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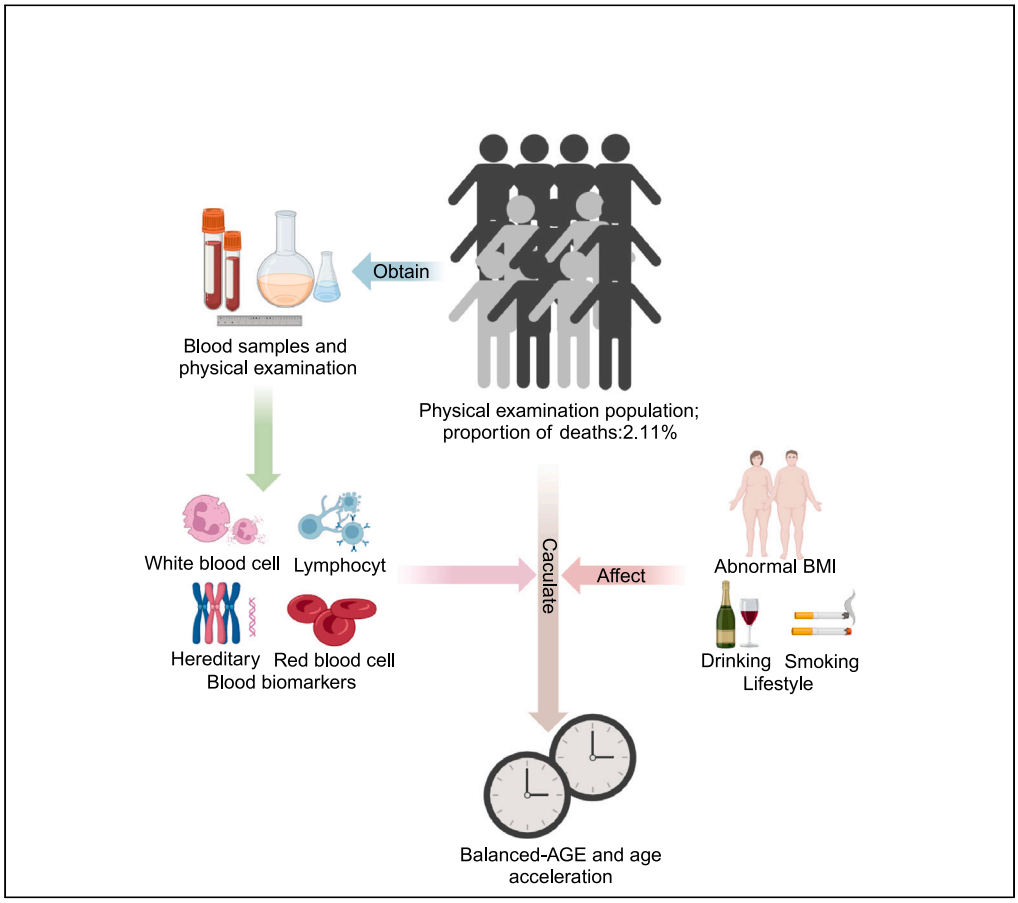

Qingqing Jia, Chen Chen, Andi Xu, ..., Huakang Tu, Ting Sun, Xifeng Wu

1195037@zju.edu.cn (T.S.)
xifengw@zju.edu.cn (X.W.)

Highlights

A valid method for estimating biological age using physical examination data is proposed

The Balanced-AGE is a promising predictor of mortality

The smoking, drinking, and BMI people have different aging acceleration

The Balanced-AGE can be used to identify individuals at high risk of aging people

Jia et al., iScience 27, 108891
March 15, 2024
https://doi.org/10.1016/j.isci.2024.108891

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A biological age model based on physical examination data to predict mortality in a Chinese population

Qingqing Jia,[1,7] Chen Chen,[1,7] Andi Xu,[1] Sicong Wang,[1] Xiaojie He,[2] Guoli Shen,[2] Yihong Luo,[1] Huakang Tu,[1] Ting Sun,[2,*] and Xifeng Wu[1,3,4,5,6,8,*]

SUMMARY

Biological age could be reflective of an individual's health status and aging degree. Limited estimations of biological aging based on physical examination data in the Chinese population have been developed to quantify the rate of aging. We developed and validated a novel aging measure (Balanced-AGE) based on readily available physical health examination data. In this study, a repeated sub-sampling approach was applied to address the data imbalance issue, and this approach significantly improved the performance of biological age (Balanced-AGE) in predicting all-cause mortality with a 10-year time-dependent AUC of 0.908 for all-cause mortality. This mortality prediction tool was found to be effective across different subgroups by age, sex, smoking, and alcohol consumption status. Additionally, this study revealed that individuals who were underweight, smokers, or drinkers had a higher extent of age acceleration. The Balanced-AGE may serve as an effective and generally applicable tool for health assessment and management among the elderly population.

INTRODUCTION

Aging leads to the occurrence of a variety of diseases.[1–4] China is expected to have the largest aging population by 2050.[5] Identifying those who are "aging faster than normal rate" would help early intervention and promotion of healthy aging.[6] Human aging is affected by a variety of molecular mechanisms, as well as the factors that are resulted from gene-environment interactions.[2,7–11] Generally, individuals who have the same chronological age may have completely different aging processes and longevity.[12] Therefore, identification of an individual's "true age" based on specific biological and environmental factors[13–15] would allow targeted early interventions with individualized preventive measurements as well as better health care management in the general population.

Specific biomarkers that are associated with human physical function, morbidity, and mortality have been identified, and thus can be used to estimate the process of aging from the biological perspective.[14,15] The concept of biological age,[16] defined by selected biomarkers and specific modeling algorithms, can more objectively and precisely reflect the biological changes of the human body due to the process of aging.[17] From body functional-related[7] (e.g., muscle mass, anthropometry, body mass index) to blood biomarkers (e.g., red blood cell distribution width),[10] a wide range of biomarkers have been selected for the calculation of biological age.

