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Association of high serum β2-microglobulin levels with poor functional outcomes in patients with acute ischemic stroke

A cohort study

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Abstract

We evaluated the association between serum β2-microglobulin (β2M) levels and prognosis in patients with acute ischemic stroke (AIS) and determined whether the association was affected by any clinical variables. This prospective study included 533 patients with AIS who were admitted to the Hospital of Nanhua Affiliated with the University of South China for treatment from June 1, 2021, to July 31, 2022. Using multiple regression modeling, the association between serum β2M levels and poor functional outcomes—which were classified as being modified Rankin Scale scores of 3 to 6 (composite score of death and major disability), 3 to 5 (major disability), and 6 (death)—were assessed 3 months after stroke onset. At the 3-month follow-up assessment, 209 (47.39%) participants had poor functional outcomes: major disabilities in 150 (34.01%) cases and deaths in 59 (13.38%). After adjusting for important covariates, the group with serum β2M levels in the highest quartile had the highest proportion of individuals with modified Rankin Scale scores of 3 to 6 (odds ratio [OR], 3.54; 95% confidence interval [CI], 1.35–9.33), 3 to 5 (OR, 2.95; 95% CI, 1.21–7.16), or 6 (OR, 1.02; 95% CI, 0.29–3.64) compared with the group having serum β2M levels in the lowest quartiles. The risk prediction for the combined outcome of death and major disability improved after incorporating β2M levels into models that included conventional risk factors. Subgroup analysis revealed a significant impact on the association between serum β2M levels and poor functional outcomes only in patients with AIS whose time from onset to hospitalization was <12 hours (*P* for interaction < .05). Elevated serum β2M levels were associated with poor functional outcomes in patients with AIS, possibly affected by the time from onset to hospitalization.

Abbreviations: AIS = acute ischemic stroke, CI = confidence interval, CNS = central nervous system, Cr = creatinine, CRP = C-reactive protein, cysC = cystatin C, eGFR = estimated glomerular filtration rate, HDL-C = high-density lipoprotein cholesterol, mRS = modified Rankin Scale, NIHSS = The National Institutes of Health Stroke Scale, OR = odds ratio, UA = uric acid, β2M = β2-microglobulin.

Keywords: acute ischemic stroke, poor functional outcome, prognosis, β2-microglobulin

1. Introduction

Stroke is the second leading cause of disability and death in humans due to the continually increasing burden of cardiovascular risk factors and an aging population worldwide. Recent estimates place the lifetime risk of stroke at 25%.[1] In China, 70% of stroke survivors are unable to live alone due to a poor prognosis.[2] An acute ischemic stroke (AIS) event is likely to result in neuronal damage and functional disability, with inflammation and immune responses possibly driving such outcomes.[3,4] Currently, known risk factors and blood biomarkers cannot fully explain the poor prognosis of AIS.[5,6] The expression levels of β2-microglobulin (β2M), a commonly used clinical blood marker, have potentially been overlooked as

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a contributing factor in terms of functional outcomes in patients with AIS.

β2M, comprising a single polypeptide chain of approximately 100 amino acids, is primarily expressed in all nucleated cells. This small protein is eliminated from the blood via the kidneys; however, patients with renal dysfunction have elevated levels of β2M.[7,8] β2M is a structural component of the major histocompatibility complex class 1 molecule that plays an important role in the development and plasticity of the central nervous system (CNS).[9,10] Abnormal levels of β2M are associated with the changes that occur during inflammation and infection or in processes related to immune disorders.[11] Elevated β2M levels are independently associated with cardiovascular disease outcomes,[12] and its presence has been investigated in atrial fibrillation,[13] Alzheimer disease,[14] and peripheral arterial disease.[15] Recently, elevated serum β2M levels have been associated with cognitive impairment, mediated via neuroinflammation and synaptic damage within the CNS.[16,17]

Although some studies have indicated high serum β2M levels might be associated with the severity of brain injury and the risk of ischemic stroke among women[18,19]; however, the effects of β2M on functional prognosis poststroke remain largely unknown. Little data are available to determine whether other factors influence the association between β2M levels and stroke outcomes. Therefore, our research evaluated the relationship between serum β2M levels and poor functional outcomes in patients with AIS, observing that the time from onset to hospitalization influences the association between β2M levels and adverse functional outcomes. This adds a temporal dimension to our understanding of the significance of this biomarker. Moreover, it aids in the early identification of high-risk AIS patients, facilitating closer monitoring and more proactive management strategies to prevent or mitigate functional decline.

