Research Article

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Circulating Beta-2 Microglobulin and Risk of Cancer: The Atherosclerosis Risk in Communities Study (ARIC)

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Abstract

Background: Serum β-2 microglobulin (B2M), a major histocompatibility complex class I molecule that is a biomarker of kidney filtration and increased cell turnover, is elevated at the time of diagnosis in hematological and some solid cancers. However, serum B2M was not examined prospectively as a marker for cancer risk. We hypothesized that in a population without a prior cancer diagnosis, serum B2M is associated with risk of cancer ($n = 2,436$), including colorectal ($n = 255$), lung ($n = 298$), breast ($n = 424$), and prostate ($n = 524$) cancers, and hematological ($n = 176$) malignancies.

Methods: The analytical cohort ($n = 12,300$) was followed for incident cancers from 1990 through 2006. B2M (range, 0.9–57.8 mg/L) was measured in stored serum collected in 1990–1992. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals for cancer incidence and mortality in relation to quartiles of B2M.

Results: Adjusting for age, sex, race, center, education, body mass index, smoking, aspirin, and hormone therapy (in women) and comparing highest to lowest B2M quartiles, HRs were 1.25 (1.06–1.47; $P_{trend} = 0.002$) for total cancer risk and 2.21 (1.32–3.70; $P_{trend}=0.001$) for colorectal cancer risk, with similar HRs for colon and rectal cancers. These associations remained after adjustment for an inflammatory biomarker, C-reactive protein, and after excluding the first three years of follow-up. Significant associations were also observed for mortality from total, lung, and hematological cancers.

Conclusions: These findings provide the first evidence that higher serum B2M is associated with increased colorectal cancer risk.

Impact: This study supports B2M as a potential biomarker for colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev; 25(4); 657–64.*

Introduction

Beta-2 microglobulin (B2M) is a subunit of the major histocompatibility complex (MHC) class I molecule; it is found on the surface of all nucleated cells and is especially abundant in lymphocytes and monocytes (1). Under normal physiological conditions, some amount of B2M may be secreted into circulation from cell surfaces or as a result of intracellular release (2–4) and is removed from blood predominantly by the kidneys (5). Thus, the net concentration of serum B2M is determined by its generation and secretion into serum, as well as its elimination by the kidneys, so that serum B2M concentration increases with elevated turnover of cells and/or reduced kidney function (6). Therefore, in people without kidney disease, elevated B2M serves as a marker of altered cell proliferation. Elevated serum B2M concentrations have been observed in many pathological conditions, including renal disease, immunodeficiency, and autoimmune diseases (1, 7–9). Further, B2M concentrations have been reported as elevated at the time of diagnosis in many solid (3, 10–14) and hematological cancers (15, 16).

There is also evidence to suggest that elevated serum B2M concentrations are associated with risk of all-cause death. In a prospective cohort study of nondisabled people 65 years and older, B2M concentrations better predicted death than high sensitivity C-reactive protein (CRP), an established inflammatory marker (1). In addition, several cohort studies, including the Atherosclerosis Risk in Communities Study (ARIC; ref. 7), have reported positive associations between B2M and aging, various cardiovascular conditions, and mortality from diabetes, CVD and all causes, suggesting that serum B2M may also serve as an inflammatory biomarker (7, 17, 18).

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However, despite the evidence showing increased B2M concentrations in cancer patients, there have been no published studies that examined serum B2M in relation to the risk of subsequent cancer in the general population. Using ARIC data, we examined the risk of all cancers combined, the four most common solid cancers (colorectal, lung, prostate, and breast cancers) individually, and hematological cancers combined. We hypothesized that the strongest associations would be observed for colorectal and hematological cancers, but the mechanisms underlying these associations would be different. Given that colorectal cancer has been associated with immune response and inflammation (19–22), we reasoned that higher serum B2M would be an early biomarker associated with increased risk for future colorectal cancer. While this hypothesis might also be relevant for breast and prostate cancers (23, 24), associations between circulating immune and inflammatory markers and these cancers have been less consistently reported (23, 25) than those for colorectal cancer. In contrast, we hypothesized that B2M may serve as a marker of early detection for hematological cancers, because elevated B2M concentrations are consistently observed in multiple myeloma, malignant lymphomas, and leukemia and associated with progression and worse prognosis (4, 5, 15, 16). Similarly, B2M could be a potential early detection biomarker for lung cancer, which is often diagnosed at an advanced stage, given that B2M was reported to be higher and occur more frequently in lung cancer cases compared with unaffected controls (26).

Materials and Methods

ARIC, a multicenter population-based prospective cohort study of atherosclerosis, was initiated in 1987–1989 when 15,792 volunteers ages 45 to 64 years were recruited from 4 communities: suburban Minneapolis (MN), Forsyth County (NC), Jackson (MS), and Washington County (MD; refs. 27, 28). Baseline and four follow-up visits in 1990–1992, 1993–1995, 1996–1998, and 2011–2013 included interviews, laboratory measurements, and clinic examinations (27, 28). At each visit, participants were asked about their demographic characteristics, education, lifestyle behaviors, and reproductive and medical histories. Trained personnel collected anthropometric measures, blood (serum and plasma), and urine samples.

