Beta 2 Microglobulin and the Risk for Cardiovascular Events in Patients With Asymptomatic Carotid Atherosclerosis

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Background and Purpose—Atherosclerosis is a chronic inflammatory disease. Ongoing inflammation is associated with elevated levels of beta 2 microglobulin (B2M). We investigated B2M levels in a large cohort of patients with carotid atherosclerosis for the occurrence of major adverse cardiovascular events.

Methods—One thousand five of 1286 consecutive, neurologically asymptomatic patients with carotid atherosclerosis were followed for a median of 3 years (interquartile range, 2.5 to 3.5) for the occurrence of major adverse cardiovascular events, a composite of myocardial infarction, percutaneous coronary intervention, coronary bypass graft, stroke, and death.

Results—we recorded 359 major cardiovascular events in 271 (27%) patients. B2M was significantly associated with the occurrence of major adverse cardiovascular events. With increasing quartiles of B2M, the adjusted hazard ratios were 1.19 (95% CI, 0.81 to 1.73), 1.51 (95% CI, 1.05 to 2.18), and 1.88 (95% CI, 1.26 to 2.79) compared with the lowest quartile, respectively (P<0.001). Adjusted hazard ratios for the occurrence of death, myocardial infarction, and stroke for increasing quartiles of B2M were 1.25 (95% CI, 0.92 to 1.70), 1.52 (95% CI, 1.12 to 2.06), and 1.62 (95% CI, 1.16 to 2.67) compared with the lowest quartile, respectively (P<0.001). Through statistical estimation of improvement in risk stratification, addition of B2M to baseline risk factors improved the risk stratification for major cardiovascular events, at least as much as high-sensitivity C-reactive protein or even better.

Conclusions—B2M was independently and significantly associated with adverse cardiovascular outcome in patients with prevalent asymptomatic carotid atherosclerosis. (Stroke. 2011;42:1826-1833.)

Key Words: beta 2 microglobulin ■ carotid atherosclerosis ■ inflammation ■ major adverse cardiovascular events ■ risk stratification

Atherosclerosis is a chronic inflammatory process. Serum biomarkers of inflammation are associated with morbidity and mortality in patients with atherosclerotic disease. Previously, it has been demonstrated that levels of high-sensitivity C-reactive protein (hs-CRP) are prognostic parameters for the occurrence of major adverse cardiovascular events (MACE). Patients with peripheral or cerebral artery disease have a high cardiovascular risk and exhibit increased levels of inflammatory parameters compared with patients without the disease.

Beta 2 microglobulin (B2M), a nonglycosylated polypeptide with a molecular weight of 11.7 kDa, is a part of the major histocompatibility complex, which is found on all nucleated human cells and on thrombocytes. It is eliminated by the kidney and can be used to estimate the glomerular filtration rate and in renal transplantation. A correlation between an inflammatory response during hemodialysis and elevated serum B2M has been already described. Increased levels of B2M are seen in patients with hematologic and autoimmune disorders but also seem to be elevated under conditions of high cell turnover, ischemia, or cardiomyopathy. In 1 study in uremic patients on hemodialysis, B2M was related to carotid intima-media thickness. Shinkai et al have demonstrated that serum B2M is an independent predictor of mortality in older adults, possibly better than other inflammatory markers like C-reactive protein or cystatin C. Furthermore, it has been shown that B2M is associated with coronary artery disease and more strongly with peripheral...
artery disease (PAD). Wilson et al demonstrated that B2M is elevated in patients with PAD and correlates with the severity of disease independently of other risk factors, presenting B2M as a first possible biomarker for PAD, detectable through blood testing and thus possibly increasing the identification and diagnosis of PAD.

The prognostic impact of B2M in asymptomatic cerebral arterial disease with regard to cardiovascular events and mortality has to our knowledge not been investigated yet. We hypothesized that B2M levels are elevated in patients with prevalent carotid atherosclerosis verified by duplex sonography and associated with poor cardiovascular outcome in this patient group.