2. Materials and methods

2.1. Study population

In this prospective study, we sequentially screened and collected the baseline data of 533 patients with AIS who were admitted to the Hospital of Nanhua Affiliated with the University of South China for treatment from June 1, 2021, to July 31, 2022. Patients were eligible for inclusion if they had been admitted to the hospital within 48 hours of stroke onset and had undergone magnetic resonance imaging evaluations that revealed evidence of AIS. The recruited patients were newly diagnosed by 2 professional neurologists. The exclusion criteria were as follows: (1) history of stroke within 6 months; (2) history of brain surgery and/or trauma, cancer, or autoimmune diseases; (3) presence of severe renal diseases (estimated glomerular filtration rate [eGFR] ≤ 30 mL/min per 1.73 m^2); and (4) incomplete clinical data or loss to follow-up. Ultimately, 441 patients were included in the analysis (Fig. 1).

2.2. Ethics statement

This study protocol was approved by the Institutional Review Board of the Hospital of Nanhua Affiliated with the University of South China (approval number, 2021-ky-51), and registered at clinicaltrials.gov (registration number, ChiCTR2100045815 and registration date, April 25, 2021). This study was performed in accordance with the Declaration of Helsinki and all study participants provided written informed consent for inclusion.

2.3. Baseline data collection

All baseline data were collected at the time of admission by professional neurologists. Data were obtained either from hospital records or through face-to-face interviews with patients, their relatives, or their family physicians; the variables for which data were collected included age, sex, cigarette smoking status, alcohol consumption, body mass index (the weight in kilograms divided by the square of the height in meters), baseline National Institutes of Health Stroke Scale (NIHSS) score, ischemic stroke subtype, time from onset to hospitalization, diastolic and systolic blood pressures on admission, and medical history of hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation, and coronary artery disease.

Clinical laboratory testing for all enrolled patients was conducted in the hospital within 8 hours of admission; the

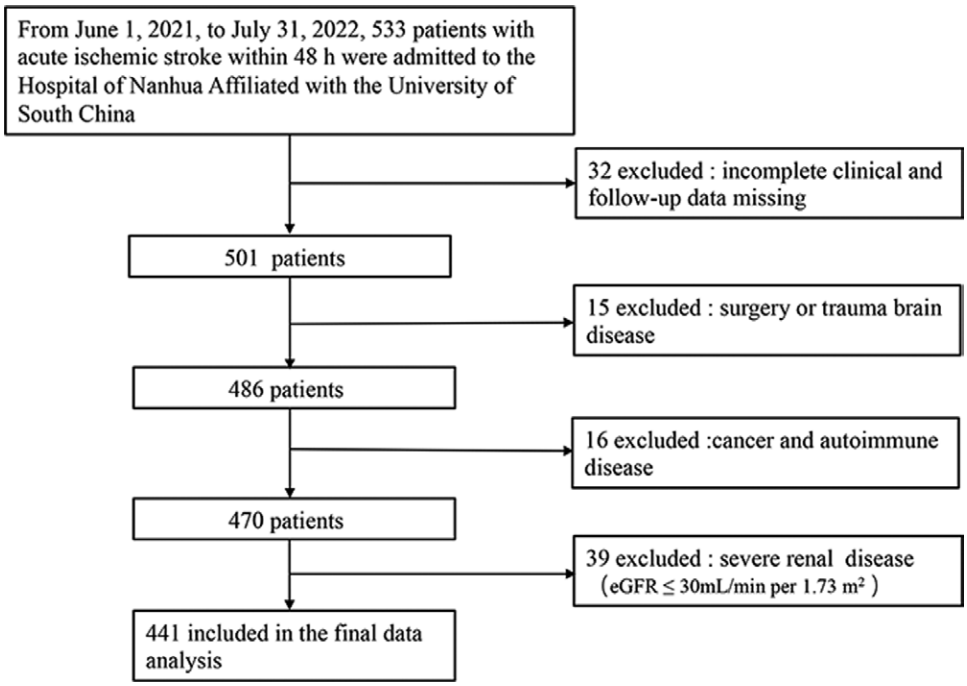

Figure 1. Flowchart of patient selection.

quantified variables included the levels of fasting blood glucose, creatinine (Cr), blood urea nitrogen, uric acid (UA), cystatin C (cysC), triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), and C-reactive protein (CRP). All these serum biochemical parameters were assayed using automatic biochemical cobas® 8000 analyzer series cobas® c 702 modules (Roche Diagnostics, Mannheim, Germany).