Several cohort studies have explored the association of biological age and chronological age with mortality.[18–20] Overall, biological age outperformed chronological age in terms of predicting mortality and health risks,[21,22] and can be used to better reflect the senescent change across multiple tissues and cells, providing insight into important pathways in aging. However, the majority of biological age models were constructed based on the cohorts in Europe or U.S.[10] Due to differences in race, lifestyle, and other factors across regions, biological age models may have large differences in predictive accuracy in different regions and are not generalizable in different populations. While some biological aging algorithms have been developed in Asian populations, most biological age models utilize complex genetic[23–25] and/or molecular[26] markers, which greatly limit their applicability and cost-effectiveness in the general population.

Physical examination data generally include comprehensive health information over years with longitudinal data.[27] Circulating biomarkers and physical examination information on body function, biological process, disease history, lifestyle, and living environment are readily

[1]Department of Big Data in Health Science School of Public Health, Center of Clinical Big Data and Analytics of The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310058, China
[2]Health Management Center, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, China
[3]National Institute for Data Science in Health and Medicine, Zhejiang University, Hangzhou, Zhejiang, China
[4]The Key Laboratory of Intelligent Preventive Medicine of Zhejiang Province, Hangzhou, Zhejiang, China
[5]Cancer Center, Zhejiang University, Hangzhou, Zhejiang, China
[6]School of Medicine and Health Science, George Washington University, Washington, DC, USA
[7]These authors contributed equally
[8]Lead contact
*Correspondence: 1195037@zju.edu.cn (T.S.), xifengw@zju.edu.cn (X.W.)
https://doi.org/10.1016/j.isci.2024.108891

Table 1. Distribution of select host characteristics of the study participants (n = 54,796)

Characteristic	Total 54796	Death n (%) 1158 (2.1)	Alive n (%) 53638 (97.9)	p Value	Balanced-AGE (mean ± sd, years)	p value	Balanced-AGE-ACC (mean ± sd, years)	p Value	Difference in Balanced-AGE-ACC[a] (years) (95% CI)	p value
Age group										
50-59years	37154	366 (1.0)	36788 (99)	<0.0001						
60–69 years	13730	324 (2.0)	13406 (98)							
≥70 years	3912	468 (14)	3444 (88)							
Sex										
Female	31390	842 (3.0)	30548 (97)	<0.0001	49.16 ± 9.12	<0.0001	−8.55 ± 6.70		Ref.	<0.0001
Male	23406	316 (1.0)	23090 (99)		55.79 ± 9.17		−2.04 ± 5.88	<0.0001	5.36 (5.21, 5.51)	
BMI										
Underweight	1103	56 (5.0)	1047 (95)	<0.0001	54.41 ± 12.5	<0.0001	−5.40 ± 8.22	<0.0001	0.66 (0.26, 1.06)	0.0012
Normal	24795	537 (2.0)	24258 (98)		52.42 ± 10.0		−5.32 ± 7.09		Ref.	
Overweight	23066	442 (2.0)	22624 (98)		53.22 ± 9.32		−4.49 ± 6.73		−0.12 (−0.24, 0)	0.0420
Obese	5832	123 (2.0)	5709 (98)	<0.0001	53.91 ± 9.12		−3.93 ± 6.70		0.36 (0.18, 0.54)	<0.0001
Smoking										
Never	25657	423 (2.0)	25234 (98)	<0.0001	53.38 ± 9.53	<0.0001	−4.96 ± 6.18	<0.0001	Ref.	
Current	14914	368 (3.0)	14546 (98)		55.21 ± 8.41		−1.50 ± 5.79		0.60 (0.33, 0.87)	<0.0001
Former	2243	75 (3.0)	2168 (97)		57.70 ± 9.40		−1.54 ± 5.59		0.76 (0.62, 0.9)	<0.0001
Drinking										
Never	21362	413 (2.0)	20949 (98)	<0.0001	53.66 ± 9.60	<0.0001	−4.77 ± 6.30	<0.0001	Ref.	
Current	20748	408 (2.0)	20340 (98)		54.65 ± 8.72		−2.44 ± 5.90		0.55 (0.06, 1.03)	0.0266
Former	608	33 (6.0)	575 (95)		59.49 ± 10.6		−0.92 ± 7.02		−0.45 (−0.58, −0.32)	<0.0001

BMI, body mass index.
Balanced-AGE-ACC: The difference between Balanced-AGE and chronological age (Balanced-AGE minus chronological age).
p values are based on independent t-test or ANOVA test. All p values are two-sided.
[a]Based on multivariable linear regression model including sex, BMI, smoking, and drinking.

available from health examination data. Generally, in some cohort studies using physical examination data, the mortality rate is low, resulting in imbalance between the number of the dead and the number of the living by the end of follow-up. Modeling with imbalanced data will affect the prediction accuracy of the model.[28,29] At the same time, although some Asian studies constructed biological age using physical examination data,[30,31] they did not deal with the imbalance issue in the data. Therefore, it is necessary to develop a measure of aging in a general Chinese health physical examination population, taking into account the imbalance of the data, so as to construct a more accurate biological aging model.

Therefore, this study aimed to develop a Chinese population-tailored age evaluation measure (Balanced-AGE), with the consideration of imbalance issue in physical examination data to optimize the model. We further evaluated the associations of biological age with all-cause mortality and quantified the impact of different demographic characteristics and lifestyles on the aging acceleration process.

RESULTS

Participants' characteristics

As shown in Table 1, the females and males accounted for 42.71% and 57.29% of the participants, respectively. The groups aged 50–59 years, 60–69 years, and ≥70 years accounted for 67.80%, 25.06%, and 7.14% of the study population, respectively. Nearly half of the study population had either a normal or overweight BMI. 45.25% of participants were in normal BMI. The mean age was 57.78 ± 695 years (Table 2). The mean of Balanced-AGE was 56.90 ± 9.09 years in the general population, 58.13 ± 8.87 years in males, and 55.21 ± 9.13 years in females.