Materials and Methods

Study Design and Patient Selection

A total of 1286 consecutive patients with carotid atherosclerosis were prospectively enrolled between March 2002 and March 2003 in the Inflammation in Carotid Arteries Risk for Atherosclerosis Study (ICARAS). Patients with prevalent carotid atherosclerosis, indicated by the presence of carotid stenosis or nonstenotic plaques verified by duplex sonography, who were clinically asymptomatic at the time of enrollment, were eligible to enter the study. The main indications for referral for performing carotid ultrasound were carotid bruits, known atherosclerotic disease in other vessel areas (coronary or peripheral artery disease), prevalence of risk factors, and patients scheduled for major cardiac surgery. All patients had to sign an informed consent; the study was approved by the local ethics committee.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria have been reported previously. In summary, patients with prevalent atherosclerotic carotid artery disease as identified in the ultrasound laboratory of our angiography department who were clinically asymptomatic at the time of enrollment were eligible. Patients who had a recent cardiac event (myocardial infarction, stroke, coronary revascularization, peripheral vascular surgery) within 6 months before screening were excluded. Neurologically “asymptomatic” was defined as the absence of transient ischemic attacks, amaurosis fugax, or stroke in patients’ recent history. Patients with a history of prior neurological event were only eligible if the event occurred at least 6 months before inclusion and no residual or recurrent symptoms were identified by a neurologist. We also excluded patients with known active malignant disease and low life expectancy.

Study End Point

The primary study end point was defined as the occurrence of a first MACE, a composite of myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, and death defined by all-cause mortality during the follow-up period. We defined a secondary composite end point consisting of death, myocardial infarction, and stroke.

Clinical Data

After enrollment, medical history and clinical results from physical examination were recorded. This included age; gender; height; weight; blood pressure; arterial hypertension; smoking status; diabetes mellitus; hyperlipidemia; family history of atherosclerotic disease; history of coronary, peripheral, and cerebrovascular disease; as well as current medication. General laboratory baseline data were obtained in all patients. All demographic and vital parameters were checked on completeness and accuracy by 2 independent observers.

Definitions

Definitions of traditional cardiovascular risk factors and end points were published previously.

Briefly, hypertension was defined as blood pressure >140/90 mm Hg examined repeatedly or documented in the patient’s history and was considered to be present in patients taking antihypertensive therapy. Diabetes mellitus was defined as a fasting blood glucose level of >126 mg/dL (7.0 mmol/L) measured at least twice and was considered to be present in all patients taking antidiabetic medication. Furthermore, a glycosylated hemoglobin HbA1c >6.5% was accepted as indicative of diabetes mellitus. Hyperlipidemia was defined as hypercholesterolemia (total cholesterol >200 mg/dL or low-density lipoprotein cholesterol >130 mg/dL) and was assumed to be present in all patients taking statins or other lipid-lowering medications. Family history of atherosclerotic disease was considered positive if present in first-degree relatives. Coronary artery disease was classified according to the Canadian Cardiovascular Society classification. Serum creatinine values were obtained for estimated glomerular filtration rate (GFR) calculation as an indicator for renal function.

Color-Coded Duplex Sonography

Duplex examination criteria for this study were already published previously. Duplex grading of the carotid stenosis was done by measurements of the peak systolic and end-diastolic velocities in the internal and common carotid arteries. Combining these velocities according to an algorithm the degrees of stenosis can be obtained corresponding to the North American Symptomatic carotid Endarterectomy Trial (NASCET) angiographic degree. In summary, carotid arteries were categorized as 0% to 29%, 30% to 69% (moderate), 70% to 89% (high grade), and >90% stenosed or occluded (highest grade). Plaque was defined as a focal thickening of the sonographic intima media complex of the vessel wall exceeding at least 50% of the surrounding vessel wall irrespective of irregularities in density or calcification. Nonstenotic plaques were defined as 0% to 29% narrowing of the carotid arteries with absence of both relevant narrowing and change in flow and velocities.

