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Association of proBNPage with all-cause and cardiovascular mortality among US adults: an analysis of data from the National Health and Nutrition Examination Survey

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ABSTRACT

Objective Biological age assessed by the Klemera and Doubal method (KDM) and phenotypic age (PhenoAge) was considered as a marker for ageing-related outcomes because it reflects different aspects of biological ageing and health, which are associated with increased risk of death. proBNPage based on N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a novel index for biological age estimation. However, the independence of its relationship with clinical outcomes from established risk factors, KDM or PhenoAge remains uncertain. Their identification could provide valuable information to prognosis.

Design, setting and participants This study analysed data from the general population included in the National Health and Nutrition Examination Survey (NHANES). Participants who took part in the cross-sectional survey from 1999 to 2004 were included, and all-cause as well as cardiovascular mortality was recorded (up to 31 December 2019).

Outcome measures All-cause and cardiovascular mortality were considered as outcomes. Clinical risk factors were collected, and biological age was estimated by proBNPage, KDM and PhenoAge. Cox proportional hazards models were used to determine the relationship between proBNPage and outcomes with adjustment for risk factors or other biological age indexes. Restricted cubic spline (RCS) analysis based on multivariate Cox regressions was performed to examine whether there was a non-linear relationship between proBNPage and outcomes.

Results A total of 9 925 participants were included in this study. The association between proBNPage and outcomes remained significant after adjusting for risk factors, including NT-proBNP (for all-cause mortality, HR 1.14; 95% CI 1.10 to 1.17; for cardiovascular mortality, HR 1.20; 95% CI 1.14 to 1.27). Similar results were obtained after adjusting for KDM plus NT-proBNP (for all-cause mortality, HR 1.31; 95% CI 1.22 to 1.41; for cardiovascular mortality, HR 1.21; 95% CI 1.11 to 1.28) or PhenoAge plus NT-proBNP (for all-cause mortality, HR 1.21; 95% CI 1.16 to 1.28; for cardiovascular mortality, HR 1.35; 95% CI 1.24 to 1.47). These findings were confirmed in most subgroups. A non-linear relationship was observed between proBNPage and all-cause and cardiovascular mortality with an inflection point.

Conclusions A non-linear positive relationship was observed between proBNPage and clinical outcomes. After adjusting for established risk factors and other biological age estimation indices (KDM or PhenoAge), proBNPage was significantly associated with mortality. The results remain similar after further adjustment for NT-proBNP. These results suggest that proBNPage is a useful surrogate for biological age estimation.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study used a large dataset and a weighted design, which significantly strengthens the reliability of the findings.
⇒ This study investigated novel biological ageing markers (proBNPage) in the general population rather than exclusively in the elderly or a specific ethnicity.
⇒ Comprehensive data collection methods, including standardised interviews and laboratory assessments, enhance the reliability of the findings.
⇒ Other ageing-related events were not assessed, and the research was conducted in a single cohort of US adults, which limits its generalisability.
⇒ The inability to perform repeated measurements of NT-proBNP in the study population affects our ability to determine whether changes in proBNPage affect clinical outcomes.

INTRODUCTION

Population ageing has become a major public health challenge worldwide.[1] Ageing signifies prolonged exposure to risk factors and accumulation of damage, resulting in severe disease burden and event risk,[2] but certain individuals are exposed to more risk factors at an earlier age, causing them to exhibit signs of ageing sooner. To better describe

this phenomenon, the concept of biological age has been proposed, which was demonstrated as a more precise gauge than chronological age in assessing our proximity to mortality.[3] Besides, biological age also proved useful as a surrogate endpoint in the development of anti-ageing treatment.[4]

At present, numerous studies have explored the indexes capable of estimating biological age.[5–11] Among these, the method proposed by Petr Klemera and Stanislav Doubal (Klemera and Doubal method, or KDM)[10] is generally accepted as the optimal method for biological age estimation since it provides useful information in predicting mortality and diseases.[12–15] In addition, Morgan E. Levine *et al*[11] proposed phenotypic age (PhenoAge) as a useful epigenetic biomarker in estimating biological age, which strongly outperformed previous measures regarding predictions for various ageing outcomes.[11 16] Both of them use physiology-based algorithms, providing clinically usable biological age estimation; characteristically, they both require a set of clinical variables and complex calculations, impeding their clinical application to some extent.

Moreover, neither KDM nor PhenoAge considered the effect of N-terminal pro-B-type natriuretic peptide (NT-proBNP), a biomarker commonly used for heart function assessment and easily measurable in clinical practice. Recently, research found that NT-proBNP is the most effective clinical marker for indicating biological age.[17] Based on this, proBNPage was proposed as a novel surrogate index of biological age with simple formulas using NT-proBNP.[17] Further analysis showed that proBNPage was related to cardiovascular disease (CVD) and physical and mental dysfunction in the elderly.[18] According to these, proBNPage may provide an easily used biological age estimation method. However, these studies did not provide information about the association of proBNPage with other ageing-related outcomes, such as CVD mortality. They were conducted mainly on elderly white people, and the data for other ethnicities and younger individuals were limited. Although the relationship between proBNPage and ageing-related outcomes remains significant and was independent of clinical risk factors,[17] only a few risk factors were adjusted in the model. Furthermore, we still do not know whether the association between proBNPage and outcomes is significant after adjusting for other traditional biological age indexes, such as KDM or PhenoAge. Considering that KDM and PhenoAge did not include NT-proBNP in the calculation formula, such analysis may provide additional information.

