

# **Serum Beta-2 Microglobulin Concentration Predicts Cardiovascular and All-cause Mortality**

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## **Main Text**

Beta-2 microglobulin (B2M) is the light chain in the major histocompatibility complex (MHC) Class 1 molecule [1], which is present in all nucleated cells. It has been suggested as an initiator of inflammation [2], and is an early marker of diseases related to inflammation such as cardiovascular and renal disease [3, 4]. B2M is renally excreted and so the serum level increases in renal dysfunction. We and others have reported that serum B2M level predicts mortality in certain patient populations [3, 5-9]. We therefore examined whether serum B2M level predicted cardiovascular and all-cause mortality in a large nationally representative cohort.

Data from the Third National Health and Nutrition Examination Survey (NHANES III) were used [10]. NHANES III was conducted by the National Center for Health Statistics from 1988 to 1994 using a stratified multistage probability sample which represented the civilian non-institutionalized U.S. population. Participants gave written consent before participation, and ethical approval was obtained from the Human Subjects Committee of the U.S. Department of Health and Human Services. Detailed study designs and measurements are provided in Supplementary Appendix.

The median B2M level in study participants was 2.01 mg/L (IQR=1.66-2.55). Supplementary Table 1 shows their characteristics. During a median follow-up of 13.5 years (range 0.1 to 18.2 years) and 79,528 person-years, 1,150 and 2,524 participants died from cardiovascular causes and all causes, respectively. Higher B2M levels were associated with cardiovascular and all-cause mortality. Kaplan-Meier survival curves showed that quartile 4 had a much higher mortality compared to the other quartiles (log-rank test <0.001; Figure 1a and 1b). Moreover, the survival curves diverged from

the beginning and continued through 18.2 years.

The results from Cox-regression analysis are shown in Supplementary Table 2. For all-cause mortality, a 1-unit increase of B2M was associated with HR of 1.89 (95% CI: 1.79-2) for crude model, 1.38 (95% CI: 1.32-1.44) after adjustment for lifestyle factors, and 1.45 (95% CI: 1.35-1.56) after further adjustment for cardiometabolic risk factors. In multicategorical and threshold models, participants in highest quartile of B2M were at higher risk for all-cause mortality compared to quartile 1 and quartile 1 through 3, respectively (Models 1-3, Supplementary Table 2). Examination of regression splines suggests an almost linear relationship between serum B2M and mortality risk (Figure 1c). Adjustment for additional renal function biomarkers (Supplementary Table 3) or excluding participants who died during first 2 years of follow-up (Supplementary Table 4) showed similar findings. The association of B2M level with all-cause mortality was consistent in selected subgroups (Figure 2). The HR was higher in those younger than 65 years of age and lower in those with less than high school education.

For cardiovascular mortality, a 1-unit increase of B2M was associated with HR of 1.93 (95% CI: 1.8-2.08) for the crude model, 1.4 (95% CI: 1.29-1.52) after adjustment for lifestyle factors, and 1.46 (95% CI: 1.31-1.64) after further adjustment for cardiometabolic risk factors. In multicategorical and threshold models, participants with the highest quartile of B2M were at higher risk of cardiovascular mortality compared with quartile 1 and quartile 1 through 3, respectively (Models 1-3, Supplementary Table 2). Examination of regression splines suggests an almost linear relationship between serum B2M and mortality (Figure 1d). Sensitivity analyses

(Supplementary Tables 3 and 4) showed similar findings, except that the association became insignificant after further adjustment for additional renal function biomarkers in the multicategorical model. Association of B2M level with cardiovascular mortality was consistent in the subgroups examined, except in the subgroups of participants with education level lower than high school, diabetes, hypertension, and current smoker (Figure 2).

Thus, the main finding of this study was that serum B2M concentration predicted cardiovascular and all-cause mortality in a large cohort representative of the American general population. This was independent of renal function, cancer, history of heart disease, and cardiometabolic and mortality risk factors.

The association of B2M with mortality has been noted before [3, 5-9]. In our recent study, we showed that B2M is a predictor of mortality in diabetes patients [9]. As B2M reflects renal function, three studies examined its association of B2M with mortality in dialysis [6-7] or uremic [3] patients. These studies showed that B2M is a predictor of all-cause [3, 7] and infectious disease [6] mortality in people with renal impairment, although the association with cardiovascular mortality was inconsistent [3, 6]. In 1,034 individuals aged 65 or above, B2M was shown to be associated with all-cause mortality [8], with the middle (HR of 2.02 [95% CI: 1.35-3.04]) and upper (HR of 2.84 [95% CI: 1.92-4.20]) tertiles of B2M concentration being associated with higher mortality. Our study extends the finding to people younger than 65 years, in whom a single biomarker to identify at risk persons would be especially valuable. The Atherosclerosis Risk in Communities (ARIC) Study examined several biomarkers of renal dysfunction (cystatin-C, BTP, and eGFR) and found that B2M had the strongest association with all-cause mortality in the general population [5]. In our

analysis, we adjusted for renal function and showed that B2M was independently associated with cardiovascular and all-cause mortality (Supplementary Table 3).