B2M was measured in serum samples collected at Visit 2 (93% of the living cohort was reexamined); therefore, most of the covariates shown in Table 1 and tested in the multivariable regression models were also collected at Visit 2, i.e., body mass index (BMI), waist-to-hip ratio (WHR), smoking status, pack-years, alcohol intake (g/week), use of hormone replacement therapy (HRT), and aspirin use during the 2 weeks before the examination. Education, consumption of red meat, and dietary fiber were collected at Visit 1. The analytical cohort was followed from Visit 2 until the end of 2006, for a median of 14.8 years of follow-up (maximum was 16.9 years).

Local institutional review boards approved the ARIC protocol and all participants provided their informed consent, which included permission for follow-up for the occurrence of noncardiovascular diseases.

Ascertainment of cancer incidence and mortality

Incident cancers in 1990–2006 were identified by linkage to the state cancer registries of MD, MN, MS, and NC and were supplemented by active surveillance of the cohort, which included recording of hospital discharges and hospitalizations that were self-reported during annual follow-up telephone calls (29, 30). Additional cancer cases (not reported by cancer registries) were included after hospital records were obtained, reviewed, and verified for cancer. Primary site, date of cancer diagnosis, and source of diagnostic information (e.g., pathology report) were recorded (29, 30). Data on cancer stage were not uniformly collected.

Deaths from cancer as the underlying cause were obtained from death certificates in 1990–2011. The follow-up for death was five years longer than for incidence (1990–2006), which explains the higher number of deaths from lung cancer ($n = 352$) compared with the number of incident lung cancers ($n = 298$). Also, although the inclusion of only first primary incident cancers did not affect cancer deaths, it resulted in a decrease of incident cancer cases.

Table 1. Characteristics across quartiles of serum B2M in 12,300 participants; ARIC, 1990–1992

Characteristics, mean (SD) or prevalence %	Quartiles of B2M (mg/L)			
	≤1.66	1.67–1.88	1.89–2.15	≥2.16
	$N = 3{,}063$	$N = 3{,}140$	$N = 3{,}046$	$N = 3{,}051$
Age at Visit 2 (years)	54.3 (5.1)	56.2 (5.5)	57.6 (5.6)	59.1 (5.5)
Female, %	59.0	53.2	53.3	56.1
White, %	63.7	75.4	80.2	78.5
BMI, kg/m^2	26.7 (4.8)	27.3 (5.0)	28.0 (5.3)	28.9 (5.9)
Education, %				
<High school	18.3	19.6	21.9	27.7
High school	31.4	33.1	34.3	33.8
>High school	50.4	47.3	43.8	38.6
Pack-years of smoking	12.7 (18.6)	14.4 (19.9)	15.6 (20.9)	16.7 (22.3)
Alcohol intake, g/week	6.1 (13.4)	5.6 (13.4)	5.1 (13.8)	4.4 (13.6)
Aspirin use, % yes	44.5	45.3	47.7	48.2
Diabetes, % yes	11.4	8.8	9.5	14.3
HRT use, % ever, for females	38.8	34.1	29.6	25.0
eGFR, mL/min/1.73 m^2 (SD)	110 (11)	100 (10)	92 (11)	78 (16)
Cystatin C, mg/dL (SD)	0.7 (0.1)	0.8 (0.1)	0.9 (0.1)	1.1 (0.5)
Creatinine, mg/dL (SD)	1.1 (0.2)	1.1 (0.2)	1.2 (0.2)	1.3 (0.7)
hs-CRP[a], mg/L (SD)	1.8 (1.8)	2.1 (2.1)	2.6 (2.6)	3.7 (4.0)

[a]Geometric mean for hs-CRP was calculated because of the non-normal distribution.

Measurements of biological markers

WBC count was measured in local centers at the time of the Visit 2 exams (1990–1992). Serum concentrations of B2M, high-sensitivity CRP (hs-CRP) as well as cystatin (a marker of kidney function), were measured in 2012–2013 using stored serum from Visit 2. B2M and CRP were measured using the Roche B2M reagent and Roche latex-particle enhanced immunoturbidimetric assay kits, respectively, and read on the Roche Modular P Chemistry analyzer (Roche Diagnostics). Cystatin C was measured using the Gentian immunoassay (Gentian Technology AS). Another marker of kidney function, creatinine, was measured by the modified kinetic Jaffé method (31) in 1990–1992. Estimated glomerular filtration rate (eGFR), an index of kidney function, was calculated in ARIC using standardized cystatin C and standardized creatinine (32).

The analytical sample for this analysis ($n = 12{,}300$) excluded the participants who did not consent to participate in studies of non-cardiovascular diseases ($n = 187$); were not black or white ($n = 48$); were blacks from Minneapolis and Washington County ($n = 55$); did not participate in Visit 2 ($n = 1{,}323$), self-reported history of cancer at baseline ($n = 882$); had cancer diagnosed before Visit 2 ($n = 214$), or had missing information about B2M ($n = 786$; some individuals were excluded for more than one reason).

Statistical analysis

Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for incident cancers in relation to B2M modeled as quartiles. Tests for trends were calculated by fitting quartile numbers as an ordinal variable into the Cox regression model.

The proportional hazards assumption was tested by adding an interaction term between follow-up time and B2M concentration, and the assumption was not violated for any individual cancer or total cancer.