Laboratory Analysis

All standard laboratory analyses were determined according to local standard procedures. B2M was measured from frozen samples (-80°C) collected at the baseline study visit with the use of a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) with a particle-enhanced immunonephelometric assay. Monoclonal antibodies to B2M were coated on polystyrene particles that agglutinate to increase the intensity of scattered light in proportion to the concentration of B2M. The intra-assay coefficient of variation ranges from 2% to 3.2%, and the interassay coefficient of variation ranges from 1.5% to 1.9%. The normal range of B2M in our laboratory is 0.7 to 1.8 mg/L. Laboratory estimation of hs-CRP was performed and published previously. Serum creatinine was determined by the Jaffe method on the Olympus AU 5400 System (Olympus Europa Holding GmbH, Hamburg, Germany) with a reference cutoff <1.2 mg/dL. For estimated GFR calculation as an indicator for renal function, the Modification of Diet in Renal Disease (MDRD) equation was used. Briefly, GFR = 186 × (serum creatinine ^(-1.154)) × (age ^(-0.203)) × (0.742 if female) × (1.21 if black).

Surveillance Protocol

Patients were scheduled for follow-up visits to our outpatients ward in 6- to 9-month intervals after the initial presentation for clinical re-examination. Additionally, a follow-up questionnaire was sent to each patient after 3 years, re-evaluating the occurrence of MACE. The questionnaire was kept simple with boxes to tick and space to add additional details. Information from the questionnaire was validated by reviewing our outpatients’ ward documentation reports and the original hospital discharge reports of the corresponding readmissions due to cardiovascular events. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or to the treating physicians was established. The
performance of percutaneous coronary interventions or coronary bypass graft was validated by review of the procedure protocols. If information about death was not available, contact with the municipality was established. Outcome was assessed by 2 independent observers who were blinded with respect to patients’ baseline clinical and laboratory data.

Statistical Methods
Continuous data are presented as the median and the interquartile range (from the 25th to the 75th percentile) or the total range. Discrete data are given as counts and percentages. We used $\chi^2$ tests, exact tests, and Spearman correlation coefficients for univariate analyses, as appropriate. Time-dependent variables were analyzed using the Kaplan–Meier method and compared by the log rank test. For this purpose, levels of B2M were categorized in quartiles. Multivariable Cox proportional hazards models were applied to assess the association between B2M and the occurrence of a first cardiovascular event. Results of the Cox models are presented as the hazards ratio and the 95% CIs. We assessed the overall model fit using Cox-Snell residuals. Furthermore, we tested the proportional hazard assumption for all covariates using Schoenfeld residuals (overall test) and the scaled Schoenfeld residuals (variable-by-variable testing). According to the tests, the proportional hazards assumption was not violated. A 2-sided $P<0.05$ was considered statistically significant. Calculations were performed with Stata (Release 8.0; Stata) and SPSS for Windows (Version 15.0; SPSS Inc.).

We also performed a statistical estimation of improvement in risk stratification. Differences in C statistics after the addition of the biomarker B2M to a model with established risk factors (age, gender, body mass index, smoking, hypertension, low-density lipoprotein cholesterol level, glycohemoglobin A1 level, estimated GFR, history of myocardial infarction, peripheral artery disease, history of stroke, baseline degree of carotid stenosis, and statins) were estimated with the method described by Delong. The increased discriminative value of the biomarker B2M was further examined with the method described by Pencina and D’Agostino Jr.20 The area under the receiver operating characteristic curve, net reclassification improvement (NRI), and Integrated Discrimination Improvement (IDI) are presented with the 95% CIs. Similar calculations were performed with hs-CRP and compared with B2M. Calculations were performed with R Version 2.11.1 (the R Project for Statistical Computing, 2002, Vienna, Austria). For model comparison between B2M and hs-CRP, Akaike and Bayesian Information Criterion are given.