All participants had complete laboratory biomarker records (Table 2), including renal, hematological, inflammation, diabetes, lipid, liver, cardiovascular, cancer-related biomarkers, and physical anthropometry.

The scatterplots between the two estimated biological ages (Balanced-AGE and Imbalanced-AGE) and chronological age, and the residuals are shown in Figure 1. Chronological age was highly correlated with Balanced-AGE (Figure 1A, $r = 0.701$, $p < 0.001$) and Imbalanced-AGE (Figure 1B, $r = 0.873$, $p < 0.001$). Distributions of the Balanced-AGE-ACC estimations in the different sub-groups are shown in Table 1. The AGE-ACC of Balanced-AGE and Imbalanced-AGE was reasonably normally distributed (Figures 1E and 1F).

Table 2. Physical examination items and model weights for Balanced-AGE and Imbalanced-AGE

Marker Category	Variable	Mean ± Sd	Unit	Biomarkers in Balanced-AGE model	Biomarkers in Imbalanced-AGE model
Age	Age	57.78 ± 6.95	Year	0. 0455	0.1052
Sex	Sex	NA	N/A	−0.3421	
Renal	Albumin (ALB)	44.55 ± 3.00	G/L	−0.0266	
	Uric acid (UA)	341.48 ± 85.00	Umol/L	–	
	Serum creatinine (CR)	65.22 ± 15.93	Umol/L	–	0.0068
	Urea nitrogen (BUN)	5.43 ± 1.32	Mmol/L	–	
Hematological	Mean corpuscular hemoglobin concentration (MCHC)	335.6 ± 11.65	U/L	−0.0131	
	Red blood cell distribution width (RDW)	12.98 ± 0.92	U/L	0.1093	0.2650
	White blood cell count (WBC)	6.01 ± 1.58	G/L	–	–
	Hematocrit (HCT)	42.72 ± 4.16		–	–
	Mean corpuscular volume (MCV)	91.8 ± 4.95	Fl	–	–
	Mean platelet volume (MPV)	9.93 ± 1.52	FL	–	–
Inflammation	Lymphocyte percentage (LY%)	33.49 ± 7.84	%	−0.0151	−0.0316
	Blood platelet count (PLT)	202.25 ± 54.48	%	–	–
	Basophil percentage (BA%)	0.51 ± 0.24	%	–	–
	Monocytes percentage (MO%)	6.77 ± 1.76	%	–	–
	Neutrophil percentage (NE%)	56.82 ± 8.35	%	–	–
	Eosinophil percentage (EO%)	2.40 ± 1.99	%	–	–
Diabetes	Hemoglobin (HGB)	138.55 ± 28.36	G/L	0.0034	–
	Fasting blood glucose (FPG)	5.56 ± 1.38	G/L	–	–
	Fasting insulin (FINS)	59.47 ± 41.61	Mmol/L	–	–
Lipid	Apolipoprotein A1(Apo A1)	1.33 ± 0.21	G/L		–
	High-density lipoprotein (HDL)	1.36 ± 0.35	Mmol/L	–	–
	Total cholesterol (TC)	5.19 ± 1.01	Mmol/L	–	–
	Triglycerides (TG)	1.80 ± 1.45	Mmol/L	–	–
	Low-density lipoprotein (LDL)	2.97 ± 0.78	Mmol/L	–	–
	Apolipoprotein B (Apo B)	1.00 ± 0.24	G/L	–	–
	Direct bilirubin (DBIL)	2.86 ± 1.54	Mmol/L	–	–
	Total bilirubin (TBIL)	14.37 ± 5.54	Mmol/L	–	–
	Indirect bilirubin (IBIL)	11.51 ± 4.51	Mmol/L	–	–
Liver	Alkaline phosphatase (ALP)	84.79 ± 24.96	U/L	0.0027	0.0045
	Gamma-glutamyl transpeptidase (GGT)	36.46 ± 44.69	U/L	0.0008	0.0018
	Alpha fetoprotein (AFP)	3.37 ± 7.02	UG/L		–
	Lactic dehydrogenase (LDH)	190.29 ± 35.61	U/L	0.0013	–
	Aspartate transaminase (AST)	25.62 ± 12.85	U/L	–	–
	Alanine transaminase (ALT)	24.72 ± 19.52	U/L	–	–
	Total protein (TP)	72.20 ± 4.32	G/L	–	–
	Globulin (Glob)	27.62 ± 3.69	G/L	–	–
	Total protein/globulin (A/G)	1.64 ± 0.26		0.3636	–
Cancer	CA199	11.40 ± 24.15	KU/L	0.0011	0.0032
	CA125	11.80 ± 11.12	KU/L	0. 0018	0.0063
	Alpha fetoprotein(AFP)	3.58 ± 30.12	UG/L		

(*Continued on next page*)

Table 2. *Continued*

Marker Category	Variable	Mean ± Sd	Unit	Biomarkers in Balanced-AGE model	Biomarkers in Imbalanced-AGE model
Cardiovascular	Creatine kinase (CK)	111.25 ± 215.10	U/L	–	–
	Systolic blood pressure (SBP)	131.34 ± 18.39	Mmhg	–	–
	Diastolic blood pressure (DBP)	78.36 ± 11.86	Mmhg	–	–
	Pulse	77.07 ± 11.38	Times		
	Constant			−7.6540	−22.2614
	γ			0.0007	0.0004

Balanced-AGE, Biological age calculated based on balanced model.
Imbalanced-AGE, Biological age calculated based on Imbalanced model.
The model weight, Constant, γ is the constant terms and parameters obtained by Gompertz regression.