For each cancer, we analyzed two multivariable-adjusted models. Model 1 was adjusted for age (continuous), sex, and a 5-level variable combining race and ARIC field center. Model 2 was additionally adjusted for education (less than high school, high school, more than high school), BMI (<25, 25–29.9, $\geq$30 kg/m^2), smoking status (yes, no) and pack-years of smoking (continuous), use of aspirin (yes, no), sex-HRT variable (men, women never taking HRT, and women who were former or current HRT users), and eGFR (continuous). We adjusted for eGFR for two reasons: (i) serum B2M concentration depends on its elimination by the kidneys (Spearman correlation coefficient between B2M and eGFR $\rho = -0.72$) and (ii) previous studies found that reduced kidney function and chronic kidney diseases were associated with poor prognosis for several cancers, including cancers of the lung (33), bladder (33), kidney (34, 35), and several others (36–39). Therefore, we aimed to understand whether the B2M–cancer associations were independent of reduced kidney function. Additional covariates that were tested for confounding but were not included in final models included consumption of alcohol, red meat, and dietary fiber.

The fully adjusted breast cancer models also included age at menarche (continuous), number of live births (0, 1–2, 3–4, and $\geq$5), and menopausal status (yes, no). The colorectal cancer analysis was also run separately by subsite (colon, rectum). In addition, we conducted several supplementary analyses for all cancer types. We further adjusted for WHR, in addition to BMI, to decrease the likelihood of residual confounding by body fatness (the Spearman correlations were: between B2M and BMI $\rho = 0.15$; B2M and WHR $\rho = 0.18$; BMI and WHR $\rho = 0.46$). We also assessed the independence of the B2M and colorectal cancer association from hs-CRP, which we previously found to be associated with colorectal cancer in ARIC (30). To do so, we first determined the Spearman correlation between B2M and hs-CRP ($\rho = 0.27$). In addition, we conducted several sensitivity analyses: we excluded participants ($\sim$10% of participants) with evidence of acute inflammation (hs-CRP >10 mg/L) and those with reduced kidney function measured as eGFR $\leq$ 90 mL/min/1.73 m^2 ($\sim$33% of participants), i.e., the cutoff point recommended by the National Kidney Foundation (40). To minimize the impact of undiagnosed disease, we excluded people with less than 3 years of follow-up (4% of participants). Although we were not able to study associations across cancer stage, we examined associations between B2M and risk of death from cancer.

Given that each biomarker is measured with some degree of error, we sought to determine whether combining B2M and hs-CRP would better capture the association with colorectal cancer than either biomarker measurement alone; we restricted this analysis to colorectal cancer due its stronger association with B2M. To do so, we created a combined score, by summing hs-CRP and B2M as categorical variables (where the quartiles for each variable were coded 0–3). The resulting combined score, which ranged from 0 to 6, was categorized into 4 groups (0–1, 2–3, 4–5, and 6). In addition, we tested the risk of colorectal cancer in relation to two other combined scores based on hs-CRP and B2M that were created as (i) a sum of the normalized log-transformed continuous biomarkers; (ii) a linear combination of normalized log-transformed continuous biomarkers with weights equal to beta coefficients derived from Cox regression, when hs-CRP and B2M were included simultaneously.

We also tested whether the multivariable models that included both B2M and hs-CRP (normalized log-transformed) provided better fit than the model with B2M alone; we tested for differences in model fit using the likelihood ratio χ^2 test. Analyses were performed using SAS v9.3 (SAS Institute Inc.); all statistical tests were two sided.

Results

The analytical cohort ($n = 12{,}300$; 55% women, 26% black and 74% white) was followed for incident cancer from 1990–1992 through 2006. During follow-up, 2,436 incident cancers were ascertained, including 255 colorectal, 298 lung, 424 breast, 524 prostate, and 176 hematological cancers. Serum B2M concentrations at the start of follow-up ranged from 0.9 to 57.8 mg/L, with a mean (SD) of 2.0 (1.5) mg/L. B2M was unrelated to sex, but higher B2M quartiles were associated with older age, higher proportions of whites (versus blacks), less than high school education, higher BMI, greater pack-years of smoking, recent aspirin use, and less ever use of hormone therapy (Table 1). These findings were mostly consistent with previous studies (41–43).

In the minimally adjusted Model 1, for the highest versus lowest B2M quartile, there were significant positive associations of B2M with the risk of total (HR, 1.20; 95% CI, 1.07–1.36; $P_{trend} = 0.0003$) and colorectal cancers (HR, 1.55; 95% CI, 1.04–2.30; $P_{trend} = 0.02$) and a possible association with hematological cancers (HR, 1.42; 95% CI, 0.92–2.21; $P_{trend} = 0.07$; Table 2). In adjusted Model 2, compared with the lowest quartile, people in

Table 2. HR (95% CI) for the incidence of total cancer and cancer types by quartiles of B2M; ARIC, 1990–2006