Results
Patients Characteristics at Baseline
We enrolled a total of 1286 consecutive patients into ICARAS, of which 221 patients (17%) were lost to follow-up. Furthermore, in 60 patients (5%), there were no adequate frozen samples available for B2M determination, leaving 1005 patients with complete data for the final analysis. The median age was 69 years (interquartile range, 61 to 76 years) and 633 (63%) patients were male. Baseline demographic and clinical data of the 1005 patients are given in Table 1. For comparison of baseline demographic data and B2M levels, we randomly selected a subgroup of 68 patients of the patients lost to follow-up and thus formerly excluded from the analysis. We did not detect any significant differences in clinical baseline characteristics (age, gender, frequency of cardiovascular risk factors, and comorbidities) and B2M in the randomly selected subgroup of patients who were excluded compared with the study population of 1005 patients (data not shown).

B2M in our collective was median 1.96 mg/L (interquartile range, 1.49 to 2.58 mg/L; Table 1). B2M was significantly higher in hypertensive patients (data not shown).

Follow-Up for Cardiovascular Events
During a median of 3 years of clinical follow-up (interquartile range, 2.5 to 3.5 years), we recorded 359 cardiovascular events in 271 (27%) patients, including 38 myocardial infarctions (3.8%), 75 percutaneous coronary interventions (7.5%), 43 coronary bypass grafts (4.3%), 52 strokes (5.2%), and 151 deaths (15%). Cumulative event-free survival rates at 1-, 2-, and 3-year follow-up were 89% (95% CI, 0.88 to 0.92), 82% (95% CI, 0.80 to 0.85), and 73% (95% CI, 0.69 to 0.77).

B2M and MACE
B2M levels (in quartiles) were significantly associated with the occurrence of a first cardiovascular event by univariate analysis (log rank $P<0.001$). To account for potential confounding effects, we calculated the risk for MACE by multivariable Cox proportional hazards analysis adjusting for age, gender, body mass index (kg/m$^2$), smoking, hyperten-
sion, low-density lipoprotein cholesterol level (mg/dL), glycohemoglobin A1 level (%) as an indicator of diabetes, estimated GFR (mL/min/1.73 m²), history of myocardial infarction, PAD, history of stroke, baseline degree of carotid stenosis (in categories), and statin treatment (Figure 1). Adjusted hazard ratios for a first MACE for increasing quartiles of B2M were 1.19 (95% CI, 0.81 to 1.73), 1.51 (95% CI, 1.05 to 2.18), and 1.88 (95% CI, 1.26 to 2.79) compared with the lowest quartile, respectively, indicating a gradual, significant, and independent association between B2M and MACE (P < 0.001). We observed similar effect sizes when we adjusted additionally for hs-CRP in our analysis (P = 0.027). The results from the multivariate model for the risk for MACE for the risk factors we adjusted for are shown in Table 2. B2M was also independently and linearly associated with an increased risk for MACE when used as a continuous variable. The adjusted hazard ratios were 1.04 (95% CI, 1.01 to 1.07) per 1-mg/L increase of B2M (P = 0.019).

B2M and Death, Myocardial Infarction, and Stroke

Analyzing the association between B2M and the occurrence of death, myocardial infarction, and stroke, we observed virtually identical effect sizes as observed for MACE (Figure 2). Adjusted hazard ratios for the occurrence of death, myocardial infarction, and stroke for increasing quartiles of B2M were 1.25 (95% CI, 0.92 to 1.70), 1.52 (95% CI, 1.12 to 2.06), and 1.62 (95% CI, 1.16 to 2.67) compared with the lowest quartile, respectively (P < 0.001; Figure 2). We observed similar effect sizes when we adjusted additionally for hs-CRP in our analysis (P = 0.016). B2M was also independently and linearly associated with an increased risk for death, myocardial infarction, and stroke when used as a continuous variable. The adjusted hazard ratios were 1.04 (95% CI, 1.01 to 1.08) per 1-mg/L increase of B2M (P = 0.003). The results from the Kaplan–Meier estimates for the individual end points death, myocardial infarction, and stroke according to quartiles of B2M are consistent, particularly for death and stroke (http://stroke.ahajournals.org).