Using data from a large, nationally representative sample with comprehensive variable collection in the National Health and Nutrition Examination Surveys (NHANES), this study aimed to explore whether the relationship between proBNPage and clinical outcomes is significant after adjusting for clinical risk factors or traditional biological age indicators (KDM or PhenoAge), and this was further investigated across different subgroups. Additionally, the dose–effect relationship between proBNPage and outcomes was tested. NHANES, as a nationally representative dataset, addresses the limitation of generalisability by encompassing a diverse population sample, making the findings more applicable to broader populations. Furthermore, its standardised interviews and laboratory assessments allow for comprehensive adjustment of potentially confounding variables, thereby reducing the risk of bias and enhancing the robustness of our results.

METHODS

Study population

This study analysed data from the adults participating in NHANES conducted in 1999–2004. These years were chosen because they provide useable NT-proBNP data for proBNPage calculation. Since minors (under 20 years of age) did not undergo blood biochemical and NT-proBNP tests, only adults were chosen in this study. Using mobile examination centres to ensure a representative sample of the population across various locations in the United States, NHANES researchers' recruitment involved a comprehensive assessment of participants' health and nutritional status through interviews, physical examinations and laboratory tests. The follow-up status (mortality) of participants was obtained from the national death index database.

We opted for complete-case analysis rather than imputation due to concerns about the potential bias introduced by imputing critical covariates and the lack of clear guidance on how survey weights might affect imputation accuracy in NHANES. This approach ensures that all analyses are based on fully observed data, minimising the risk of misclassification. In total, 12 310 individuals with NT-proBNP information and aged over 20 years were initially eligible for inclusion. To ensure the robustness of our covariate adjustments, we excluded participants missing essential demographic or medical information (n=2328) because this information was necessary for adjusting covariates in the analysis of the relationship between proBNPage and mortality. These included participants missing data on body mass index (BMI) (n=340), blood pressure (n=426), smoking status (n=14), diagnoses of CVD, hyperlipidaemia and diabetes mellitus (n=655), history of cancer (n=13), education information (n=13) and poverty-to-income ratio (n=867). Next, we excluded individuals missing biochemical examination data, such as uric acid (UA) (n=1), C-reactive protein (CRP) (n=37), glycated haemoglobin A1C (HbA1c) (n=11) and total cholesterol (TC) (n=6). Finally, we excluded two individuals without follow-up information, resulting in the inclusion of a total of 9925 individuals (online supplemental figure S1).

Covariate assessment

Chorological age, sex, ethnicity (Mexican American, non-Hispanic black, non-Hispanic white, other Hispanic and other races), education level (less than high school, high

school or over than high school), Poverty-to-income index, smoking status (never, former or current), NT-proBNP, TC, HbA1c, UA and CRP were recorded. BMI was calculated as weight (kg)/height squared (m^2), and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.[19] Hypertension, diabetes mellitus, hyperlipidaemia, cancer and CVD history (including angina, coronary heart disease, congestive heart failure or stroke) were defined by participants' self-report. Most of these covariates were considered as potentially confounding the association between proBNP and mortality, and some of these variables were included in the calculation of KDM and PhenoAge.

Measurement of biological age

The biological age was estimated by proBNPage, KDM and PhenoAge in different ways.

proBNPage was calculated with NT-proBNP, and the formulas were presented as follows[17]:

Male: proBNPage= [log (NT-proBNP)+1.2068]/0.0827.

Female: proBNPage= [log (NT-proBNP) – 1.5258]/0.0478.

KDM was calculated using eight biomarkers, including CRP, serum creatinine, haemoglobin, serum albumin, serum TC, serum urea nitrogen, serum alkaline phosphatase (ALP) and systolic blood pressure. The counts of biomarkers and samples were denoted by j and i values, respectively. The slope, intercept and root mean square error of the biomarkers' regression against chronological age are represented by k, q and s values, respectively. The variance explained by the regression of biomarkers against chronological age is depicted as rj^2. The formula is presented as follows[10]:

$$KDM_E = \frac{\sum_{j=1}^{m}\left(x_j, -, q_j\right)\left(\frac{k_j}{s_j^2}\right)}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j^2}\right)^2}$$

$$r_{char} = \frac{\sum_{j=1}^{m}\frac{r_j^2}{\sqrt{1-r_j^2}}}{\sum_{j=1}^{m}\frac{r_j}{\sqrt{1-r_j^2}}}$$

$$s_{KDM}^2 = \frac{\sum_{j=1}^{n}\left(\left(KDM_{Ei}, -, CA_i\right), -, \frac{\sum_{i=1}^{n}\left(KDM_{Ei}, -, CA_i\right)}{n}\right)}{n} - \left(\frac{1-r_{char}^2}{r_{char}^2}\right) \times \left(\frac{\left(CA_{max}, -, CA_{min}\right)^2}{12m}\right)$$

$$KDM = \frac{\sum_{j=1}^{m}\left(x_j, -, q_j\right)\left(\frac{k_j}{s_j^2}\right) + \frac{CA}{s_{KDM}^2}}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j^2}\right)^2 + \frac{1}{s_{KDM}^2}}$$

CA indicates chronological age.