		Quartiles of B2M (mg/L)				
Cancer	**Cases, *n***	**≤1.66**	**1.67–1.88**	**1.89–2.15**	**≥2.16**	**P_{trend}**
Total cancer	2,436	512	583	659	682	
Person-years	162,617	42,356	42,688	40,069	37,503	
Incidence rates[a]	14.98	12.09	13.66	16.45	18.18	
Model 1[b]		1 (reference)	1.01 (0.89–1.14)	1.14 (1.02–1.29)	1.20 (1.07–1.36)	0.0003
Model 2[c]		1 (reference)	1.02 (0.89–1.17)	1.20 (1.05–1.38)	1.25 (1.06–1.47)	0.002
Colorectal cancer	255	44	64	77	70	
Person-years	162,617	42,356	42,688	40,069	37,503	
Incidence rates	1.57	1.04	1.50	1.92	1.87	
Model 1		1 (reference)	1.35 (0.91–1.99)	1.66 (1.13–2.43)	1.55 (1.04–2.30)	0.02
Model 2		1 (reference)	1.37 (0.89–2.09)	1.93 (1.25–2.99)	2.21 (1.32–3.70)	0.001
Lung cancer	298	59	72	66	101	
Person-years	162,617	42,356	42,688	40,069	37,503	
Incidence rates	1.83	1.39	1.69	1.65	2.69	
Model 1		1 (reference)	0.99 (0.70–1.40)	0.88 (0.61–1.26)	1.31 (0.93–1.84)	0.12
Model 2		1 (reference)	1.09 (0.74–1.61)	0.98 (0.64–1.50)	1.64 (1.03–2.61)	0.06
Breast cancer (women only)	424	103	91	103	127	
Person-years	92,554	25,434	23,206	22,114	21,801	
Incidence rates	4.58	4.05	3.92	4.66	5.83	
Model 1		1 (reference)	0.88 (0.66–1.17)	0.99 (0.75–1.31)	1.19 (0.90–1.56)	0.14
Model 2*[d]		1 (reference)	0.84 (0.58–1.23)	1.13 (0.78–1.64)	1.01 (0.64–1.59)	0.62
Prostate cancer	524	119	153	149	103	
Person-years	70,062	16,922	19,482	17,955	15,703	
Incidence rates	7.48	7.03	7.85	8.30	6.56	
Model 1		1 (reference)	1.12 (0.87–1.42)	1.15 (0.90–1.48)	0.82 (0.62–1.08)	0.21
Model 2		1 (reference)	1.14 (0.88–1.48)	1.20 (0.89–1.62)	0.95 (0.66–1.36)	0.98
Hematological cancers	176	35	38	43	60	
Person-years	162,617	42,356	42,688	40,069	37,503	
Incidence rates	1.08	0.83	0.89	1.07	1.60	
Model 1		1 (reference)	0.92 (0.58–1.47)	1.02 (0.65–1.62)	1.42 (0.92–2.21)	0.07
Model 2		1 (reference)	1.06 (0.62–1.79)	1.17 (0.67–2.00)	1.50 (0.80–2.81)	0.18

[a]Per 1,000 person-years.
[b]Model 1: adjusted for age, sex, race-study center.
[c]Model 2: all variables in Model 1 + education, BMI, smoking status and pack-years of smoking, use of aspirin, hormone replacement therapy (in women), and eGFR.
[d]Model 2*: all variables in Model 2 + menopausal status, age at menarche, and number of live births.

the highest quartile were more likely to develop total (HR, 1.25; 95% CI, 1.06–1.47; $P_{trend} = 0.002$) and colorectal cancers (HR, 2.21; 95% CI, 1.32–3.70; $P_{trend} = 0.001$). When the analysis for colorectal cancer was stratified by subsite, HRs were similar for colon ($n = 206$) and rectal ($n = 67$) cancers: 1.72 and 1.98 for the highest versus lowest B2M quartile (not presented in the tables). After multivariable adjustment (Model 2), the HRs for hematological cancers did not change substantively. For lung cancer, there was a statistically significant association in the fourth versus first quartile only: HR = 1.64 (95% CI, 1.03–2.61) in Model 2 (Table 2). B2M was not associated with risk of breast or prostate cancer in either Model 1 or 2.

Additional adjustment of B2M–cancer associations for WHR or log-transformed hs-CRP did not markedly change any of the observed associations (Supplementary Table S1, Models 3 and 4, respectively). After excluding participants with a possible acute inflammatory response (CRP >10 mg/L), associations for colorectal and total cancer remained statistically significant (Supplementary Table S1, Model 5). Likewise, the associations for total and colorectal cancer also did not appreciably change after excluding those with eGFR $\leq$ 90 mL/min/1.73 m^2: for the highest versus lowest B2M quartile, the HRs were 1.21 (95% CI, 0.96–1.51; $P_{trend} = 0.004$) for total cancer and 2.18 (95% CI, 1.18–4.04; $P_{trend} = 0.004$) for colorectal cancer (Supplementary Table S1, Model 6). After excluding people with less than 3 years of follow-up, the positive associations with total or colorectal cancer did not change, whereas the hazard ratios for lung and hematological cancers for the fourth quartiles (versus the first quartiles) diminished (Supplementary Table S1, Model 7). Further, there was no evidence of multiplicative interactions of serum B2M with age, BMI, smoking, HRT or aspirin use in relation to any cancer (P values for all interactions were > 0.1). Stratified associations for colorectal and lung cancers are presented in Supplementary Tables S2a and 2b, respectively.

Further, we analyzed serum B2M and risk of death from cancer during follow-up through 2011 and showed that the patterns of associations were similar to the associations for incident total cancer and each cancer type, respectively (Table 3). The association for death from hematological cancers was stronger than that for incidence and was statistically significant, while death from colorectal cancer was not significantly associated with B2M (with only 85 deaths observed). The association for death from lung cancer was of the same magnitude as for incidence and was significant.