Statistical Estimation of Improvement in Risk Stratification

We observed a minor but not statistically significant increase in C statistics for the prediction of MACE when B2M (in quartiles) or hs-CRP was incorporated into a model with our established baseline risk factors but was slightly better for B2M (P = 0.18 for B2M versus P = 0.28 for hs-CRP; Table 3). The IDI estimates, however, suggest that the addition of B2M improved substantially the discriminatory property model for the prediction of risk in comparison to hs-CRP (P = 0.002 versus 0.048, respectively). The NRI for B2M was estimated at 0.298 (0.1630 to 0.4320; P = 0.0001) versus 0.201 (0.0667 to 0.3360) for hs-CRP (P = 0.0034). Addition of B2M to hs-CRP increased IDI and NRI to hs-CRP alone (Table 3).

We observed similar results for B2M with a trend for statistical significance for prediction of the occurrence of
Table 2. Multivariable Cox Proportional Hazard Analysis Assessing the Risk for Major Adverse Cardiovascular Events

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.0</td>
<td>0.99–1.02</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.16</td>
<td>0.89–1.52</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.0</td>
<td>0.97–1.03</td>
</tr>
<tr>
<td>HbA1c</td>
<td>1.01</td>
<td>0.98–1.04</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.86</td>
<td>0.65–1.14</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>1–10 cigarettes daily</td>
<td>0.85</td>
<td>0.55–1.3</td>
</tr>
<tr>
<td>11–20 cigarettes daily</td>
<td>0.65</td>
<td>0.37–1.14</td>
</tr>
<tr>
<td>&gt;20 cigarettes daily</td>
<td>0.8</td>
<td>0.45–1.41</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>1.00</td>
<td>0.99–1.0</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>1.00</td>
<td>0.99–1.0</td>
</tr>
<tr>
<td>History of MI</td>
<td>1.79</td>
<td>1.38–2.33</td>
</tr>
<tr>
<td>History of stroke</td>
<td>1.13</td>
<td>0.83–1.53</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>0.97</td>
<td>0.76–1.24</td>
</tr>
<tr>
<td>Statins</td>
<td>1.08</td>
<td>0.84–1.39</td>
</tr>
<tr>
<td>Carotid stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;29% (nonstenotic plaques)</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>30–69%</td>
<td>0.6</td>
<td>0.29–1.22</td>
</tr>
<tr>
<td>70–89%</td>
<td>1.2</td>
<td>0.91–1.59</td>
</tr>
<tr>
<td>≥90%</td>
<td>0.94</td>
<td>0.66–1.33</td>
</tr>
<tr>
<td>Beta 2 microglobulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First quartile</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>Second quartile</td>
<td>1.19</td>
<td>0.81–1.73</td>
</tr>
<tr>
<td>Third quartile</td>
<td>1.51</td>
<td>1.05–2.18</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>1.88</td>
<td>1.26–2.79</td>
</tr>
</tbody>
</table>

HbA1c indicates glycated hemoglobin A1; GFR, glomerular filtration rate; MI, myocardial infarction.

Discussion

The present study shows for the first time that B2M levels are strongly and gradually associated with a significantly increased occurrence of MACE and mortality. Through statistical estimation of improvement in risk stratification as evaluated by IDI and NRI, our data suggest that addition of B2M to baseline risk factors improves the risk stratification for major cardiovascular events, at least as much as hs-CRP or even better. These results may encourage one to further evaluate B2M as a possible novel biomarker for elevated risk in patients with carotid atherosclerosis.

Patients with cerebrovascular disease, although asymptomatic, have advanced atherosclerosis, which is a major cause of morbidity and mortality in the Western world. Therefore, they constitute a population prone to have early and severe major adverse cardiovascular events, as confirmed again through the cumulative event-free survival rates, the event rate of 27%, and the death rate of 15% during the median 3 years of follow-up in our study, compatible with the literature. In this context, Vidula et al have published that increasing levels of inflammatory markers are higher in the years close to death than in periods more remote from death in patients with PAD.9 We did not investigate the course of B2M during follow-up, but our data demonstrate lower survival consequently higher death rates for patients with elevated B2M.