PhenoAge was calculated by serum albumin, creatinine, glucose, ALP, CRP, lymphocyte percentage, mean cell volume, erythrocyte distribution width, leucocyte count and chronological age. The formula is presented as follows[11]:

$$\text{PhenoAge} = 141.50 + \frac{\ln\left[-0.00553\ln\left(\exp\left(\frac{-1.51714\times exp(xb)}{0.0076927}\right)\right)\right]}{0.09165}$$

Detailed information on the measurement of biological age, including the sample collection procedure, relevant lab analysis and limits of detection is provided in the online supplemental material for the methods.

Outcome ascertainment and follow-up

All-cause mortality was determined by the record in NHANES. We used the International Classification of Diseases 10th revision codes and a probabilistic record matching method with the National Death Index to identify cases as suggested elsewhere.[20 21] Cardiovascular mortality was recorded using the codes I60-I69, I00-I09, I11, I13 and I20-I51. Follow-up continued until December 31, 2019.

Statistical analysis

Weighting was performed for statistical analysis based on the NHANES guidelines. Continuous variables were summarised by means and SE or median with IQR according to the distribution of variables. Categorical variables were summarised by unweighted number and percentage (%).

To facilitate the comparison among different proBNPage levels (indicating low-, intermediate- and high-risk populations), we chose tertile categorisation for proBNPage. After that, the time-to-event analysis was summarised by Kaplan–Meier estimates and analysed by the log-rank test. To investigate whether the relationship between proBNPage and mortality remained significant after adjusting for risk factors, KDM or PhenoAge, HR with 95% CI was calculated by multivariate Cox regressions. The Schoenfeld residual test indicated that the proportional hazards assumption holds (all tests, p>0.05). To minimise the risk of confounding bias, risk factors including chronological age, sex, ethnicity, BMI, education level, eGFR, CRP, UA, smoking status, poverty-to-income ratio and self-reported histories of hypertension, hyperlipidaemia, diabetes mellitus, CVD and cancer were adjusted in Model 1. To further elucidate the impact of the proBNPage formula, model 2 was adjusted for NT-proBNP in addition to the variables included in model 1. To explore whether the association between proBNPage and outcomes is significant after adjusting for traditional biological age indicators, we adjusted KDM and PhenoAge in models 3 and 4. Finally, to analyse the impact of the proBNPage formula in these contexts, NT-proBNP was added to models 3 and 4, with the results reported in models 5 and 6, respectively.

Adjusted restricted cubic spline (RCS) analysis based on multivariate Cox regressions was performed to examine whether there was a non-linear relationship between variables. If a non-linear relationship was detected, the inflection point was determined by assessing all possible values and selecting the one that produces the highest

likelihood. Using this identified inflection point, we conducted a two piece-wise Cox proportional hazards regression, following established recommendations.[22]

To explore whether the association was modified by subgroup characteristics, we conducted a stratification analysis to assess potential heterogeneity in the associations across predefined subgroups. Subgroups including chronological age stratification (<65 or ≥65 years), sex, ethnicity, smoking status, education level, hypertension, hyperlipidaemia, diabetes mellitus and CVD were constructed, and potential interaction effects were tested. Except for chronological age, the variables used for stratification were not adjusted in the models. Risk factors including NT-proBNP were used for the adjustment in model 1. KDM plus NT-proBNP and PhenoAge plus NT-proBNP were used for the adjustment in models 2 and 3, respectively. This approach complements our primary multivariable-adjusted analysis and aims to identify clinically meaningful variations in the associations.

For all analyses, a two-tailed p<0.05 was considered statistically significant. Statistical analyses were performed by R software (Version 4.2.1).

Patient and public involvement

None.

RESULTS

Baseline characteristics of the study population

A total of 9 925 participants, with a median age of 44 (IQR, 33–57; range, 20–85) years were included in the current study (online supplemental figure S1). The median with IQR of proBNPage, KDM and PhenoAge were 56.70 (38.32–68.39), 43.30 (31.47–57.41) and 38.76 (26.85–52.22) years, respectively. The baseline characteristics based on the tertials of proBNPage are presented in table 1, and the distribution of all these three biological age estimation indexes is shown in online supplemental figure S2.

Association of proBNPage with mortality outcomes

During a median follow-up time of 16.91 (IQR, 15.16–18.67) years, 2 799 all-cause mortality and 899 cardiovascular mortality events occurred. A stepwise increase in both all-cause and cardiovascular mortality rates was observed with increasing proBNPage (online supplemental figure S3, Log-rank, p<0.01). After adjusting the p value by the Bonferroni–Hochberg (BH) method, a significant difference was found between any two groups (online supplemental table S1).

To explore whether the association between proBNPage and outcome is evident after adjusting for established risk factors, Cox analysis was applied. The data in table 2 show a significant relationship between proBNPage and all-cause and cardiovascular mortality events (crude model, HR 1.51, 95% CI 1.47 to 1.56 for all-cause mortality; HR 1.63, 95% CI 1.55 to 1.72 for cardiovascular mortality). After adjusting for risk factors, there was still a significant relationship between proBNPage and all-cause and cardiovascular mortality events (model 1, HR 1.14, 95% CI 1.11 to 1.17 for all-cause mortality; HR 1.21, 95% CI 1.16 to 1.27 for cardiovascular mortality). To better describe the impact of proBNPage formula, NT-proBNP was adjusted. The results showed that the contribution of proBNPage remained consistent. (model 2, HR 1.14, 95% CI 1.10 to 1.17 for all-cause mortality; HR 1.20, 95% CI 1.14 to 1.27 for cardiovascular mortality).