Finally, we examined whether the combined score based on hs-CRP and B2M was more strongly associated with colorectal cancer incidence than was B2M alone. As shown in Table 4, the associations for combined score 1, which included categorical B2M and hs-CRP, were very similar in magnitude and behaved in a parallel fashion to the B2M–colorectal cancer association: the HR for highest versus lowest quartile was 2.49 (95% CI, 1.42–4.38; $P_{trend} = 0.0002$). The associations with two other combined scores based on continuous log-transformed hs-CRP and B2M were also similar to those with B2M and to each other (Supplementary

Table 3. HR (95% CI) of death from total cancer and cancer types by quartiles of serum B2M; ARIC, 1990–2011

		Quartiles of B2M (mg/L)				
Cause of death	**Deaths, *n***	**≤1.66**	**1.67–1.88**	**1.89–2.15**	**≥2.16**	**P_{trend}**
Total cancer	1,160	237	261	322	344	
Person-years	219,393	57,374	57,868	54,563	49,588	
Mortality rate[a]	5.29	4.13	4.51	5.90	6.94	
Model 2[b]		1 (reference)	0.92 (0.76–1.12)	1.09 (0.89–1.34)	1.30 (1.02–1.64)	0.01
Colorectal cancer	85	13	27	24	21	
Person-years	219,393	57,374	57,868	54,563	49,588	
Mortality rate	0.39	0.23	0.47	0.44	0.42	
Model 2		1 (reference)	1.60 (0.77–3.32)	1.50 (0.68–3.33)	1.73 (0.68–4.44)	0.34
Lung cancer	352	70	84	84	114	
Person-years	219,393	57,374	57,868	54,563	49,588	
Mortality rate	1.60	1.22	1.45	1.54	2.30	
Model 2		1 (reference)	1.05 (0.73–1.50)	1.05 (0.72–1.55)	1.69 (1.10–2.59)	0.02
Breast cancer (women only)	77	19	18	23	17	
Person-years	124,783	34,480	31,434	29,888	28,982	
Mortality rate	0.62	0.55	0.57	0.77	0.59	
Model 2		1 (reference)	1.12 (0.49–2.53)	1.23 (0.51–2.95)	0.80 (0.26–2.47)	0.83
Prostate cancer	58	14	15	20	9	
Person-years	94,609	22,894	26,434	24,676	20,605	
Mortality rate	0.61	0.61	0.57	0.81	0.44	
Model 2		1 (reference)	0.78 (0.36–1.68)	0.93 (0.42–1.68)	0.42 (0.14–2.07)	0.25
Hematological cancers	138	21	29	34	54	
Person-years	219,393	57,374	57,868	54,563	49,588	
Mortality rate	0.63	0.37	0.50	0.82	1.09	
Model 2		1 (reference)	1.21 (0.64–2.28)	1.48 (0.78–2.82)	2.44 (1.20–4.95)	0.001

[a]Per 1,000 person-years.

[b]Multivariable model (Model 2, Table 2): adjusted for age, sex, race, study center, education, BMI, smoking status and pack-years of smoking, use of aspirin, hormone replacement therapy (in women), and eGFR.

Table S3). However, the multivariable model with continuous hs-CRP and B2M included simultaneously in the model showed improvement in model fit compared with the multivariable model that included B2M only ($P = 0.02$).

Discussion

In this population-based cohort study of adults followed for a maximum of 17 years, participants with a serum B2M concentration in the highest versus lowest quartiles had a 25% higher risk of total cancer incidence and 121% higher risk of colorectal cancer incidence. These findings support our *a priori* hypothesis that B2M, a biomarker of increased cell turnover, is associated with colorectal cancer risk.

The robust association between B2M and colorectal cancer risk was independent of the inflammatory marker hs-CRP and persisted in all sensitivity analyses: it remained after excluding those with eGFR ≤90 mL/min/1.73 m^2 (potentially abnormal kidney function) or those with CRP <10 mg/L (acute inflammation). It also did not markedly change after excluding the first three years of follow-up, which implies that higher B2M concentrations do not reflect the subclinical presence of malignancy. The magnitude of the B2M–colorectal associations was similar to the strength of the association between hs-CRP and colorectal cancer in this study: for the highest versus lowest hs-CRP quartile, the HR was 2.17 (95% CI, 1.39–3.37; $P_{trend} = 0.0002$). This association is unlikely to be explained by residual confounding by obesity, because B2M concentration was only weakly correlated with BMI or WHR (Spearman $\rho = 0.15$ and $\rho = 0.18$, respectively). This characteristic distinguishes B2M from CRP, because CRP concentrations are moderately associated with obesity, both in our study (Spearman $r = 0.39$) and other studies (44, 45). Also, we showed that linear combinations of two biomarkers, B2M and CRP, were positively associated with the risk of developing colorectal cancer. Importantly, the linear combinations of B2M and CRP did not yield a higher HR than either biomarker put into the model alone. However, the inclusion of CRP and B2M simultaneously in the model improved the fit of the model, suggesting that the

Table 4. HR (95% CI) for colorectal cancer incidence by quartiles of biomarker (hs-CRP or B2M) or inflammatory score; ARIC, 1990–2006