The presence of elevated B2M levels has been investigated in PAD.11,15,16 In our collective, 43% of our study patients had concomitant PAD. Through adjustment in our multivariate analysis, our results concerning asymptomatic carotid disease and cardiovascular outcome should not be confounded by the presence of concomitant PAD. Our findings might rather substantiate the suspicion that elevated B2M levels are not only present in individuals with peripheral, but also carotid atherosclerosis among the large category of patients with atherothrombosis.

It remains unclear why B2M predicted cardiovascular events better compared with hs-CRP but is in line with the findings on mortality by Shinkai et al14 in older adults. As repeatedly investigated, inflammation is an integral component of the development and progression of atherosclerosis. Multiple data emphasize that progressive atherosclerosis causes inflammation, quantified by inflammatory parameters, and that vice versa inflammation triggers progressive atherosclerosis.2–4,6 Oxidative stress, endothelial dysfunction, neutrophil activation, and cytokine release are key words in this chronic process. hs-CRP is an acute-phase protein and repeatedly shown to be a marker of inflammation and cardiovascular risk.2,3 B2M has been shown to be elevated in high turnover states and in infectious and chronic inflammatory diseases.9–12,21 The surface of lymphocytes and monocytes are particularly rich in B2M, the latter being synthesized to large amounts by lymphocytes and regulated by interferons and proinflammatory monocytic cytokines,8,22 which might explain the pathophysiological “role” in the atherosclerotic process in the vascular endothelium. It has been reported that B2M induces apoptosis or necrosis in tumor cells, and Xie et al suggested that B2M could possibly also induce apoptosis and necrosis in normal cells, including fibroblasts and endothelial cells, and therefore act as a chemoattractant for mononuclear cells and potentially initiate the inflammatory response,23 but this has not been investigated to our knowledge yet. A direct proatherogenic effect for hs-CRP was controversially discussed and refuted given the available data24 but has not been investigated for B2M yet. It therefore remains to be further clarified if B2M is solely a marker of inflammation or if it has a direct pathogenic effect and if other yet unknown confounders may influence B2M levels. In a very recently published proteomic study investigating over 50 different proteins, B2M was identified as a risk marker for coronary heart disease and stroke in postmenopausal women on hormone therapy, and changes in B2M after the initiation...
of hormone therapy might potentially explain hormone therapy effects on cardiovascular disease.\textsuperscript{25}

Also, B2M is a marker for kidney function.\textsuperscript{7} Elevated levels are encountered in patients with impaired renal function and chronic kidney disease and contribute to amyloid deposition.\textsuperscript{11} Renal dysfunction is also known both to trigger atherosclerosis and to worsen cardiovascular outcome and to get aggravated by atherosclerotic processes.\textsuperscript{26} As evidenced by the fact that creatinine and GFR levels were not in a noticeable pathological level and adjustment for renal function was performed in our analysis, renal mechanisms alone seem not plausible to explain the close link between B2M and MACE in this cohort. However, GFR still remains only an approximation of renal function; thus, an underestimated, mild renal insufficiency might potentially go along with elevated B2M levels and increased atherosclerosis and risk

Figure 2. Kaplan–Meier estimates for death, myocardial infarction, and stroke according to quartiles of beta 2 microglobulin (B2M).

Table 3. Estimation of Differences in C Statistics After the Addition of Biomarker B2M and hs-CRP to a Model With Established Risk Factors\textsuperscript{*} for MACE\textsuperscript{†}