We further investigated whether the association between proBNPage and outcome is significant after adjusting for KDM or PhenoAge. The results indicated that the relationship between proBNPage and all-cause and cardiovascular mortality events remained statistically significant after adjusting for either KDM (model 3, HR 1.19, 95% CI 1.16 to 1.23 for all-cause mortality; HR 1.30, 95% CI 1.24 to 1.37 for cardiovascular mortality) or PhenoAge (model 4, HR 1.10, 95% CI 1.04 to 1.16 for all-cause mortality; HR 1.17, 95% CI 1.08 to 1.27 for cardiovascular mortality). Further adjustment for NT-proBNP in these models did not have a significant effect on the results (adjusting for KDM, model 5, HR 1.19, 95% CI 1.15 to 1.23 for all-cause mortality; HR 1.31, 95% CI 1.22 to1.41 for cardiovascular mortality; adjusting for PhenoAge, model 6, HR 1.21, 95% CI 1.16 to 1.28 for all-cause mortality; HR 1.35, 95% CI 1.24 to 1.47 for cardiovascular mortality).

Dose–response relationship between proBNPage and mortality outcomes

As demonstrated in figure 1, RCS analysis revealed a non-linear correlation between proBNPage and both all-cause and cardiovascular mortality events after adjusting for risk factors, KDM or PhenoAge (NT-proBNP was added in these models, *P* for non-linear <0.05). The inflection points were 30.50, 44.21 and 41.21 for all-cause mortality and 59.12, 56.67 and 54.21 for cardiovascular mortality, respectively.

Using these inflection points, two-piecewise Cox regression analysis was constructed. As presented in online supplemental tables S2 and S3, the results revealed that before proBNPage reached the inflection point, the risk for all-cause mortality tended to decrease, but this trend was not statistically significant. After proBNPage exceeded the inflection point, the risk for all-cause mortality increased significantly as proBNPage increased, regardless of adjustment for risk factors (model 1, HR 1.01, 95% CI 1.01 to 1.02), KDM plus NT-proBNP (model 2, HR 1.33, 95% CI 1.27 to 1.39), or PhenoAge plus NT-proBNP (model 3, HR 1.33, 95% CI 1.23 to 1.45). Similarly, the risk of cardiovascular mortality did not reach statistical significance until proBNPage crossed the inflection point. After that, it rose with increasing proBNPage value, regardless of adjustment for risk factors (model 1, HR 1.22, 95% CI 1.17 to 1.27), KDM plus NT-proBNP (model 2, HR 1.42, 95% CI 1.36 to 1.48), or PhenoAge plus NT-proBNP (model 3, HR 1.40, 95% CI 1.27 to 1.54).

Table 1 Baseline characteristics of the study population according to proBNPage tertials

Variable	Tertial 1 (–5.47 to 48.75)	Tertial 2 (48.76 to 64.70)	Tertial 3 (64.71 to 185.50)
proBNPage, years	31.35 (19.96, 40.86)	58.04 (53.94, 61.54)	73.56 (68.51, 81.64)
KDM, years	37.09 (28.67, 47.16)	41.77 (30.64, 53.72)	57.16 (39.78, 71.12)
PhenoAge, years	32.19 (22.81, 42.76)	37.00 (26.16, 48.24)	51.50 (35.66, 66.78)
Chronological age (years)	38.00 (29.00, 47.00)	43.00 (33.00, 54.00)	57.00 (43.00, 71.00)
Sex (female)	383 (9.64%)	1926 (70.72%)	2524 (77.29%)
BMI	28.27±0.09	28.04±0.18	27.71±0.16
Race/ethnicity			
Mexican American	885 (9.69%)	674 (7.12%)	668 (4.04%)
Non-Hispanic black	754 (11.68%)	504 (9.37%)	471 (6.53%)
Non-Hispanic white	1463 (66.85%)	1419 (73.45%)	2335 (82.02%)
Other Hispanic	176 (6.76%)	123 (5.43%)	134 (4.37%)
Other races	133 (5.02%)	101 (4.63%)	85 (3.04%)
Education level, %			
<High school	987 (17.83%)	835 (17.44%)	1272 (22.01%)
High school	818 (25.70%)	667 (25.98%)	894 (27.26%)
> High school	1606 (56.48%)	1319 (56.58%)	1527 (50.73%)
Poverty-to-income, %	3.13±0.06	3.00±0.06	2.93±0.07
Smoking status			
Former	802 (22.52%)	654 (22.71%)	1250 (30.62%)
Never	1647 (48.89%)	1519 (51.74%)	1810 (49.04%)
Current	962 (28.59%)	648 (25.55%)	633 (20.34%)
SBP, mmHg	120.61±0.37	119.22±0.40	128.63±0.61
DBP, mmHg	74.18±0.28	72.59±0.30	70.17±0.33
NT-proBNP, pg/mL	19.81±0.30	47.81±0.44	319.22±14.29
TC, mg/dL	203.20±0.96	200.95±0.91	202.28±0.94
HbA1c, %	5.43±0.02	5.41±0.02	5.52±0.02
Albumin, g/dL	4.49±0.01	4.32±0.01	4.23±0.01
ALP, μ/L	72.90±0.89	69.50±0.73	71.34±0.79
BUN, mg/dL	13.50±0.10	12.45±0.15	14.50±0.14
eGFR, mL/min/1.73 m2	99.47±0.43	97.39±0.45	82.96±0.58
UA, μmol/L	354.46±1.80	296.15±1.72	303.72±1.86
CRP, mg/dL	0.32±0.01	0.42±0.02	0.51±0.01
Hypertension	711 (19.62%)	782 (23.67%)	1715 (39.44%)
Diabetes mellitus	216 (4.63%)	229 (5.21%)	508 (9.34%)
Established CVD	121 (3.24%)	191 (5.29%)	814 (17.42%)
Malignancy history	115 (3.48%)	205 (7.11%)	565 (14.79%)
Hyperlipidaemia	2455 (72.59%)	2058 (71.49%)	2892 (75.2%)

