that relate to mortality. We chose a set of clinical biomarkers reflective of diverse systems including the cardiovascular, pulmonary, metabolic, renal, and inflammatory systems that are easily measured in a clinical setting. The biomarkers represent a subset of biomarkers used in previous reports with demonstrated ability to quantify biologic age even in young adults (4,5) and are among the items in the frailty indices (22,23). Accelerated aging measured using clinical biomarkers in a birth cohort of healthy young adults before the onset of disease distinguished those with evidence of physical and cognitive decline and early signs of vascular aging (5). A similar set of clinical biomarkers measured in a nationally representative sample across midlife (ages 30–59), demonstrated greater risk of death in those with older BA (4). We confirm and extend this work by examining clinical BA at different points across the life span and testing both mortality and incident age-related diseases. Clinical BA using readily available clinical biomarkers may capture underlying physiologic reserve and represent a potentially useful approach to define physical

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Table 2. Incident Events According to ΔAge: Framingham Offspring Study, Exam 7[a]

	Clinical ΔAge			Inflammatory ΔAge		
		HR (95% CI)			HR (95% CI)	
	Events/*N*	MV Adjusted	*p* Value	Events/*N*	MV Adjusted	*p* Value
Mortality	490/2,532	1.04 (1.03, 1.05)	1.0×10^{-9}	718/3,140	1.05 (1.04, 1.06)	$<2 \times 10^{-16}$
CVD	310/2,400	1.05 (1.03, 1.06)	4.7×10^{-7}	413/2,944	1.04 (1.03, 1.05)	1.8×10^{-13}
Cancer	411/2,329	1.01 (0.998, 1.03)	.08	510/2,859	1.01 (1.00, 1.02)	.014

Note: CI = confidence interval; CVD = cardiovascular disease; HR = hazard ratio; MV = multivariable. Median follow-up time 14.4–14.7 years across all outcomes. Covariates: age, sex, current smoking, diabetes, hypertension treatment, lipid treatment, mortality only additionally adjusted for prevalent CVD and prevalent cancer.

[a]Each outcome is a separate model.

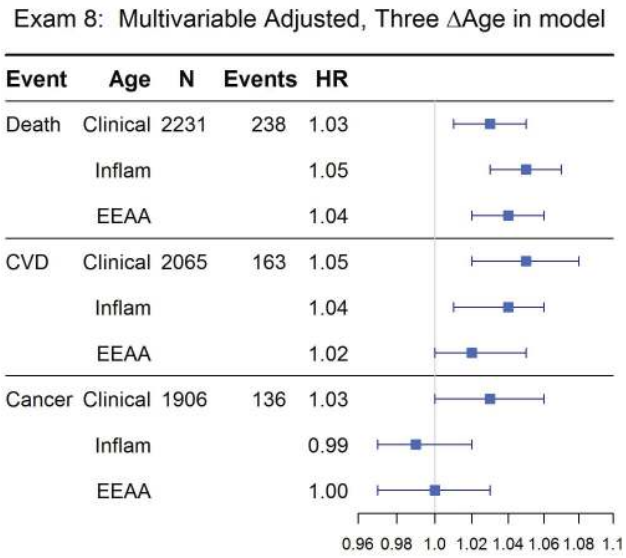

Figure 2. Incident events according to measures of Δage and epigenetic age: Framingham Offspring Study, Exam 8. Three Δage measures in the model. Median follow-up time for all outcomes was ≈8 years. Models are multivariable adjusted with covariates: age, sex, current smoking, diabetes, hypertension treatment, lipid treatment, mortality only additionally adjusted for prevalent CVD and prevalent cancer.

resilience at the whole person level (24). This may be one tool to test interventions (exercise, drug therapies) to determine whether accelerated aging or low physical resilience can be ameliorated which would have important clinical implications.

Chronic low-grade levels of inflammation occur with age, a process defined as "inflamm-aging," that is believed to accelerate biological aging (16). Multiple underlying mechanisms contribute to chronic inflammation with aging, including dysregulation of the immune system, oxidative stress, chronic infection, and so on (16). Therefore, the inflammatory markers we included in the inflammatory BA measure reflect many of these mechanisms. Older inflammatory BA is associated with risk for mortality as well as incident CVD and measures of physical function. Our results warrant replication in other independent samples. Further study of how inflammatory and other mechanisms such as epigenetics and the environment act together to accelerate or slow biologic aging are needed.

Several estimates of DNA methylation age in blood have been strongly associated with CA and shown to predict mortality (9–12). We had the opportunity to examine epigenetic measures of aging and inflammatory and clinical measures of BA in the same individuals at the same point in the adult life span. We found that EEAA was associated with all-cause mortality but not CVD and cancer. EEAA reflects both epigenetic changes and is correlated with blood cell composition (12) and may be strongly related to mortality because this measure also includes information on changes in blood cell counts with age. Although IEAA was not associated with outcomes in our study, this epigenetic measure has been associated with mortality in a larger meta-analysis that included Framingham participants (12) and with lung cancer susceptibility in women (25). IEAA is based on the Horvath epigenetic age estimate that generalizes to a broad range of tissues and cell types and is not correlated with blood cell counts. More research is needed to understand how the aging epigenome confers risk for age-related disease.

Our study has several limitations. The FHS is predominantly white; therefore, our findings may not be generalizable to other race/ethnic groups. There may be other important biomarkers of inflammation, including the senescence-associated secretory phenotype, and other biologic mechanisms (mitochondrial dysfunction, telomere length, alteration in proteostasis) not represented in this study (26). A previously reported transcriptomic BA signature was associated with indices such as grip strength but was limited in ability to examine mortality (8) and could be considered in future studies. Nonetheless, the study has several strengths including the large sample, community-based setting, ability to examine several biologic mechanisms in the same participants, and the longitudinal follow-up with careful ascertainment of events.

In summary, in our community-based sample, estimates of BA constructed from clinical, inflammatory, and DNA methylation biomarkers provide complementary information in predicting mortality and risk for age-related disease suggesting multiple aging metrics may be needed to capture the multiple dimensions of biological aging. Further study is needed to determine how the mechanisms interact to promote or delay aging.

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None reported.

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