In conclusion, serum B2M concentration is associated with a significant increased risk of cardiovascular (up to 6-fold) and all-cause (up to 4-fold) mortality. The increased risk is immediate and extends to at least 18 years, and is especially pronounced in people under the age of 65. These tantalizing findings call for validation in another cohort and a formal and preferably prospective comparison with other biomarkers predicting mortality. [?Our preliminary comparison with the Framingham Risk Score (FRS) suggested that B2M is as good as, and adds to FRS in predicting cardiovascular and all-cause mortality. [cite our abstract]] If our findings are confirmed in other populations, we may have a novel and simple way of gauging prognosis and predicting mortality.

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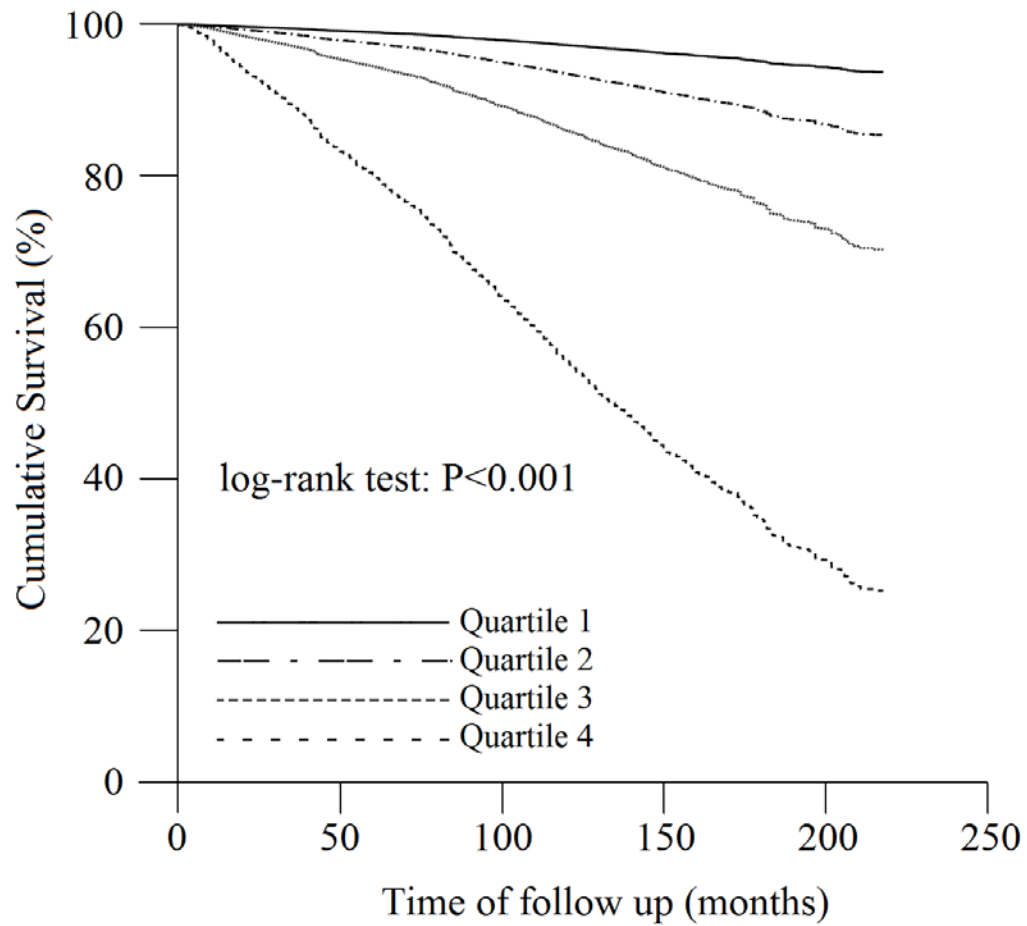
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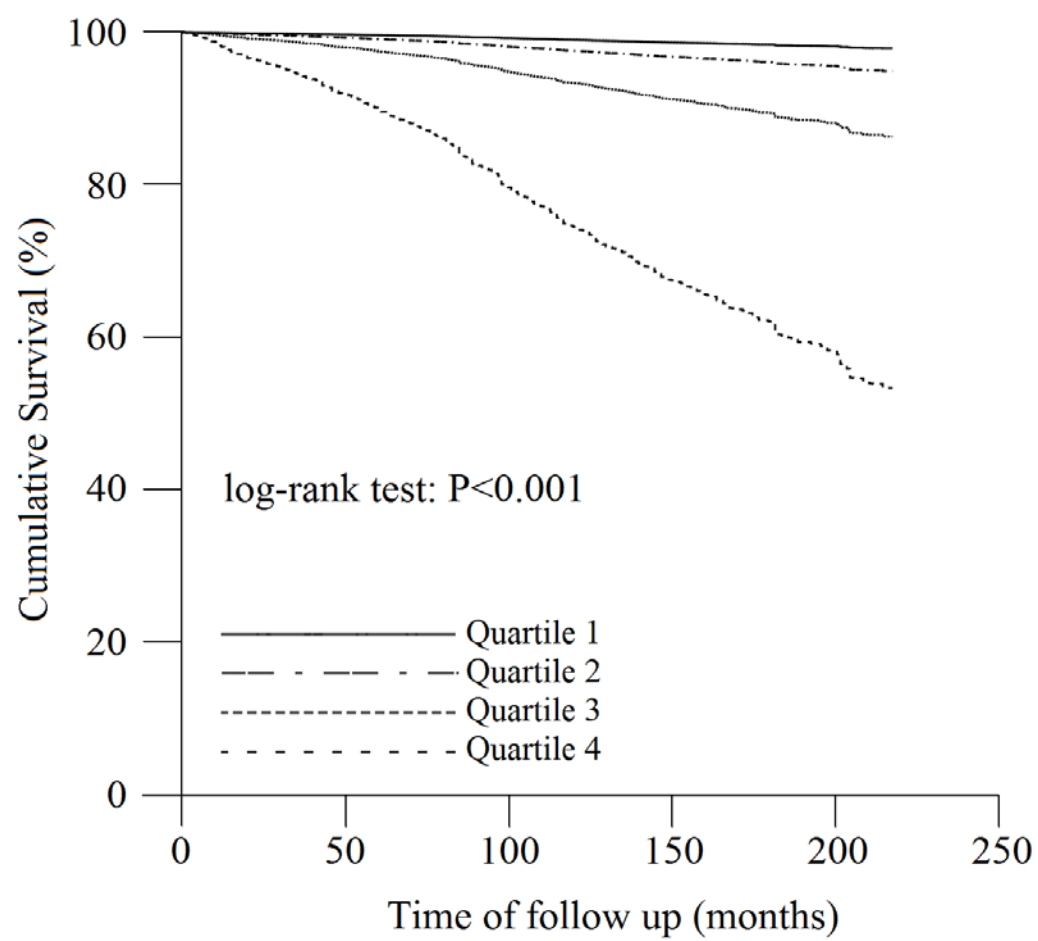
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Figure 1. Kaplan-Meier cumulative survival curves for (a) all-cause and (b) cardiovascular mortality depending on quartiles of B2M; and association between serum beta-2 microglobulin and mortality assessed via penalized regression spline (c) all-cause and (d) cardiovascular mortality.

(a)