Biomarker	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P***
B2M (mg/L)	≤1.66	1.67–1.88	1.89–2.15	≥2.16	
Number of cases/participants	44/3,063	64/3,140	77/3,046	70/3,051	
HR (95% CI)[a]	1 (reference)	1.37 (0.89–2.09)	1.93 (1.25–2.99)	2.21 (1.32–3.70)	0.001
hs-CRP (mg/L)	≤1.07	1.07–2.26	2.27–4.87	≥4.88	
Number of cases/participants	42/3,069	59/3,091	76/3,069	78/3,066	
HR (95% CI)	1 (reference)	1.36 (0.89–2.09)	1.84 (1.21–2.81)	2.17 (1.39–3.37)	0.0002
Combined score 1[b]	0–1	2–3	4–5	6	
Number of cases/participants	44/2,813	82/4,621	97/3,660	32/1,201	
HR (95% CI)	1 (reference)	1.19 (0.75–1.67)	1.84 (1.20–2.82)	2.49 (1.42–4.38)	0.0002

[a]Multivariable model (Model 2, Table 2): adjusted for age, sex, race, study center, education, BMI, smoking status and pack-years of smoking, use of aspirin, hormone replacement therapy (in women), and eGFR.

[b]Sum of hs-CRP and B2M (categorized in quartiles that ranged from to 0 to 3).

combined score may be a better marker for the development of colorectal cancer than B2M alone.

The associations for incidence and death associated with B2M behaved in parallel fashion for total cancer and each cancer type. The trends for deaths were significant for total, lung, and hematological cancers. For the lung cancer death, the association was driven by the increased hazard ratio in the highest versus lowest B2M quartile. For death from hematological cancers, the HRs in all quartiles were higher than the corresponding HRs for risk of hematological cancers, which is consistent with a strong correlation between B2M concentration and the progression of hematological cancer (4, 5). Additional studies are needed to better understand the link between B2M and risk of hematological and lung cancers.

The observed associations of elevated B2M with the total cancer incidence and death, colorectal cancer incidence and the death from lung and hematological cancers are consistent with experimental evidence that links B2M expression with carcinogenesis. In mouse studies, Josson and colleagues reported that B2M overexpression in cancer cells promoted the growth and progression of human prostate, breast, lung, and renal cancer cells and led to metastases and lethality (46). In addition, several human studies have shown that serum B2M is elevated at the time of cancer diagnosis or thereafter and increases with progression in many solid cancers, including those of the prostate (11, 12), breast (10, 13, 14), lung (26, 47), and kidneys (48), and, to a greater extent, in hematological cancers, such as lymphomas and multiple myeloma (15, 16, 49). Further, serum B2M was reported to be a strong indicator of poor prognosis and reduced survival of patients with solid and hematological cancers, including kidney, prostate and breast tumors, multiple myeloma, lymphomas and leukemia (11, 14, 16, 50–52).

Mechanisms explaining the positive associations between serum B2M concentrations and carcinogenesis have not been precisely established, but, most likely, B2M plays several roles: (i) proangiogenic; (ii) protumorigenic; (iii) it may drive innate proinflammatory cytokines (e.g., TNFα, IL1, IL6, and IL8) and growth-promoting factors (e.g., vascular endothelial growth factor, epidermal growth factor receptor, fatty acid synthase, insulin and insulin-like growth factor 1 receptor) in both cancer patients and unaffected people (3, 4, 12, 53); and (iv) may drive epithelial–mesenchymal transition (46).

Thus, B2M may have a direct pathogenic role in immune response and inflammation or may serve as an early biomarker of increased cell turnover. Its role as an inflammation marker is corroborated by studies reporting positive associations of B2M with aging, CVD, diabetes, and mortality from those diseases and all causes (1, 7–9). In a prospective cohort study of 1,034 healthy people 65 years and older followed for 8 years, the risk of all-cause death was significantly increased by 102% in the second and by 184% in the third tertile versus the first B2M tertile, independent of lifestyle factors, renal dysfunction, and preexisting comorbidities (1). Of note, B2M was a better predictor of mortality than CRP or cystatin.

The function of B2M as a marker of increased cell turnover may be particularly relevant to colorectal cancer, because the tissues in colon and rectum are characterized by rapidly dividing epithelia cells and immune response elicited by gut microbiota (22, 54). However, we do not know exactly whether B2M is secreted by immune or tumor cells, i.e., whether the elevated B2M concentrations reflect local or systemic response. Finally, B2M could be a marker of undiagnosed cancer, especially for hematological malignancies, because B2M is an established marker of progression and worse prognosis in those cancers (4, 5, 16).

A limitation of our study is that B2M was measured only once at Visit 2 (1990–92). However, a recent study using Third National Health and Nutrition Examination Survey (NHANES III) data on 795 people examined two B2M measurements taken 18 days apart and concluded that B2M has a low short-term variability (coefficient of variation was 8.4%; ref. 55). Another limitation of our study is the low number of cases ascertained in each cancer group, especially hematological cancers, which precluded both the study of leukemia and lymphoma separately and the conduct of subgroup analyses. The main strength of this study is that, to our knowledge, it is the first to examine serum B2M concentrations in relation to future cancer risk. We studied this association in a large prospective population-based study that has rigorously collected data on multiple risk factors related to B2M and cancer, including information on other markers of kidney function and inflammation. This allowed us to evaluate the association of B2M with cancer development independently of other risk factors.

In conclusion, our study provides the first evidence that higher serum B2M in people without a prior cancer diagnosis is associated with increased colorectal cancer risk. Significant associations were also observed for mortality from total, lung, and hematological cancers. Further research is needed to determine whether for these associations, circulating B2M is marking increased turnover of tumor or immune cells.