The NIHSS score was determined to assess the severity of the patients' neurological deficits upon admission.[20] In all participants, brain magnetic resonance imaging was conducted within 24 hours of admission using a 3.0 T magnetic resonance imaging scanner (SIGNA Pioneer 3.0T, GE HealthCare, Chicago, IL). Ischemic stroke subtypes were classified according to the Trial of ORG 10172 in Acute Stroke Treatment criteria as large artery atherosclerosis (thrombotic), cardiac embolism (embolic), or small artery occlusion lacunae (lacunar).[21] The eGFR was estimated according to age, sex, and serum levels of Cr and cysC using the Chronic Kidney Disease Epidemiology Collaboration equations.[22] Clinical disease history was determined for each participant from the original electronic medical records.

2.4. Blood sample collection and measurement of β2M levels in serum

Blood samples were collected upon admission and stored at -80 °C until testing. Serum levels of β2M were quantified in the clinical laboratory of the Hospital of Nanhua Affiliated with the University of South China using a commercially available β2M assay kit by immunoturbidimetric methods on the cobas® 8000 analyzer series cobas® c 702 modules. The laboratory technicians who performed these measurements were unaware of the study aims.

2.5. Follow-up and outcome assessment

Participants were followed up in person or by telephone 3 months after stroke onset by 2 professional neurologists. Patients' functional outcomes were assessed using modified Rankin Scale (mRS) scores (range, 0–6, with higher scores indicating greater disability), as previously described.[23] Three mRS ranges were used to define poor functional outcomes at 3 months, including a composite of death and major disability (mRS score, 3–6), major disability (mRS score, 3–5), and death (mRS score, 6).

2.6. Statistical analysis

Patients were divided into 4 groups according to the quartiles of the β2M serum concentrations. The Kolmogorov–Smirnov test was used to test the normality of continuous data. Continuous variables with a normal distribution are presented as the means ± standard deviations, whereas data for variables with skewed distributions are presented as medians and interquartile ranges. Categorical variables are described in terms of frequencies or percentages. Intergroup differences were assessed via one-way analysis of variance, the Kruskal–Wallis test, or the chi-square test for normally distributed, skewed continuous, and categorical variables, respectively.

The associations between serum β2M levels and poor functional outcomes were assessed using multivariate logistic regression models. The lowest quartile was defined as the reference group, and the odds ratio (OR) and 95% confidence interval (CI) were calculated for the following 3 regression models: Model I, no adjustment; Model II, adjusted for age and sex; and Model III, adjusted for age, sex, ischemic stroke subtype, baseline NIHSS score, atrial fibrillation, coronary artery disease, and the levels of HDL-C, cysC, Cr, UA, and CRP.

Confounders were identified based on their associations with poor functional outcomes or a difference from the estimated effect of more than 10%. In addition, the C-statistics, net reclassification index, and integrated discrimination improvement were used to assess the incremental prognostic value of incorporating serum β2M levels into the model compared with that based on conventional risk factors alone (covariates in multivariable models).

Interaction and sensitivity analyses were conducted in different subgroups. The interaction effect of subgroup variables on the association between serum β2M levels and poor functional outcomes was examined using likelihood ratio tests in models with interaction terms, with adjustment for the aforementioned covariates. Due to the small percentage of missing data (0–6%), no estimates have been made.

All statistical analyses were conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY) and R statistical software (version 4.1.3, the R Foundation for Statistical Computing, Vienna, Austria). Two-sided P values < .05 were considered statistically significant.