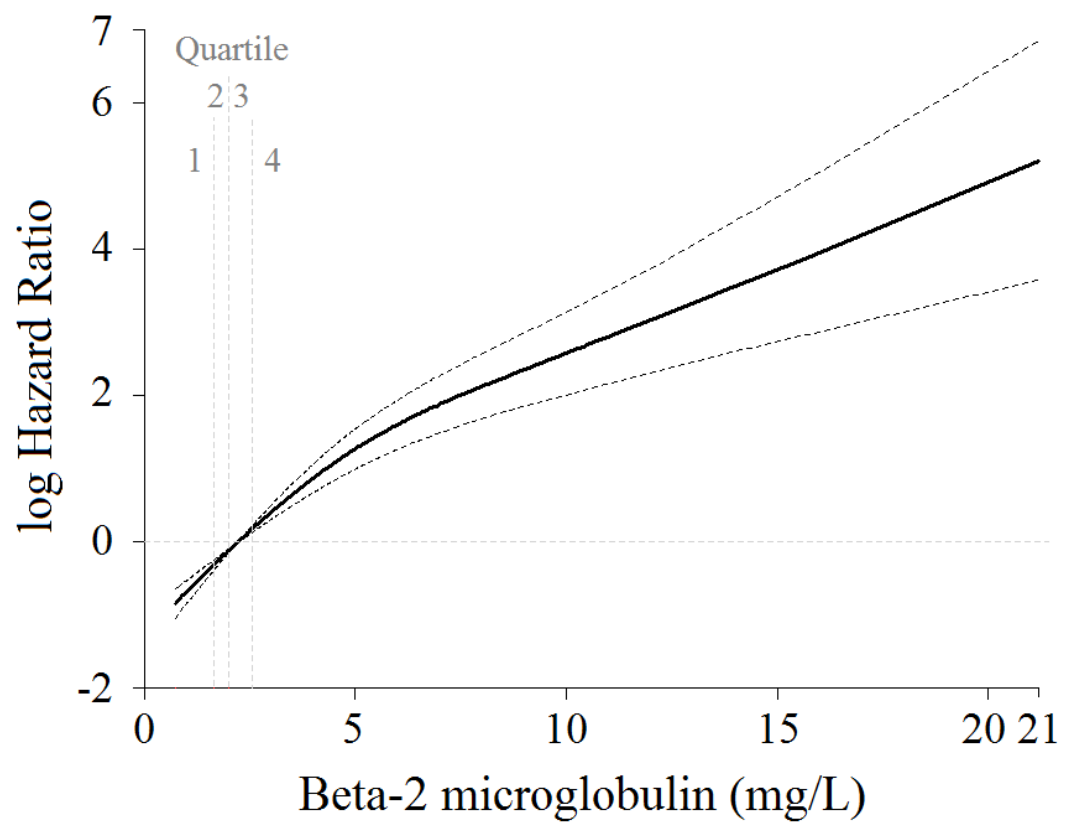


(b)





(c)



(d)