ALK, alkaline phosphatase; BMI, body mass index; BUN, blood urea nitrogen; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin A1c; KDM, Klemera and Doubal method; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PhenoAge, phenotypic age; SBP, systolic blood pressure; TC, total cholesterol; UA, uric acid.

Subgroup analysis

We first tested the association between proBNPage and all-cause mortality in subgroups. As presented in figure 2, after adjusting for risk factors (including NT-proBNP), the association of proBNPage with all-cause mortality was statistically significant in most subgroups (model 1), except for Mexican Americans, other Hispanics, individuals of other ethnicities and those without hyperlipidaemia. In addition, we included KDM plus NT-proBNP (model 2) and PhenoAge plus NT-proBNP (model 3) as

Table 2 Association of proBNPage with all-cause and cardiovascular mortality events summarised by the Cox model

	All-cause mortality		Cardiovascular mortality	
Model	HR (95% CI)	P value	HR (95% CI)	P value
Crude model	1.51 (1.47 to 1.56)	<0.001	1.63 (1.55 to 1.72)	<0.001
Model 1	1.14 (1.11 to 1.17)	<0.001	1.21 (1.16 to 1.27)	<0.001
Model 2	1.14 (1.10 to 1.17)	<0.001	1.20 (1.14 to 1.27)	<0.001
Model 3	1.19 (1.16 to 1.23)	<0.001	1.30 (1.24 to 1.37)	<0.001
Model 4	1.10 (1.04 to 1.16)	<0.001	1.17 (1.08 to 1.27)	<0.001
Model 5	1.19 (1.15 to 1.23)	<0.001	1.31 (1.22 to 1.41)	<0.001
Model 6	1.21 (1.16 to 1.28)	<0.001	1.35 (1.24 to 1.47)	<0.001

Model 1 was adjusted for chronological age, sex, ethnicity, BMI, education level, eGFR, CRP, UA, smoking status, poverty-to-income index, self-report hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease and cancer. Model 2 was additionally adjusted for NT-proBNP based on model 1. Model 3 was adjusted for KDM.
Model 4 was adjusted for PhenoAge. Model 5 was additionally adjusted for NT-proBNP based on model 3. Model 6 additionally adjusted for NT-proBNP based on model 4.
HR indicates the change in all-cause or cardiovascular mortality for every 10-year variation in proBNPage, which represents the change in the risk of all-cause or cardiovascular mortality associated with a 10-year increase in the proBNPage value.
BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KDM, Klemera and Doubal method.

confounders to examine the association of proBNPage with all-cause mortality in subgroups. The results showed that the association was statistically significant in almost all of the subgroups, with the exception of the other ethnicity group.

The association of proBNPage with cardiovascular mortality was further tested in subgroups. As demonstrated in figure 3, after adjusting for risk factors (model 1), except for the other Hispanic and other ethnicity, proBNPage was still linked to cardiovascular mortality. Additional analysis adjusted for KDM plus NT-proBNP (model 2) revealed that only individuals in the other ethnicity subgroup did not exert a significant relationship between proBNPage and cardiovascular mortality. Meanwhile, the association was compromised in Mexican Americans, other Hispanics and individuals without hyperlipidaemia when adjusted for PhenoAge plus NT-proBNP (model 3).