3. Results

3.1. Baseline characteristics of patients

A comparison of the baseline characteristics of the patients is listed in Table 1. The mean age of the patients was 65.96 ± 10.23 years, and 274 (62.13%) patients were males. Compared with the participants with lower serum β2M levels, those with higher levels of β2M were more likely to be older and males; have higher levels of total cholesterol, low-density lipoprotein cholesterol, cysC, Cr, UA, blood urea nitrogen, and CRP; to exhibit a higher prevalence of a history of coronary artery disease; and have a lower eGFR rate and HDL-C level. No significant differences were observed in any of the other observational variables included in the analysis (all $P > .05$).

3.2. Association between serum β2M levels and poor functional outcomes at 3 months

The patients who exhibited poor outcomes involving death and disability had higher β2M levels than those who exhibited good outcomes at 3 months (2.56 ± 0.69 mg/L vs 2.28 ± 0.52 mg/L, respectively; $P < .001$; Fig. 2). After adjusting for age, sex, ischemic stroke subtype, baseline NIHSS score, atrial fibrillation, coronary artery disease, and levels of HDL-C, cysC, Cr, UA, and CRP, the adjusted OR for the highest versus the lowest quartile of β2M levels was 3.54 (95% CI, 1.35–9.33) and 2.95 (95% CI, 1.21–7.16), when the poor functional outcome was defined as an mRS score of 3 to 6 and an mRS score of 3 to 5 at 3 months, respectively.

After adjusting for confounding factors, the association between serum β2M levels and the risk of death was no longer significant (OR, 1.67; 95% CI, 0.66–4.21; Table 2).

3.3. Incremental predictive value of serum β2M levels

We determined whether incorporating serum β2M levels would increase the predictive value for poor functional outcomes after AIS compared with that of the conventional model. As shown in Table 3, the C-statistics and risk reclassification appeared substantially better, based on a significantly improved continuous net reclassification index of 7.73% (95% CI, 3.21–12.25%; $P = .001$) for the composite outcome of death or major disability. However, the discriminatory power of the conventional model was not significantly improved, based on an integrated discrimination improvement of 0.07% (95% CI, –5.29–6.71%; $P = .816$).

Table 1

Baseline characteristics of participants according to quartiles of serum β2-microglobulin levels (N = 441).