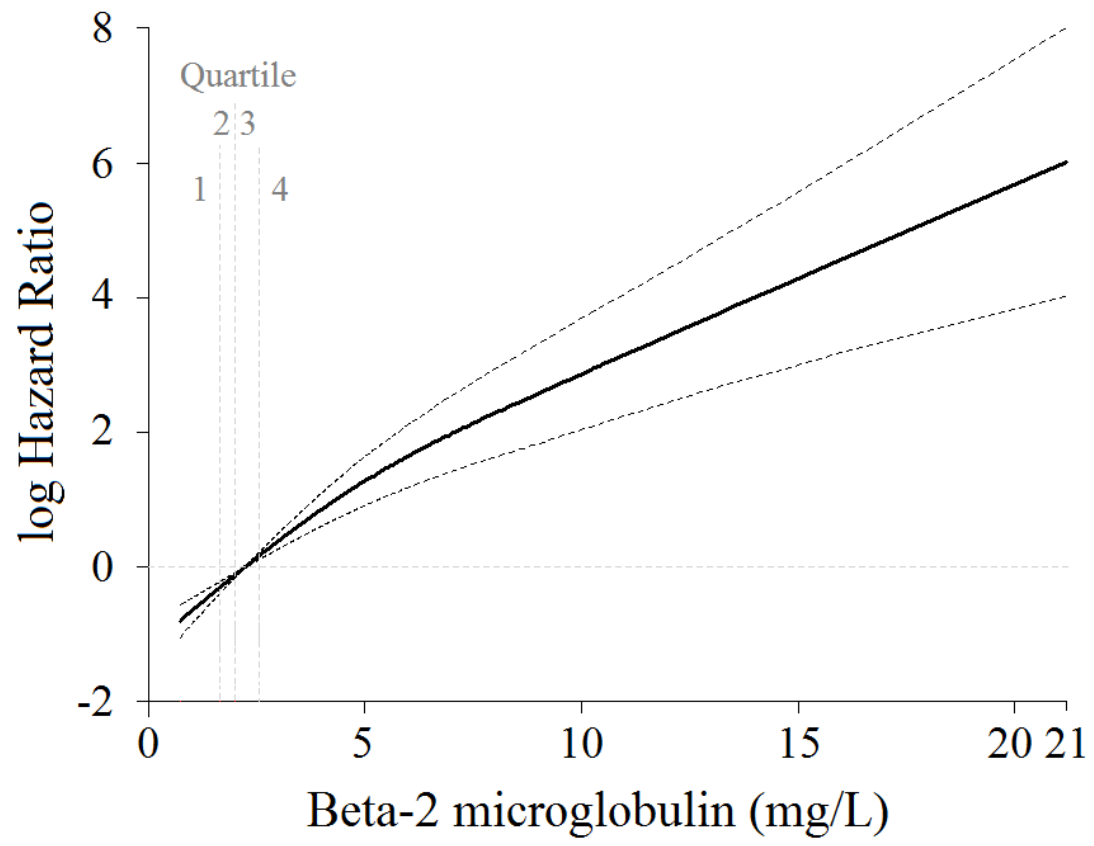
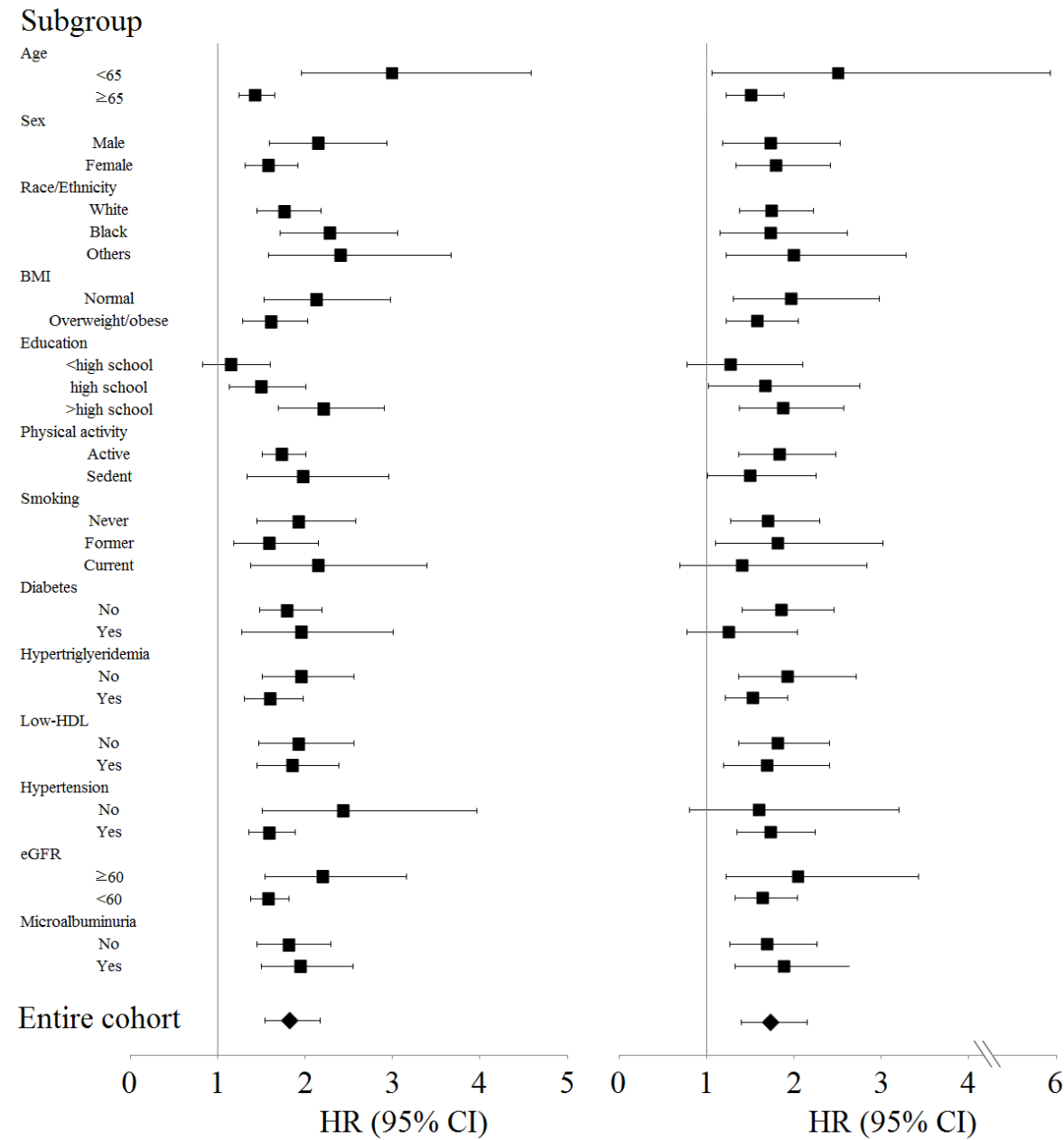


Figure 2. Adjusted hazard ratios for all-cause and cardiovascular mortality associated with beta-2 microglobulin above the median (>2.54 mg/L) in selected subgroups



BMI: body mass index; eGFR: estimated glomerular filtration rate.