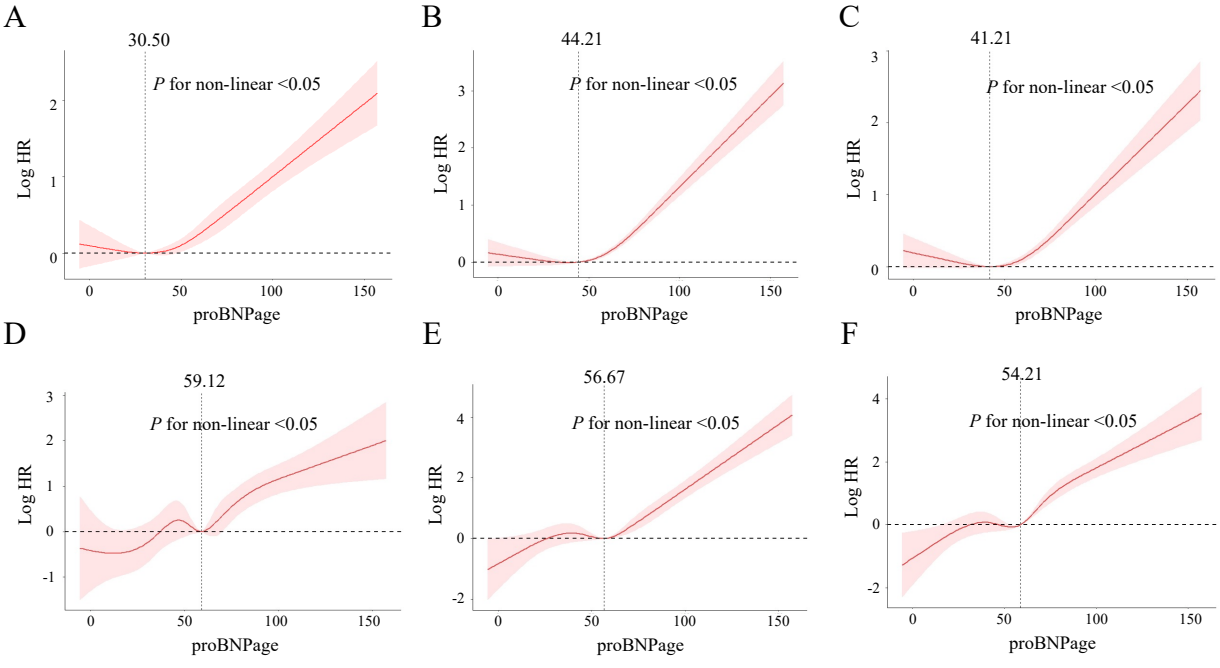

Figure 1 Restricted cubic spline fitting for the association between proBNPage and all-cause (A–C), cardiovascular mortality (D–F). A and D, adjusted for risk factors, including chronological age, sex, ethnicity, BMI, education level, eGFR, CRP, UA, smoking status, poverty to income index, NT-proBNP, self-report hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease, and cancer; B and E, adjusted for KDM and NT-proBNP; C and F, adjusted for PhenoAge and NT-proBNP