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>aROC (95% CI)</th>
<th>Pt</th>
<th>AIC/BIC</th>
<th>IDI (95% CI) Pt</th>
<th>NRI (95% CI) Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline risk factors*</td>
<td>0.6580 (0.6219–0.6941)</td>
<td>Reference</td>
<td>1199.558/1287.662</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Baseline risk factors* + B2M</td>
<td>0.6706 (0.6350–0.7062)</td>
<td>0.1837</td>
<td>1190.475/1293.263</td>
<td>0.0147 (0.0070–0.0224)</td>
<td>0.2976 (0.1630–0.4320)</td>
</tr>
<tr>
<td>in quartiles</td>
<td></td>
<td></td>
<td></td>
<td>P=0.002</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP</td>
<td>0.6633 (0.6276–0.6991)</td>
<td>0.2760</td>
<td>1196.954/1289.953</td>
<td>0.0044 (0.0037–0.0087)</td>
<td>0.2010 (0.0667–0.3360)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.0481</td>
<td>P=0.0004</td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP</td>
<td>0.6633 (0.6276–0.6991)</td>
<td>Reference</td>
<td>1196.954/1289.953</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP + B2M in quartiles</td>
<td>0.6720 (0.6367–0.7072)</td>
<td>0.3388</td>
<td>1189.604/1297.287</td>
<td>0.0126 (0.0053–0.0198)</td>
<td>0.2959 (0.1612–0.4310)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.0007</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

B2M indicates beta 2 microglobulin; hs-CRP, high-sensitivity C-reactive protein; MACE, major adverse cardiovascular events; AIC/BIC, Akaike and Bayesian Information Criteria.

*Baseline risk factors: age, gender, body mass index (kg/m\textsuperscript{2}), smoking, hypertension, low-density lipoprotein cholesterol level (mg/dL), glycated hemoglobin A1 level (%) as an indicator of metabolic control, estimated glomerular filtration rate (mL/min/1.73 m\textsuperscript{2}), history of myocardial infarction, peripheral artery disease, history of stroke, baseline degree of carotid stenosis (in categories), and statin treatment.

†The area under receiver operating characteristic curve (aROC), integrated discrimination improvement (IDI), and net reclassification improvement (NRI) are presented with the according 95% CI.

‡P values are for the comparison with the model with baseline risk factors.
Table 4. Estimation of Differences in C Statistics After the Addition of Biomarker B2M and hs-CRP to a Model With Established Risk Factors* for Death, Myocardial Infarction, and Stroke

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>aROC (95% CI)</th>
<th>P†</th>
<th>AIC/BIC</th>
<th>IDI (95% CI)</th>
<th>P†</th>
<th>NRI (95% CI)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline risk factors*</td>
<td>0.6923 (0.6546–0.7301)</td>
<td>Reference</td>
<td>1016.135/1100.2</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline risk factors* + B2M in quartiles</td>
<td>0.7078 (0.6711–0.7445)</td>
<td>0.0941</td>
<td>1008.072/1110.861</td>
<td>0.0127 (0.0051–0.0202)</td>
<td>0.3184 (0.1969–0.4670)</td>
<td>P = 0.0010</td>
<td>P = 0.0277</td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP</td>
<td>0.6949 (0.6573–0.7326)</td>
<td>0.4339</td>
<td>1015.517/1108.516</td>
<td>0.0031 (–0.0006–0.0068)</td>
<td>0.2010 (0.0067–0.3360)</td>
<td>P = 0.1023</td>
<td>Reference</td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP + B2M in quartiles</td>
<td>0.6949 (0.6573–0.7326)</td>
<td>Reference</td>
<td>1015.517/1108.516</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline risk factors* + hs-CRP + B2M in quartiles</td>
<td>0.7081 (0.6715–0.7447)</td>
<td>0.1432</td>
<td>1008.399/1116.082</td>
<td>0.0113 (0.0040–0.0186)</td>
<td>0.2911 (0.1424–0.4398)</td>
<td>P = 0.0023</td>
<td>P = 0.00010</td>
</tr>
</tbody>
</table>

B2M indicates beta 2 microglobulin; hs-CRP, high-sensitivity C-reactive protein; MACE, major adverse cardiovascular events; AIC/BIC, Akaike and Bayesian Information Criteria.