Characteristics*	Total	Quartiles of β2M, mg/L				*P* value
		<1.98	1.99–2.27	2.28–2.72	≥2.73	
No. of patients	441	110	106	111	114	
Demographics						
Age, years	65.96 ± 10.23	59.92 ± 8.98	65.57 ± 8.56	67.31 ± 9.97	70.84 ± 10.16	<.001
Male, n (%)	274 (62.13%)	60 (54.55%)	62 (58.49%)	80 (72.07%)	72 (63.16%)	.046
Body mass index, kg/m^2	22.04 (20.31–23.92)	21.50 (16.85–29.06)	22.22 (16.14–32.47)	22.86 (15.04–33.30)	22.04 (16.63–29.90)	.073
Cigarette smoking, n (%)	100 (22.68%)	22 (20.00%)	28 (26.42%)	29 (26.13%)	21 (18.42%)	.358
Alcohol drinking, n (%)	46 (10.43%)	10 (9.09%)	11 (10.38%)	14 (12.61%)	11 (9.65%)	.837
Medical history						
Hypertension, n (%)	363 (82.31%)	91 (82.73%)	87 (82.08%)	86 (77.48%)	99 (86.84%)	.333
Diabetes mellitus, n (%)	134 (30.39%)	25 (22.73%)	34 (32.08%)	33 (29.73%)	42 (36.84%)	.141
Dyslipidemia, n (%)	86 (19.50%)	27 (24.55%)	20 (18.87%)	19 (17.12%)	20 (17.54%)	.477
Atrial fibrillation, n (%)	80 (18.14%)	14 (12.73%)	19 (17.92%)	24 (21.62%)	23 (20.18%)	.334
Coronary artery disease, n (%)	122 (27.66%)	19 (17.27%)	31 (29.25%)	28 (25.23%)	44 (38.60%)	.004
Clinical features						
Time from onset to hospitalization, h	7.00 (4.00–24.00)	8.00 (1.00–48.00)	7.00 (1.00–48.00)	9.00 (1.00–48.00)	7.50 (1.00–48.00)	.898
Baseline NIHSS score	4.00 (2.00–10.00)	4.00 (0.00–20.00)	4.50 (0.00–20.00)	4.00 (0.00–20.00)	5.00 (0.00–35.00)	.701
Admission systolic BP, mm Hg	155.33 ± 23.62	154.14 ± 20.18	154.53 ± 22.71	155.28 ± 24.11	157.26 ± 26.99	.762
Admission diastolic BP, mm Hg	88.41 ± 14.62	89.85 ± 15.45	87.79 ± 12.61	87.75 ± 15.09	88.25 ± 15.16	.474
Ischemic stroke subtype, n (%)						
Thrombotic	140 (31.75%)	42 (38.18%)	33 (31.13%)	35 (31.53%)	30 (26.32%)	
Embolic	67 (15.19%)	13 (11.82%)	18 (16.98%)	16 (14.41%)	20 (17.54%)	
Lacunar	234 (53.06%)	55 (50.00%)	55 (51.89%)	60 (54.05%)	64 (56.14%)	
Clinical laboratory test						
TC, mmol/L	4.55 (3.89–5.19)	4.67 (2.53–9.99)	4.64 (2.34–11.89)	4.55 (2.27–6.82)	4.33 (2.60–8.27)	.046
TG, mmol/L	1.44 (1.03–2.08)	1.43 (0.26–5.97)	1.54 (0.60–5.78)	1.30 (0.52–5.69)	1.56 (0.01–5.62)	.136
HDL-C, mmol/L	1.16 (0.97–1.35)	1.25 (0.66–2.56)	1.11 (0.70–2.32)	1.15 (0.65–2.08)	1.08 (0.43–2.02)	<.001
LDL-C, mmol/L	2.89 (2.27–3.45)	3.01 (1.03–7.47)	2.99 (1.20–5.65)	2.99 (0.90–5.33)	2.67 (1.03–4.96)	.002
eGFR, mL/min per 1.73 m^2	81.62 (68.50–96.35)	102.70 (49.57–162.67)	88.22 (50.91–117.55)	76.19 (55.80–110.11)	61.00 (39.64–104.01)	<.001
cysC, mg/L	1.17 (1.03–1.34)	0.95 (0.62–2.85)	1.11 (0.87–1.50)	1.25 (0.97–1.58)	1.48 (0.99–2.15)	<.001
Cr, mmol/L	77.10 (64.30–90.30)	64.70 (35.50–97.90)	70.30 (45.20–147.10)	79.20 (45.20–131.62)	93.65 (53.60–170.20)	<.001
UA, μmol/L	338.00 (267.00–397.50)	282.00 (90.00–537.00)	342.00 (128.00–621.00)	350.00 (118.00–611.00)	357.00 (170.00–719.00)	<.001
BUN, mmol/L	5.13 (4.10–6.29)	4.44 (1.89–10.99)	4.94 (1.79–12.32)	5.18 (1.78–11.82)	5.79 (2.57–10.57)	<.001
FBG, mmol/L	6.69 (5.46–8.79)	6.56 (2.74–22.69)	6.55 (3.02–22.82)	6.85 (3.29–19.79)	6.69 (2.13–35.53)	.178
CRP, mg/L	2.30 (1.33–5.17)	1.88 (0.07–112.58)	2.31 (0.05–59.73)	2.33 (0.26–55.53)	3.16 (0.40–198.23)	<.001

BUN = blood urea nitrogen, Cr = creatinine, CRP = C-reactive protein, cysC = cystatin C, eGFR = estimated glomerular filtration rate, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, NIHSS = The National Institutes of Health Stroke Scale, TC = total cholesterol, TG = triglyceride, UA = uric acid, β2M = β2-microglobulin.

*Continuous variables were expressed as mean ± SD, or as median (interquartile range). Categorical variables were expressed as frequency (percent).

3.4. Subgroup analysis

The results of the subgroup analyses are shown in Figure 3. The subgroup analyses were conducted according to age (<65 and ≥65 years); sex; time from onset to hospitalization (<12 and ≥12 hours); baseline NIHSS score (<5 and ≥5); ischemic stroke subtype; history of hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation, or coronary artery disease; and eGFR (<90 mL/min per 1.73 m^2 and ≥90 mL/min per 1.73 m^2). The only variable that significantly impacted the association between serum β2M levels and poor functional outcomes was a shorter time from onset to hospitalization (<12 hours): *P* for interaction = .001 for an mRS score of 3 to 6 and =.009 for an mRS score of 3 to 5, with adjusted ORs of 4.89 (95% CI, 2.00–11.95) and 2.32 (95% CI, 1.11–4.85), respectively.