Disclosure of Potential Conflicts of Interest

E. Selvin is a consultant/advisory board member for Roche Diagnostics. No potential conflicts of interest were disclosed by the other authors.

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References

1. Shinkai S, Chaves PHM, Fujiwara Y, Watanabe S, Shibata H, Yoshida H, et al. Beta2-microglobulin for risk stratification of total mortality in the elderly population: Comparison with cystatin C and C-reactive protein. Arch Intern Med 2008;168:200–6.
2. Tikidzhieva A, Benner A, Michel S, Formentini A, Link K, Dippold W, et al. Microsatellite instability and Beta2-microglobulin mutations as prognostic markers in colon cancer: Results of the FOGT-4 trial. Br J Cancer 2012;106:1239–45.
3. Shi C, Shi Y, Zhu Y, Su LWK, Chung T, Cheng. B2-microglobulin: Emerging as a promising cancer therapeutic target. Drug Discov Today 2009;14: 25–30.
4. Nomura T, Huang WC, Zhau HE, Josson S, Mimata H, Chung LW. β2-Microglobulin-mediated signaling as a target for cancer therapy. Anticancer Agents Med Chem 2014;14:343–52.
5. Karlsson FA, Wibell L, Evrin PE. Beta 2-microglobulin in clinical medicine. Scand J Clin Lab Invest Suppl 1980;154:27–37.
6. Bianchi C, Donadio C, Tramonti G, Consani C, Lorusso P, Rossi G. Reappraisal of serum beta2-microglobulin as marker of GFR. Ren Fail 2001;23:419–29.
7. Astor BC, Shafi T, Hoogeveen RC, Matsushita K, Ballantyne CM, Inker LA, et al. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. Am J Kidney Dis 2012;59:653–62.
8. Zissis M, Afroudakis A, Galanopoulos G, Palermos L, Boura X, Michopoulos S, et al. B2 microglobulin: Is it a reliable marker of activity in inflammatory bowel disease? Am J Gastroenterol 2001; 96:2177–83.
9. Liabeuf S, Lenglet A, Desjardins L, Neirynck N, Glorieux G, Lemke H, et al. Plasma beta-2 microglobulin is associated with cardiovascular disease in uremic patients. Kidney Int 2012;82:1297–303.
10. Teasdale C, Mander AM, Fifield R, Keyser J W, Newcombe RG, Hughes LE. Serum beta2-microglobulin in controls and cancer patients. Clinica chimica acta 1977;78:135–43.
11. Zhang YX, Wang L, Ji PY, Zhao GG, Zhong GP, Wang ZP. Correlation of serum beta-2 microglobulin levels with prostate-specific antigen, Gleason score, clinical stage, tumor metastasis and therapy efficacy in prostate cancer. Arch Med Res 2013;44:259–65.
12. Huang W, Wu D, Xie Z, Zhau H, Nomura T, Zayzafoon M, et al. Beta2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis. Cancer Res 2006;66:9108–16.
13. Li K, Du H, Lian X, Yang S, Chai D, Wang C, et al. Characterization of ß2-microglobulin expression in different types of breast cancer. BMC Cancer 2014;14:750.
14. Klein T, Levin I, Niska A, Koren R, Gal R, Schachter J, et al. Correlation between tumour and serum beta 2m expression in patients with breast cancer. Eur J Immunogenet 1996;23:417–23.
15. Yang J, Qian J, Wezeman M, Wang S, Lin P, Wang M, et al.et al. Targeting beta2-microglobulin for induction of tumor apoptosis in human hematological malignancies. Cancer cell 2006;10:295–307.
16. Rossi D, Fangazio M, De Paoli L, Puma A, Riccomagno P, Pinto V, et al. Beta2microglobulin is an independent predictor of progression in asymptomatic multiple myeloma. Cancer 2010;116:2188–200.
17. Cheung C, Lam KSL, Cheung BMY. Serum Beta-2 Microglobulin predicts mortality in people with diabetes. Eur J Endocrinol 2013;169:1–7.
18. Amighi J, Hoke M, Mlekusch W, Schlager O, Exner M, Haumer M, et al. Beta 2 microglobulin and the risk for cardiovascular events in patients with asymptomatic carotid atherosclerosis. Stroke 2011;42:1826–33.
19. Puntoni M, Marra D, Zanardi S, Decensi A. Inflammation and cancer prevention. Ann Oncol 2008;19:vii225–9.
20. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883–99.
21. Grizzi F, Bianchi P, Malesci A, Laghi L. Prognostic value of innate and adaptive immunity in colorectal cancer. World J Gastroenterol 2013;19: 174–84.
22. Rhodes JM, Campbell BJ. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. Trends Mol Med 2002;8:10–6.
23. Jun W, Lee I, Tworoger SS, Buring JE, Ridker PM, Rosner B, et al. Plasma C-reactive protein and risk of breast cancer in two prospective studies and a meta-analysis. Cancer Epidemiol Biomarkers Prev 2015;24:1199–206.
24. Toriola AT, Laukkanen JA, Kurl S, Nyyssönen K, Ronkainen K, Kauhanen J. Prediagnostic circulating markers of inflammation and risk of prostate cancer. Int J Cancer 2013;133:2961–7.
25. Sutcliffe S, Platz EA. In: Inflammation in the etiology of prostate cancer: an epidemiologic perspective. Urologic oncology: Seminars and original investigations ; Elsevier; 2007. p. 242–9.
26. Shuster J, Gold P, Poulik MD. Beta 2-microglogulin levels in cancerous and other disease states. Clin Chim Acta 1976;67:307–13.
27. Atherosclerosis Risk in Communities (ARIC) study [Internet]. ARIC documentation. [cited on 2015 May 16]. Available from: www2.cscc.unc.edu/aric//ARIC.
28. The Atherosclerosis Risk in Communities (ARIC) study: design and objectives. The ARIC investigators. Am J Epidemiol 1989;129:687–702.
29. Mink PJ, Shahar E, Rosamond WD, Alberg AJ, Folsom AR. Serum insulin and glucose levels and breast cancer incidence: The Atherosclerosis Risk in Communities Study. Am J Epidemiol 2002;156:349–52.
30. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities (ARIC) study. Cancer Epidemiol Biomarkers Prev 2011:29–307.
31. Rebholz CM, Grams ME, Matsushita K, Selvin E, Coresh J. Change in novel filtration markers and risk of ESRD. Am J Kidney Dis 2015:47–54.
32. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20–9.
33. Jørgensen L, Heuch I, Jenssen T, Jacobsen BK. Association of albuminuria and cancer incidence. J Am Soc Nephrol 2008;19:992–8.
34. Maisonneuve P, Agodoa L, Gellert R, Stewart JH, Buccianti G, Lowenfels AB, et al. Cancer in patients on dialysis for end-stage renal disease: An international collaborative study. The Lancet 1999;354:93–9.
35. Christensson A, Savage C, Sjoberg DD, Cronin AM, O'Brien FM, Lowrance W, et al. Association of cancer with moderately impaired renal function at baseline in a large, representative, population-based cohort followed for up to 30 years. Int J Cancer 2013;133:1452–8.
36. Cengiz K. Increased incidence of neoplasia in chronic renal failure (20-year experience). Int Urol Nephrol 2002;33:121–6.
37. Wong G, Hayen A, Chapman JR, Webster AC, Wang JJ, Mitchell P, et al. Association of CKD and cancer risk in older people. J Am Soc Nephrol 2009;20:1341–50.
38. Vajdic CM, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M, et al. Cancer incidence before and after kidney transplantation. JAMA 2006;296:2823–31.
39. Stengel B. Chronic kidney disease and cancer: A troubling connection. J Nephrol 2010;23:253–62.
40. Eknoyan G, Levin NW. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1–266.
41. Schardijn GH, Statius van Eps LW. Beta 2-microglobulin: Its significance in the evaluation of renal function. Kidney Int 1987;32:635–41.