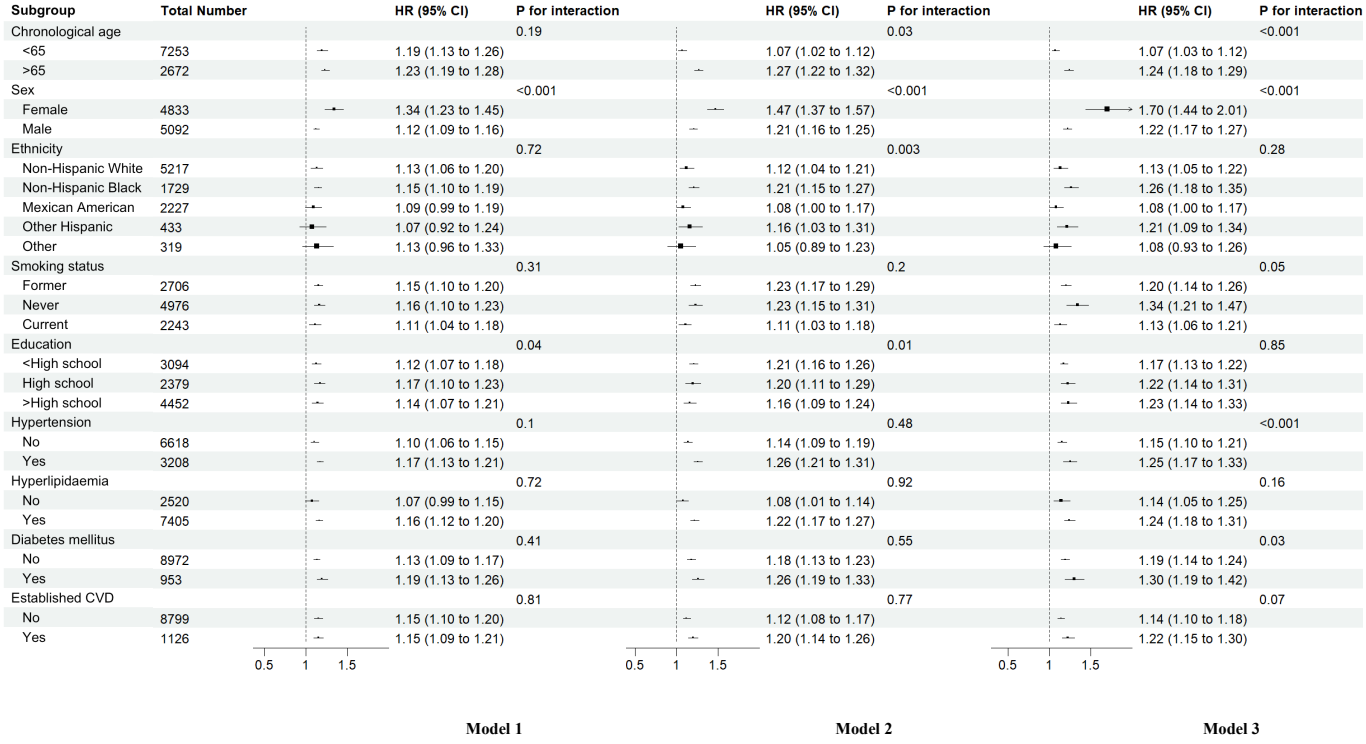

Figure 2 Forest plot for the subgroup analysis of the association of proBNPage with all-cause mortality. Model 1 was adjusted for risk factors, including chronological age, sex, ethnicity, BMI, education level, eGFR, CRP, UA, smoking status, poverty-to-income index, NT-proBNP, self-report hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease and cancer. The variables used for the stratification were not adjusted in the model, except for chronological age. Model 2 was adjusted for KDM and NT-proBNP. Model 3 was adjusted for PhenoAge and NT-proBNP. BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KDM, Klemera and Doubal method; NT-proBNP, N-terminal pro B-type natriuretic peptide; UA, uric acid.

DISCUSSION

Main results

This study observed that proBNPage is significantly associated with all-cause and cardiovascular mortality in the general population, after adjusting for clinical risk factors and traditional biological age estimation indexes. Further analysis showed a non-linear relationship between proBNPage and outcomes with inflection points (for all-cause mortality, the inflection points were 30.50 after adjusting for risk factors, 44.21 after adjusting for KDM and NT-proBNP and 41.21 after adjusting for PhenoAge and NT-proBNP; for cardiovascular mortality, the inflection points were 59.12 after adjusting for risk factors, 56.67 after adjusting for KDM and NT-proBNP and 54.21 after adjusting for PhenoAge and NT-proBNP). Notably, when proBNPage is low, its increase does not lead to significant changes in all-cause or cardiovascular mortality; however, an increase in proBNPage beyond the inflection point will significantly increase clinical risks, demonstrating that proBNPage is a novel index to estimate biological age.

The concept of biological age is used to identify an individual's overall health status and predict their lifespan, which is reflected by DNA methylation[23 24] and telomere length.[25] However, DNA methylation and telomere length are not routinely measurable. Thus, to better measure biological age in clinical settings, scholars have studied the relationship between them and various clinical variables. Based on the results, alternative formulas for estimating biological age have been proposed. Among these, the applications of KDM and PhenoAge are the most extensive. Both of them require conducting multiple blood tests, and the formula is difficult to use to some extent. Moreover, KDM and PhenoAge do not consider the effect of NT-proBNP on the ageing process. Considering the great contribution of CVD in ageing-related outcomes[26 27] and the value of NT-proBNP on cardiac function assessment[28 29] as well as CVD[30–32] and non-CVD prognoses,[33–35] NT-proBNP may serve as a biomarker reflecting systemic ageing processes. Specifically, high NT-proBNP levels are associated with cardiac stress, endothelial dysfunction and systemic inflammation—key pathways implicated in ageing and mortality risk.[36–38] Building on this, proBNPage, a novel biological age estimator based on NT-proBNP, has been proposed. This index correlates not only with clinical outcomes[17] but also with ageing-related diseases.[18] However, the results of these studies were derived from a community-based elderly population (72.8±5.5 years), and the participants were mainly of white ethnicity. Therefore, we cannot be certain whether this conclusion can be extrapolated to other populations; thus, further confirmation is necessary. Furthermore, we