After adjustment for sex, smoking status, drinking status, and BMI (Table 1), we found that males were physiologically older than females (difference in Balanced-AGE-ACC = 5.36 years, $p < 0.0001$). Compared with never smokers, Balanced-AGE-ACC of former smokers and current smokers increased by 0.76 and 0.60 years ($p < 0.0001$), respectively. Compared with never drinkers, the Balanced-AGE-ACC for former drinkers and current drinkers increased by −0.45 and 0.55 years ($p < 0.0001$), respectively. Compared with those with normal BMI, those with Obese or underweight BMI had higher Balanced-AGE-ACC; particularly, those with underweight BMI had the greatest acceleration in Balanced-AGE-ACC by of 0.66 years ($p < 0.0001$).

Model performance

As shown in Figure 2, in general, Balanced-AGE and Imbalanced-AGE outperformed chronological age in predicting the risk of mortality. Balanced-AGE, chronological age and Imbalanced-AGE had small differences in AUC values of predicting mortality at 3 years (AUC: 0.778 vs. 0.734 vs. 0.798, Bonferroni $p < 0.0001$) and 5 years (AUC: 0.802 vs. 0.763 vs. 0.803), yet the predictive precision of the Balanced-AGE effect achieved its optimum with an AUC of 0.908 (AUC: 0.908 vs. 0.773 vs. 0.811, Bonferroni, $p < 0.0001$) at 10 years. Overall, Balanced-AGE had the optimal discrimination for predicting the risk of mortality at longer follow-up period, compared with Imbalanced-AGE and chronological age. Therefore, further analyses focused on Balanced-AGE.

Risk estimates of association with mortality

As shown in Figure 3A, the Balanced-AGE was strongly related to all-cause mortality. Participants with a higher Balanced-AGE at baseline had a higher risk of mortality (Figure 3A, per 5 years increase). After adjusting for chronological age, sex, smoking, drinking, and BMI, each 5-year increase in Balanced-AGE was associated with a 45% higher risk of mortality (HR = 1.45, 95%CI: 1.38–1.52, $p < 0.0001$). Given the need to generalize measures of aging across various populations, we examined the associations in subgroups. For example, each 5-year increase in Balanced-AGE was associated with a 53% higher risk of mortality in the 50–59 years age group (HR = 1.53, 95%CI: 1.41–1.65, $p < 0.0001$), in the 60–69 years and >70 years age groups the associated increase in risk of mortality was 46% and 38%, respectively. Furthermore, when examining the associations within subgroups by sex, smoking, drinking, and BMI, we found that Balanced-AGE was predictive in all subgroups. Among the subgroups, the association of Balanced-AGE with mortality was the strongest in former drinkers (HR: 1.70, 95%CI: 1.40–2.06, $p < 0.0001$).

Balanced-AGE-ACC was also used to predict the risk of all-cause mortality. Figure 3B presents the association between Balanced-AGE-ACC and mortality. Overall, each quartile increase in Balanced-AGE-ACC was associated with a 36% higher risk of mortality (HR = 1.36, 95%CI: 1.26–1.46) after adjusting chronological age, sex, smoking, drinking, and BMI. In stratified analysis by sex, the association was more pronounced in males than in females (per quartile increase, HR = 1.62 vs. HR = 1.30). The associations were consistent among various subgroups by smoking, drinking, and BMI.

DISCUSSION

We developed and validated a novel aging measure (Balanced-AGE) based on a large-scale physical examination database. This is the first modeling study to consider data imbalance in the development of biological age in a Chinese population. After using the repeated sub-sampling to address the problem of imbalanced data, we significantly improved the performance of biological age (Balanced-AGE) in predicting all-cause mortality. Meanwhile, we found that this predictive measure for mortality remained effective across different subgroups by age, sex, smoking status, and alcohol consumption status. In addition, we verified that people who were overweight, smokers, or drinkers had accelerated biological age. Our results have important clinical implications for the identification of at-risk aging populations for healthy aging counseling and intervention.

Ideally, all possible biomarkers should be available for the estimation of biological age, but the optimal biomarker panel should include the representative biomarkers.[13,32] The majority of the selected aging biomarkers are related to multiple physiological functions, including renal, hematological, inflammatory, diabetes, lipid, liver, and cancer. In this study, we considered the effect of aging on mortality and used the