42. Stanga Z, Nock S, Medina-Escobar P, Nydegger UE, Risch M, Risch L. Factors other than the glomerular filtration rate that determine the serum beta-2-microglobulin level. PLoS ONE 2013;8:e72073.
43. Juraschek SP, Coresh J, Inker LA, Levey AS, Köttgen A, Foster MC, et al. Comparison of serum concentrations of β-trace protein, β2-microglobulin, cystatin C, and creatinine in the US population. Clin J Am Soc Nephrol 2013;8:584–92.
44. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131–5.
45. Choi J, Joseph L, Pilote L. Obesity and Creactive protein in various populations: a systematic review and metaanalysis. Obes Rev 2013; 14:232–44.
46. Josson S, Josson T, Nomura J-, Lin W-, Huang D, Wu HE, et al. β2-microglobulin induces epithelial to mesenchymal transition and confers cancer lethality and bone metastasis in human cancer cells. Cancer Res 2011;71:2600–10.
47. Anand R, Mathur M, Acharya N. Serum beta-2 microglobulin levels in bronchogenic carcinoma. J Thorac Cardiovasc Surg 1993;9:96–9.
48. Nomura T, Huang WC, Zhau HE, Wu D, Xie Z, Mimata H, et al. Beta2-microglobulin promotes the growth of human renal cell carcinoma through the activation of the protein kinase A, cyclic AMP-responsive element-binding protein, and vascular endothelial growth factor axis. Clin Cancer Res 2006;12:7294–305.
49. Yoo C, Yoon D, Kim S, Huh J, Park C, Lee S, et al. Serum beta-2 microglobulin as a prognostic biomarker in patients with mantle cell lymphoma. Hematol Oncol 2015.
50. Rasmuson T, Grankvist K, Ljungberg B. Serum beta 2-microglobulin and prognosis of patients with renal cell carcinoma. Acta Oncol 1996;35: 479–82.
51. Tsimberidou A, Kantarjian H, Wen S, O'Brien S, Cortes J, Wierda W, et al. The prognostic significance of serum beta2 microglobulin levels in acute myeloid leukemia and prognostic scores predicting survival: analysis of 1,180 patients. Clin Cancer Res 2008;14: 721–30.
52. Nakajima Y, Tomita N, Watanabe R, Ishiyama Y, Yamamoto E, Ishibashi D, et al. Serum beta-2 microglobulin level as a useful prognostic marker of Hodgkin lymphoma. Blood 2013;122:4239.
53. Xie J, Yi Q. Beta2-microglobulin as a potential initiator of inflammatory responses. Trends Immunol 2003;24:228–9.
54. Clevers H. At the crossroads of inflammation and cancer. Cell 2004;118:671–4.
55. Selvin E, Juraschek S, Eckfeldt J, Levey A, Inker L, Coresh J. Within-person variability in kidney measures. Am J Kidney Dis 2013;61:716–22.