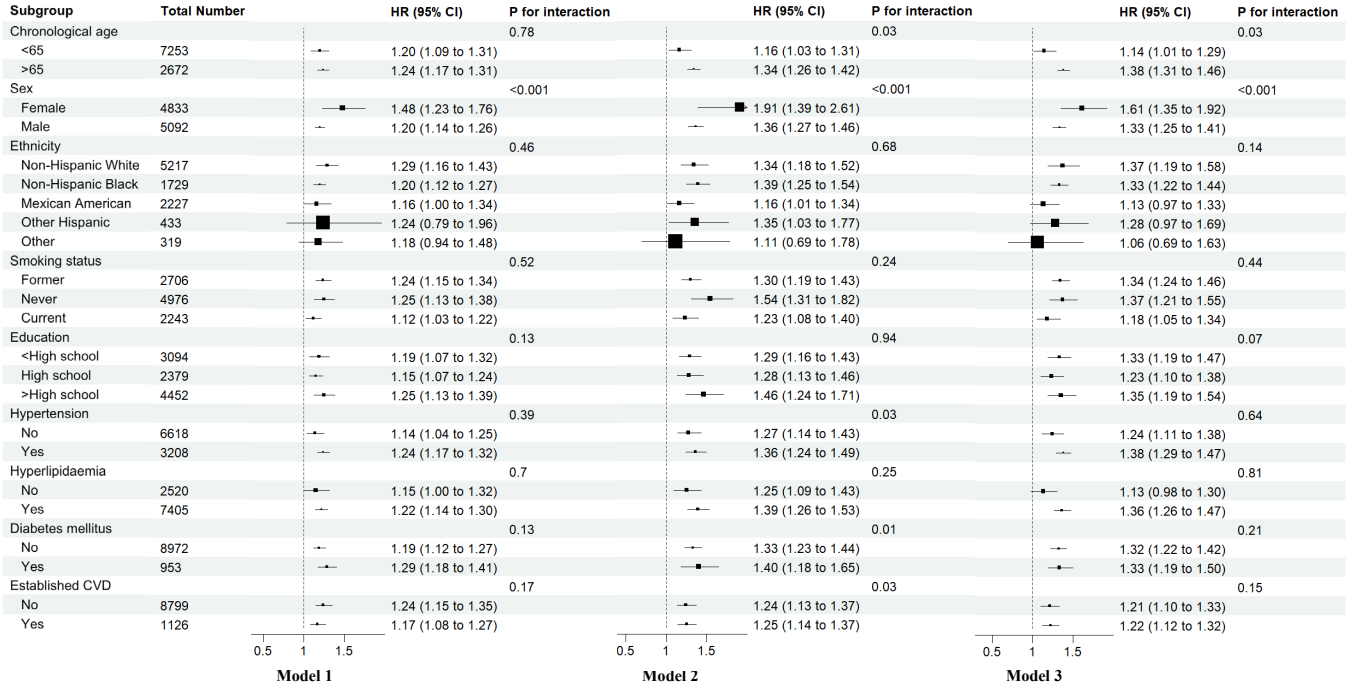

Figure 3 Forest plot for the subgroup analysis of the association of proBNPage with cardiovascular mortality. Model 1 was adjusted for risk factors, including chronological age, sex, ethnicity, BMI, education level, eGFR, CRP, UA, smoking status, poverty-to-income index, NT-proBNP, self-report hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease and cancer. The variables used for stratification were not adjusted in the model, except for chronological age. Model 2 was adjusted for KDM and NT-proBNP. Model 3 was adjusted for PhenoAge and NT-proBNP. BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; KDM, Klemera and Doubal method; NT-proBNP, N-terminal pro B-type natriuretic peptide; UA, uric acid.

still do not know whether the association of proBNPage with prognosis is significant after adjusting for traditional biological age indexes, namely, KDM and PhenoAge.

proBNPage is significantly associated with mortality

In the present study, using a nationwide representative sample in the United States, we found that the association of proBNPage with all-cause mortality, as well as cardiovascular mortality, remains significant after adjusting for risk factors and traditional biological age indexes. Interestingly, our findings suggested a non-linear relationship between proBNPage and all-cause mortality, with a significant increase in risk only after proBNPage exceeds a critical inflection point. While the exact biological basis for this inflection remains to be fully elucidated, it may reflect a threshold of proBNPage-associated pathologies, such as irreversible cardiac dysfunction and systemic inflammation.[36–38] It may also reflect a transition in the ageing process, where the cumulative impact of proBNPage becomes clinically significant only after surpassing a critical level. Future studies integrating longitudinal biomarker data and experimental models are needed to clarify these mechanisms.

The association of proBNPage with mortality was further confirmed in most of the subgroups, indicating that proBNPage has significant importance in the assessment of future ageing-related events. The association remains similar in younger individuals, suggesting proBNPage elevation provides useful information in the whole lifecycle. Apart from that, only the white and black subgroups achieve favourable results across all models, whereas variations in proBNPage are not significantly associated with prognosis among other racial groups, particularly among minority populations (categorised as "others"). Although previous research evidence indicates common characteristics in the ageing process among different racial groups,[39 40] there are significant differences in the biological age characteristics between different races,[41 42] as well as their associations with ageing-related events.[42 43] These differences may be related to various factors, including genetics,[44 45] environment,[46] lifestyle[45] and socioeconomics.[47] Thus, conducting more in-depth research on the biological age characteristics and their associations with ageing-related events among different ethnicities is of great significance for developing effective prevention and treatment strategies.[48] Besides, the difference in NT-proBNP levels among different racial populations was reported as significant,[49] and the proBNPage derived from Caucasians may not be sufficient to fully represent biological ageing. In the future, a revised proBNPage based on the changes in NT-proBNP characteristics in different

ethnicities may perform better in predicting age-related outcomes.

Strengths and limitations

To the best of our knowledge, this is the first study to uncover a significant relationship between proBNPage and all-cause mortality, as well as cardiovascular mortality. Our results revealed a non-linear relationship with an inflection point between proBNPage and outcomes, indicating a clear cut-off value for risk stratifying for proBNPage.

Nonetheless, we must acknowledge several limitations. First, we did not assess other ageing-related events beyond all-cause and cardiovascular mortality, and the study was conducted in a single cohort in the United States. Future studies are needed to validate these findings in diverse populations with multiple outcome measures to ensure their generalisability. Second, the inability to perform repeated measurements of NT-proBNP in the dataset limits our ability to determine whether changes in proBNPage affect clinical outcomes. Moreover, constrained by the nature of observational studies, we cannot determine a causal relationship between proBNPage and clinical outcomes. Third, we are unable to accurately assess the effect of unmeasured potential confounders on the study findings, and measurement error of laboratory tests, as well as residual confounding due to measurement error, may introduce information bias. Despite these limitations, this study still provides valuable information regarding proBNPage and the occurrence of adverse events.

CONCLUSIONS

proBNPage was positively associated with all-cause and cardiovascular mortality, and this association remains significant after adjustment for covariates or traditional biological age estimation indexes. The dose–response analysis showed a non-linear relationship between proBNPage and outcomes with an inflection point, indicating a cut-off value for risk stratification based on proBNPage. These results suggest that proBNPage is a useful surrogate for biological age estimation.

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