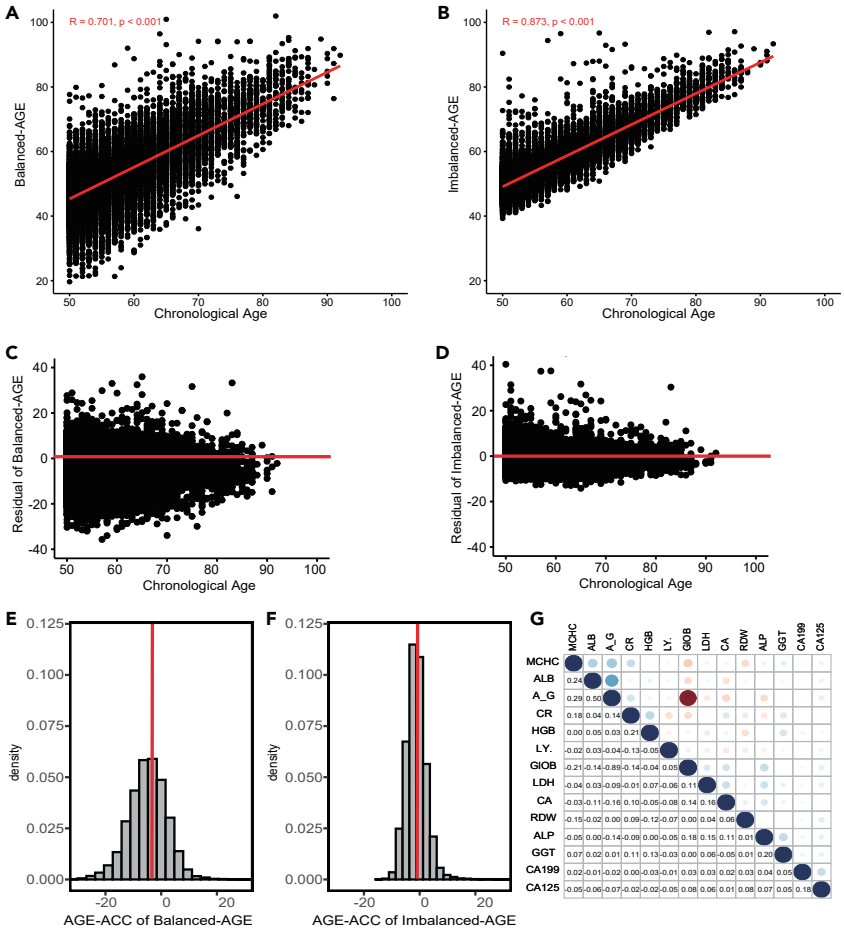

Figure 1. Characterization of CA, BA, AGE-ACC and included predictors

(A)The correlation between Balanced-AGE and CA, (B) the correlation between Imbalanced-AGE and CA, (C) Residual plot of Balanced-AGE on CA, (D) Residual plot of Imbalanced-AGE on CA, (E) Distribution of AGE-ACC of Balanced-AGE, (F) Distribution of AGE-ACC of Imbalanced-AGE, (G) Pearson correlation matrix of the biomarkers included for predictive model construction. Since AGE-ACC is defined as the difference between BAs and CA, a score of 0 indicates no difference between individuals' BA and CA; positive values mean that the individuals' BAs are larger than their CAs, indicating accelerated aging, while negative values indicate decelerated aging.

(LASSO)cox method to select 13 biological markers (ALB, ALP, A/G, GGT, HGB, LDH, LY%, MCHC, RDW, CA125, CA129, CA, and Sex). RDW[33] appeared to be one of the most significant biomarkers of aging and mortality accessed by Gompertz weight (weight = 0.1093). Recent studies have shown that markers associated with red blood cells, such as RDW, MCHC, and HGB are associated with a significant increase in multiple indicators of adverse health status such as multimorbidity, cognitive impairment,[34] disability, and mortality in the elderly.[35] A previous study found that CA125 and CA199, known tumor biomarkers, were significantly associated with risk of cancer-related mortality[36] and a composite mortality endpoint of cardiovascular (CV).[37] Elevated ALP and GGT levels were associated with higher all-cause mortality, consistent with linear dose-response relationships.[38] ALB and A/G are among the potential indicators to reflect chronic liver injury.[39] In the context of life extension and health promotion, those biomarkers can be used in clinical research and practice to discover new molecular targets of aging, to subsequently delay aging, and to identify participants at increased risk of multiple ageing-related conditions.

The concept of biological age has been applied and validated in a large number of population cohort studies. Examples include biological age derived from principal component analysis (PCA)[40] and multiple linear regression (MLR).[21] Klemera and Double method (KDM)[22] and Levine method[17] both construct corresponding biological age models based on different sets of aging biomarkers. These measures were constructed with the relative weight of each marker given by its ability to predict chronological age rather than the risk of incident diseases or mortality. Levine et al. selected chronological age and nine clinical blood markers based on the penalty Cox regression model, and then constructed PhenoAge by the parametric proportional hazards model of the Gompertz distribution of mortality risk. PhenoAge correlated well (r = 0.94) with chronological age. We therefore applied the Levine method to construct our Balanced-AGE.

Although many studies have shown that biological ages are valid independent predictors of mortality with good predictive performance, these studies ignored the problem of data where the number of non-events is much larger than the number of the events, which is defined as