4. Discussion

In this study, we examined the associations between serum β2M levels and various clinical variables and determined whether β2M levels could serve as a prognostic indicator of poor functional outcomes in patients with AIS. The results demonstrated that higher serum β2M levels at the time of admission were independently associated with poor functional outcomes in patients with AIS at the 3-month follow-up. Incorporating β2M levels into the conventional risk model improved the predictive power for the composite outcome of major disability and death. The subgroup analyses revealed that this association was affected by the time from onset to hospitalization, and these findings were not affected by the degree of renal function or neurological dysfunction post-stroke. Though the underlying mechanisms remain obscure, this finding might offer fresh perspectives on the correlation between β2M levels and the prognosis of AIS patients, thereby aiding in the discernment of those at heightened risk for AIS and facilitating the formulation of preemptive intervention strategies.

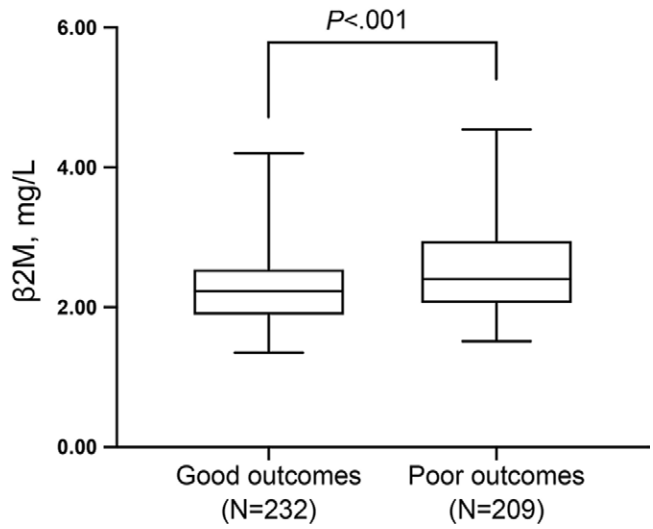

Figure 2. Serum levels of β2M in acute ischemic stroke patients with good and poor outcomes. Data are shown as mean ± standard deviation. *P* values refer to Kruskal–Wallis tests for differences between groups. Good outcomes were defined as mRS scores 0 to 2, while poor outcomes as mRS scores 3 to 6. β2M = β2-microglobulin.

During stroke, cerebral ischemia and hypoxia aggravate the injury through a time-dependent series of mechanisms that drive changes in neuronal excitability, induce microglial activation, and promote oxidative stress, eventually resulting in neuronal death.[4] The extent of functional recovery is affected by local inflammatory changes and the plasticity of neural circuitry post-stroke.[24] β2M is a well-known biomarker of kidney function that reflects the degree of chronic inflammation in the body.[25] β2M also forms the light chain of the major histocompatibility complex class 1 molecule that plays an important role in neural regeneration and synaptic plasticity in the CNS[9,26] and may regulate CNS synapses to influence stroke prognosis.[27]

Using animal models, Smith et al[16] reported that β2M might induce age-related cognitive dysfunction and neurogenesis by triggering inflammatory processes. Based on a rat model of focal cerebral ischemia involving occlusion of the middle cerebral artery, Feng et al[28] also found that inhibiting β2M expression could reduce the infarct volume and improve the degree of cognitive impairment by activating neuroinflammation associated with the nucleotide oligomerization domain-like receptor family pyrin domain-containing protein 3 inflammasome. These findings indicate that higher serum β2M levels are suggestive of a more pronounced inflammatory state at the onset of stroke in the acute phase, which would be more likely to lead to neuronal injury and death and affect the functional recovery of patients.

Direct clinical evidence of a relationship between serum β2M levels and functional outcomes in patients with AIS is lacking. A nested case-control study based on data from 473 patients with ischemic stroke and 473 controls demonstrated that high

Table 2

Adjusted ORs (95% CIs) for poor functional outcomes according to serum β2-microglobulin levels.