*Baseline risk factors: age, gender, body mass index (kg/m²), smoking, hypertension, low-density lipoprotein cholesterol level (mg/dL), glycated hemoglobin A1 level (%) as an indicator of metabolic control, estimated glomerular filtration rate (mL/min/1.73 m²), history of myocardial infarction, peripheral artery disease, history of stroke, baseline degree of carotid stenosis (in categories), and statin treatment.

†The area under receiver operating characteristic curve (aROC), integrated discrimination improvement (IDI), and net reclassification improvement (NRI) are presented with the according 95% CI.

‡P values are for the comparison with the model with baseline risk factors.

for MACE and might not be adequately reflected by GFR and hs-CRP. Furthermore, a high percentage in our cohort had arterial hypertension, consistent with the literature, and we adjusted for it as a traditional risk factor in our analysis. Recently, B2M has been shown to be related to arterial stiffness in Japanese subjects,27 and it is known that arterial hypertension, arterial stiffness, endothelial dysfunction, and atherosclerosis are related. An alternative explanation might be that B2M, not covalently attached to the major histocompatibility complex, has a tendency to be released in the systemic circulation19 as a result of chronic ischemia and reperfusion damage that might occur, repeatedly, in patients with vulnerable soft plaques, small vessel disease, or vasoconstriction and reperfusion injury. This may reflect the clear need for further studies and the considerable interest in the development of effective strategies to identify persons at risk, because identifying markers for carotid/or generalized atherosclerosis might help identify people at risk and may finally lead to new and better therapies.

Limitations
We are aware of several limitations of our study. As already stated, 281 patients had to be excluded from our analysis due to loss of follow-up or loss of adequate frozen samples for B2M analysis from the initial 1286 ICARAS patients. However, comparing B2M and baseline demographics in a randomly selected subgroup of 68 of these patients lost for follow-up with our study population, we did not detect any significant differences that could possibly affect our results. Also, selection of patients was hospital-based, displayed by a rather high prevalence of atherosclerotic comorbidities, diabetes, and arterial hypertension compared with a community-based population, although we adjusted for this in our calculations. We defined death by all-cause mortality. We excluded patients with low life expectancy and active malignant disease, and according to our patient population with high prevalence of cardiovascular comorbidities, death from other than cardiovascular causes seems unlikely but cannot be excluded. The study was also not designed to compare B2M levels with persons without cerebrovascular disease; therefore, its role as a widely applicable detection tool for the presence of carotid stenosis, analog to the recent findings in PAD, still remains to be further investigated.

Conclusions
B2M levels are strongly and gradually associated with significantly increased occurrence of MACE and mortality in patients with prevalent asymptomatic carotid atherosclerosis. In our opinion, these findings may add to the significance and
use of a new future tool in the awareness and detection of high-risk patients.

Acknowledgments
We thank Patricia Bankl for excellent technical laboratory assistance.

Disclosures
None.

References
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ONLINE SUPPLEMENT

Supplemental Table
Table S1. Results from the Kaplan Meier estimates for the individual endpoints death, myocardial infarction and stroke according to quartiles of B2M.

<table>
<thead>
<tr>
<th>Quartiles of B2M mg/L</th>
<th>1 (n=245)</th>
<th>2 (n=256)</th>
<th>3 (n=252)</th>
<th>4 (n=252)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(&lt;1.49)</td>
<td>(1.5 to 1.96)</td>
<td>(1.97 to 2.58)</td>
<td>(&gt;2.59)</td>
<td></td>
</tr>
<tr>
<td>Death, No. (%)</td>
<td>17 (6.9)</td>
<td>20 (7.8)</td>
<td>35 (13.9)</td>
<td>70 (27.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial infarction, No. (%)</td>
<td>8 (3.3)</td>
<td>13 (5.1)</td>
<td>11 (4.4)</td>
<td>9 (3.6)</td>
<td>0.77</td>
</tr>
<tr>
<td>Stroke, No. (%)</td>
<td>5 (2)</td>
<td>15 (5.9)</td>
<td>21 (8.3)</td>
<td>11 (4.4)</td>
<td>0.015</td>
</tr>
</tbody>
</table>