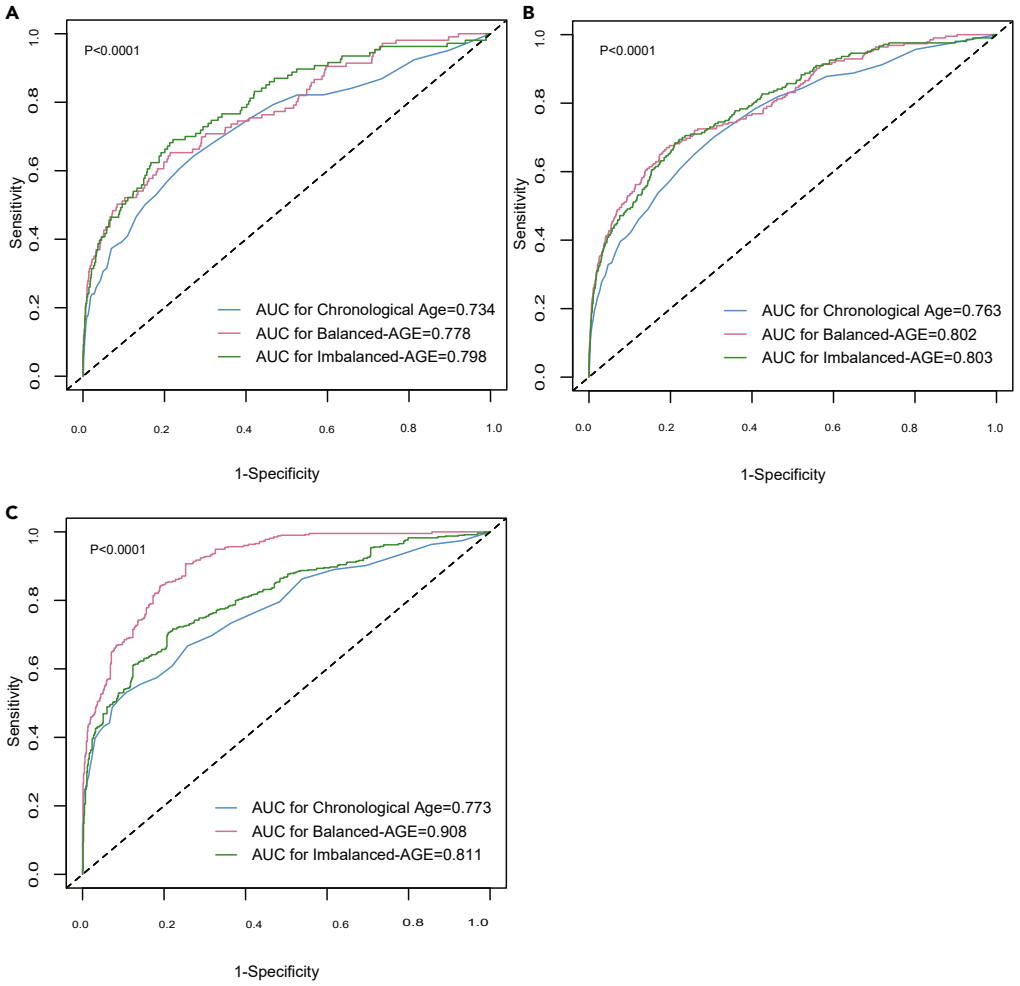

Figure 2. Receiver operating characteristic curves for 3-year, 5-year, and 10-year all-cause mortality prediction: comparison across CA, Balanced-AGE, and Imbalanced-AGE

Balanced-AGE: Biological age calculated based on a balanced model. Imbalanced-AGE: Biological age calculated based on an Imbalanced model. AUC: Area under the curve. (A) 3-year time-dependent ROC.

(B) 5-year time-independent ROC.

(C) 10-year time-independent ROC.

the phenomenon of "data imbalance."[29,41] Data imbalance severely affect the predictive power of models, and the medical field usually faces with data imbalance problem.[28] In predicting mortality risk by biological age, the 10-year AUC was 0.6177 for Levin's DNAm PhenoAge, 0.5605 for Horvath's DNAm Age, and 0.5670 for Hannum's DNAm Age, respectively.[17] Noticeably in our study, we addressed the imbalanced data issue from low mortality rate in health examination cohort data based on repeated random sub-sampling method, and greatly improved the power of biological age (Balanced-AGE) for predicting mortality with a 10-year AUC of 0.908.

In the Cox regression prediction model for the risk of mortality, the Balanced-AGE estimate predicted the risk of all-cause death independently of chronological age. The strength of the association of Balanced-AGE with risk of death was more pronounced in younger groups (50–59 years and 60–69 years), which may show that changes in physiological status, as reflected by aging biomarkers, may play a greater role in accelerating human aging in the younger groups compared to the older group (≥70 years). The National Institutes of Health found that abstaining from alcohol doubled the risk of mortality,[42] and in our study we found former drinkers had the greatest mortality risk at the same biological age level, suggesting the long-term harm of alcohol consumption on human mortality and aging. In terms of early aging health intervention, attention should be paid to this part of the population that is ignored due to alcohol abstinence.

In this study, we demonstrated that sex, smoking, alcohol consumption, and obesity can accelerate the aging process in humans. Smokers had shorter telomere lengths than never-smokers. Dose-response meta-analyses showed an inverse trend between pack-years of smoking and telomere length.[43] At the same time, studies have shown that even small amounts of alcohol consumption are associated with the premature aging of the brain,[44] which in turn affects the aging process of the organism. NAD(+) (Nicotinamide adenine dinucleotide) can slow down the

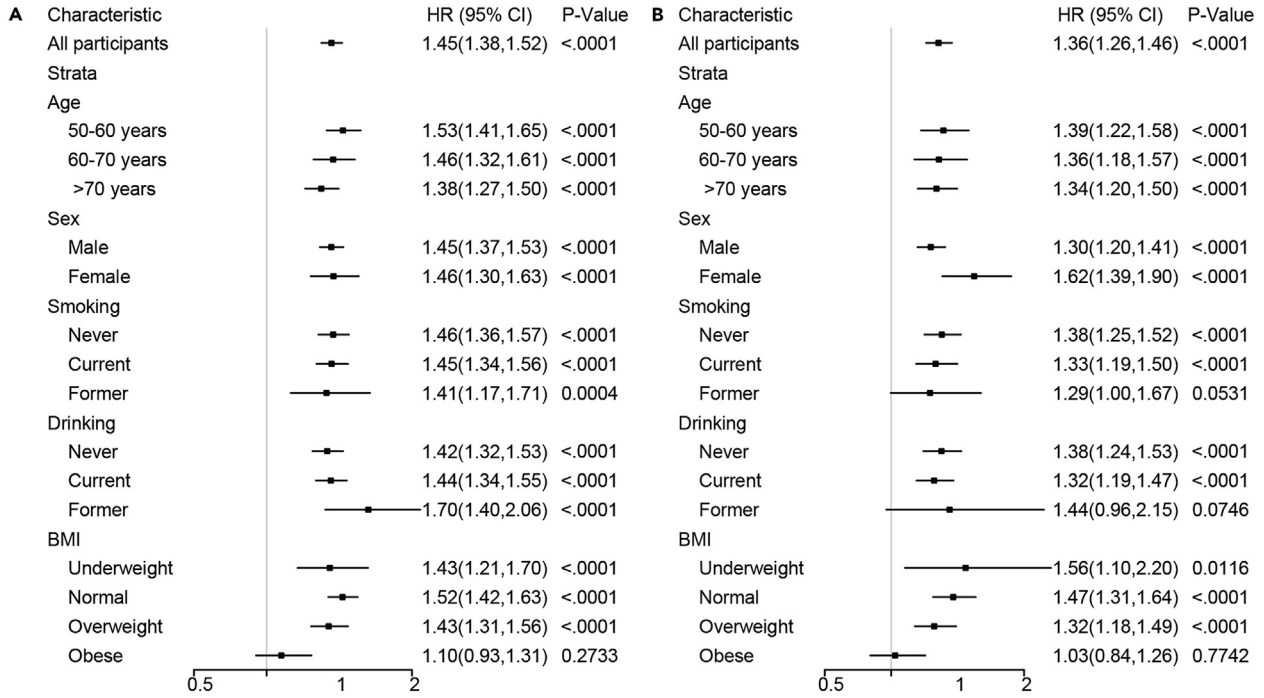

Figure 3. Stratified analysis to assess the association of Balanced-AGE and Balanced-AGE-ACC with mortality

(A) Associations of Balanced-AGE with all-cause mortality in subgroups (per 5 years increase), (B) Associations of Balanced-AGE-ACC with all-cause mortality in subgroups (per quartile years increase).

aging process, male's blood levels of NAD(+) decline faster with age compared to female, and male are more prone to acquiring poor lifestyles (e.g., smoking, drinking), so male are more susceptible to aging than female.[45] Obesity is associated with the acceleration of cellular processes observed during normal aging, and inflammation and oxidative stress appear to be important mediators of this association.[46] For example, obesity increases inflammation and oxidative stress, and one of the major hallmarks of aging is increased levels of pro-inflammatory molecules.[47]

Our study has a few strengths. First, our data came from a database of individuals who had completed at least one independent physical examination at baseline and had rich and accurate blood biomarker data. Mortality data were obtained from the Hangzhou Public Security Bureau database, which provides reliable follow-up for death. Second, this study applied a complex sampling design and maximally avoided the biased estimation that was potentially introduced by the imbalanced data problem, which greatly improved the death prediction accuracy of the model. Third, by exploring associations between Balanced-AGE and mortality risk after adjusting for health behaviors, our results showed the robustness of these associations across subgroups. Fourth, we assessed the potential impact of smoking and drinking behaviors on biological aging and verified that smokers and drinkers have accelerated biological aging than never smokers or drinkers.

Overall, we developed an effective approach to estimate biological age (Balanced-AGE) with highly imbalanced physical examination data, and this aging measure is a promising predictor of mortality. The Balance-AGE could be used as an independent indicator of the aging process. More importantly, its prediction power for mortality is robust regardless of chronological age and lifestyles. In addition, this measure revealed the impact of sex, smoking, drinking, and BMI on aging process. These findings suggest that this new aging measure may be used to identify high-risk individuals for early intervention and evaluate the efficacy of aging intervention.

Limitations of the study

However, a few limitations should be noted. This study did not exclude death due to accidents or other reasons that are not directly related aging. Also, the lack of physical activity and dietary data limited our ability to assess their impact on biological aging. Our study had no detailed information on the amount and frequency of cigarette or alcohol consumption, and additional information on such aspects could help explore their impact on biological aging more comprehensively.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.108891.

ACKNOWLEDGMENTS

This study was approved by the Institutional Review Board of the Second Affiliated Hospital of Zhejiang University School of Medicine.

This study was Funding by grants 2020E10004 from the Key Laboratory of Intelligent Preventive Medicine of Zhejiang Province, 2019R01007 from the Leading Innovative and Entrepreneur Team Introduction Program of Zhejiang, 2020C03002 from the Key Research and Development Program of Zhejiang Province, and K20230085 from the Healthy Zhejiang One Million Cohort (all to Prof Wu).

AUTHOR CONTRIBUTIONS

QQJ and CC contributed equally to this work; Conceptualization, X.F.W., T.S.; Methodology, Q.Q.J., C.C., H.K.T., A.D.X., T.S. and X.F.W.; Formal analysis, Q.Q.J., H.K.T., C.C., A.D.X., X.F.W. and T.S.; Investigation: Q.Q.J., H.K.T., S.C.W., Y.H.L., G.L.S., X.F.W. and X.J.H.; Writing–Original Draft, Q.Q.J., C.C.; Writing – Review & Editing, X.F.W., H.K.T., C.C., A.D.X., T.S. and S.C.W.; All authors critically revised and reviewed the article, and approved the final version of the article before submission. All authors read and approved the final article.

DECLARATION OF INTERESTS

There is no competing interest to declare.

Received: March 23, 2023
Revised: September 2, 2023
Accepted: January 9, 2024
Published: February 3, 2024

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
SAS 9.4	SAS	https://www.sas.com/en_us/home.html
R Studio	R-Tools Technology, Inc	https://www.rstudio.com/
STATA 16.0	StataCorp LLC	https://www.stata.com/
Levine method	Levine et al.[17]	https://doi.org/10.18632/aging.101414
Repeated sub-sampling approach	Khalilia et al.[41]	https://doi.org/10.1186/1472-6947-11-51
Gompertz algorithms	Kirkwood et al.[48]; Dey et al.[49]	https://doi.org/10.1098/rstb.2014.0379; https://doi.org/10.1016/j.chest.2020.03.015
Balanced-AGE	This paper	N/A
Imbalanced-AGE	This paper	N/A
Balanced-AGE-ACC	This paper	N/A
Imbalanced-AGE-ACC	This paper	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xifeng Wu (xifengw@zju.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study population

This study collected a wide range of baseline data through routine physical examinations from 73261 individuals aged 50 years or older at the Second Affiliated Hospital, Zhejiang University School of Medicine from January 01, 2008 and October 28, 2020. Information on mortality was collected through October 28, 2021. We excluded participants with the missing physical examination items or with incomplete records or invalid government issued IDs (n = 18465). The final valid analytic sample included 54796 participants at baseline enrollment. The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Zhejiang University, School of Medicine.

Follow-up for mortality

Information on survival status and date of death were retrieved from the Hangzhou Public Security Bureau database, and matched with participants' physical examination data through unique government issued IDs. By the end of October 28, 2021, 1158 (2.11%) out of 54796 participants died. The median follow-up time was 80 (IQR, 79.6–80.4) months.

Health measurements

BMI was categorized into four levels (underweight: <18.5 kg/m^2; normal: 18.5≤BMI<24.9 kg/m^2; overweight: 25≤ BMI< 24 kg/m^2; obese: ≥28.0 kg/m^2).[29] Age was categorized into three groups (50–59, 60–69, and ≥70 years old). According to participants' smoking and alcohol consumption history, the smoking status was classified as never smoker, former smoker and current smoker. Drinking status was classified as never drinker, former drinker and current drinker.[50]

METHODS DETAILS

Data pre-processing and biological age estimation

An imbalanced data problem is a phenomenon where there is a large gap between the number of event cases and non-event cases in training data, which will cause low prediction accuracy.[28] In this study, the proportion of mortality was 2.11%. The number of death cases was far smaller than the number of alive cases, which is extremely imbalanced. Therefore, to improve the validity and accuracy of the model adopted in this study, we used a repeated sub-sampling design[41] to address the issue of data imbalanced[51] and then construct the Balanced-AGE (Figure S1). To evaluate the effect of the complex sampling design, we randomly split the original datasets at a 7:3 ratios and formed an imbalanced training set and validation set. The imbalanced training set was used to establish Imbalanced-AGE. Before being selected, each variable was standardized [(X-mean)/SD] to ensure the objectivity of the subsequent algorithm calculation. For the balanced training set which consisted of multiple sub-samples, the (LASSO)cox algorithm was performed to select biomarkers in each sub-sample. Biomarkers that were selected in more than 80% of the subsamples were included in the construction of Balanced-AGE. Besides chronological age and sex, 12 additional biomarkers (Albumin (ALB), Alkaline phosphatase (ALP), Total protein/globulin (A/G), Globulin (Glob) Gamma-glutamyl transpeptidase (GGT), Hemoglobin (HGB), Lactic dehydrogenase (LDH), Lymphocyte percentage (LY%), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width (RDW), CA125, CA129) were selected. We did not include Glob due to its high correlation with A/G ($r = -0.89$, $p < 0.05$, Figure 1G) and relatively small contribution to the mortality. Finally, 13 biomarkers were included in the final Balanced-AGE model. For the Imbalanced training set, the (LASSO)cox algorithm was applied directly, besides chronological age, another 7 biomarkers (Alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), Serum creatinine (CR), Red blood cell distribution width (RDW), CA199, CA125, Lymphocyte percentage (LY%)) were selected to construct Imbalanced-AGE.

According to the Gompertz[48,49] (age-mortality) distribution and the Levine method,[17] 13 and 8 biomarkers obtained from the screening stage were included in the model, respectively, and finally, Balanced-AGE and Imbalanced-AGE were obtained. The formula is shown below:

$$BA = \frac{\ln[-\gamma * \ln(1 - F(t, xb))]}{\exp(\gamma t)} - \frac{b_0}{b_1}$$

Where $F(t, xb)$ is the Gompertz model based on the selected biomarkers, t denotes the longest follow-up time (months), and xb represents the linear combination of biomarkers from the Gompertz model.

In our study, $t = 165$ months. The b_0, b_1, γ are parameters estimated based on the Gompertz regression which contains only chronological age.

For Balanced-AGE:

$$\begin{aligned} F(165, xb) = (&-\mathrm{ALB} * 0.0266 + \mathrm{ALP} * 0.0027 - \mathrm{A/G} * 0.3636 + \mathrm{GGT} * 0.0008 + \mathrm{HGB} * 0.0034 \\ &+ \mathrm{LDH} * 0.0013 - \mathrm{LY\%} * 0.0151 - \mathrm{MCHC} * 0.0131 + \mathrm{RDW} * 0.1093 + \mathrm{AGE} * 0.0455 + \mathrm{CA125} * 0.0018 \\ &+ \mathrm{CA199} * 0.0011 - \mathrm{Sex} * 0.3421 - 7.654) / 0.0547 + 231.5667 \end{aligned}$$

For Imbalanced-AGE:

$$\begin{aligned} F(165, xb) = (&\mathrm{ALP} * 0.0045 + \mathrm{GGT} * 0.0018 + \mathrm{CR} * 0.0068 + \mathrm{RDW} * 0.2650 + \mathrm{AGE} * 0.1052 + \mathrm{CA199} * 0.0032 \\ &+ 0.0063 * \mathrm{CA125} - \mathrm{LY\%} * 0.0316 - 22.2614) + 125.2547 \end{aligned}$$

To eliminate the effect of chronological age on the aging process, we adopted the concept of age acceleration (AGE-ACC)[50,52] in later analysis, which is defined as the difference between Balanced-AGE (or Imbalanced-AGE) and chronological age, symbolized as Balanced-AGE-ACC (or Imbalanced-AGE-ACC). AGE-ACC represents Balanced-AGE (or Imbalanced-AGE) after adjustment for chronological age, when AGE-ACC = 0 means no difference between individuals' Balanced-AGE (or Imbalanced-AGE) and chronological age; AGE-ACC>0 means that an individual is physiologically older compared with the corresponding chronological age; AGE-ACC<0 means that an individual is physiologically younger compared with the corresponding chronological age.

QUANTIFICATION AND STATISTICAL ANALYSIS

The baseline characteristics of the participants were reported as Mean ± Sd or n (%). T-test and ANOVA analysis were used to compare the differences between groups. BMI, age, smoking, and drinking status were collected and considered for stratified analysis.

The model discrimination was assessed in the validation set. 3-year, 5-year, and 10-year-time-dependent areas under the receiver operating characteristic curves (AUC) were calculated and compared for the Balanced-AGE, Imbalanced-AGE, and chronological age.

The COX proportional hazards model was used to assess the estimated risk between Balanced-AGE and all-cause mortality. To exclude potential confounding effects, we: 1) adjusted chronological age, sex, BMI, smoking, and drinking status; 2) employed stratified analyses with chronological age, sex, BMI, smoking, and drinking status.

We also used the COX model to assess the association between Balanced-AGE-ACC and mortality with adjustment of chronological age, sex, BMI, smoking, and drinking status. Multivariable linear regression which adjusted sex, BMI, smoking, and drinking was applied to calculate the effect of different lifestyles on Balanced-AGE-ACC.

All data were processed and analyzed by software SAS 9.4; statistical graphics were plotted by R Studio; the Gompertz model was performed by STATA 16.0. p values were two-sided. p values < 0.05 and Bonferroni p values < 0.0167 were considered statistically significant.