	Quartiles of β2M, mg/L					
Poor functional outcomes	**<1.98**	**1.99–2.27**	**2.28–2.72**	**≥2.73**	***P* for trend**	**β2M, mg/L (increase per unit)**
A composite of death and major disability (mRS score 3–6)						
Cases, N (%)	37 (33.64)	54 (50.94)	44 (39.64)	74 (64.91)		209 (47.39%)
Model I	1.00	2.05 (1.18, 3.55)	1.30 (0.75, 2.24)	3.65 (2.10, 6.34)	<.001	2.18 (1.57, 3.02)
Model II	1.00	1.73 (0.98, 3.05)	1.05 (0.58, 1.89)	2.64 (1.45, 4.81)	.004	1.85 (1.29, 2.65)
Model III	1.00	2.46 (1.19, 5.07)	1.23 (0.56, 2.73)	3.54 (1.35, 9.33)	.024	2.88 (1.43, 5.82)
Major disability (mRS score 3–5)						
Cases, N (%)	29 (26.36)	42 (39.62)	26 (23.42)	53 (46.49)		150 (34.01%)
Model I	1.00	1.83 (1.03, 3.26)	0.85 (0.46, 1.57)	2.43 (1.38, 4.26)	.008	1.54 (1.12, 2.10)
Model II	1.00	1.65 (0.91, 2.99)	0.76 (0.40, 1.46)	1.99 (1.08, 3.68)	.059	1.39 (0.98, 1.96)
Model III	1.00	1.88 (1.00, 3.54)	0.91 (0.44, 1.88)	2.95 (1.21, 7.16)	.031	2.05 (1.11, 3.80)
Death (mRS score 6)						
Cases, N (%)	8 (7.27)	12 (11.32)	18 (16.22)	21 (18.42)		59 (13.38%)
Model I	1.00	1.63 (0.64, 4.16)	2.47 (1.02, 5.94)	2.88 (1.22, 6.81)	.012	1.92 (1.29, 2.86)
Model II	1.00	1.37 (0.53, 3.56)	1.97 (0.78, 4.94)	2.04 (0.81, 5.16)	.131	1.63 (1.05, 2.52)
Model III	1.00	1.18 (0.42, 3.26)	1.50 (0.53, 4.22)	1.02 (0.29, 3.64)	.920	1.67 (0.66, 4.21)

Model I: adjusted for none; Model II: adjusted for sex and age; Model III: adjusted for age, sex, ischemic stroke subtype, baseline National Institutes of Health Stroke Scale score, history of atrial fibrillation and coronary artery disease, high-density lipoprotein cholesterol, cystatin C, creatinine, uric acid, and C-reactive protein.

CI = confidence interval, mRS = modified Rankin Scale, OR = odds ratio, β2M = β2-microglobulin.

Table 3

Reclassification and discrimination statistics of β2M and 3-month poor functional outcomes after acute ischemic stroke.

	C-statistic		NRI (continuous), %		IDI, %	
Characteristics	**Estimate (95%CI)**	***P* value**	**Estimate (95%CI)**	***P* value**	**Estimate (95% CI)**	***P* value**
Death or major disability (mRS score 3–6)						
Conventional model	0.827 (0.788,0.866)		Reference		Reference	
Conventional model +β2M	0.844 (0.807,0.881)	.041	7.73 (3.21,12.25)	.001	0.07 (-5.29,6.71)	.816
Major disability (mRS score 3–5)						
Conventional model	0.688 (0.636,0.739)		Reference		Reference	
Conventional model +β2M	0.695 (0.644,0.745)	.509	18.12 (11.66,24.58)	<.001	0.76 (-8.67,7.15)	.851
Death						
Conventional model	0.861 (0.818,0.903)		Reference		Reference	
Conventional model +β2M	0.872 (0.831,0.912)	.133	6.90 (0.38,13.42)	.038	3.98 (-3.01,10.97)	.265

Conventional model included sex, age, ischemic stroke subtype, baseline NIHSS score, coronary artery disease, atrial fibrillation, time from onset to hospitalization, current smoking, body mass index, dyslipidemia, history of hypertension, history of diabetes mellitus, high-density lipoprotein cholesterol and C-reactive protein.

CI = confidence interval, IDI = integrated discrimination index, mRS = modified Rankin Scale, NIHSS = The National Institute of Health Stroke Scale, NRI = net reclassification improvement, β2M = β2-microglobulin.

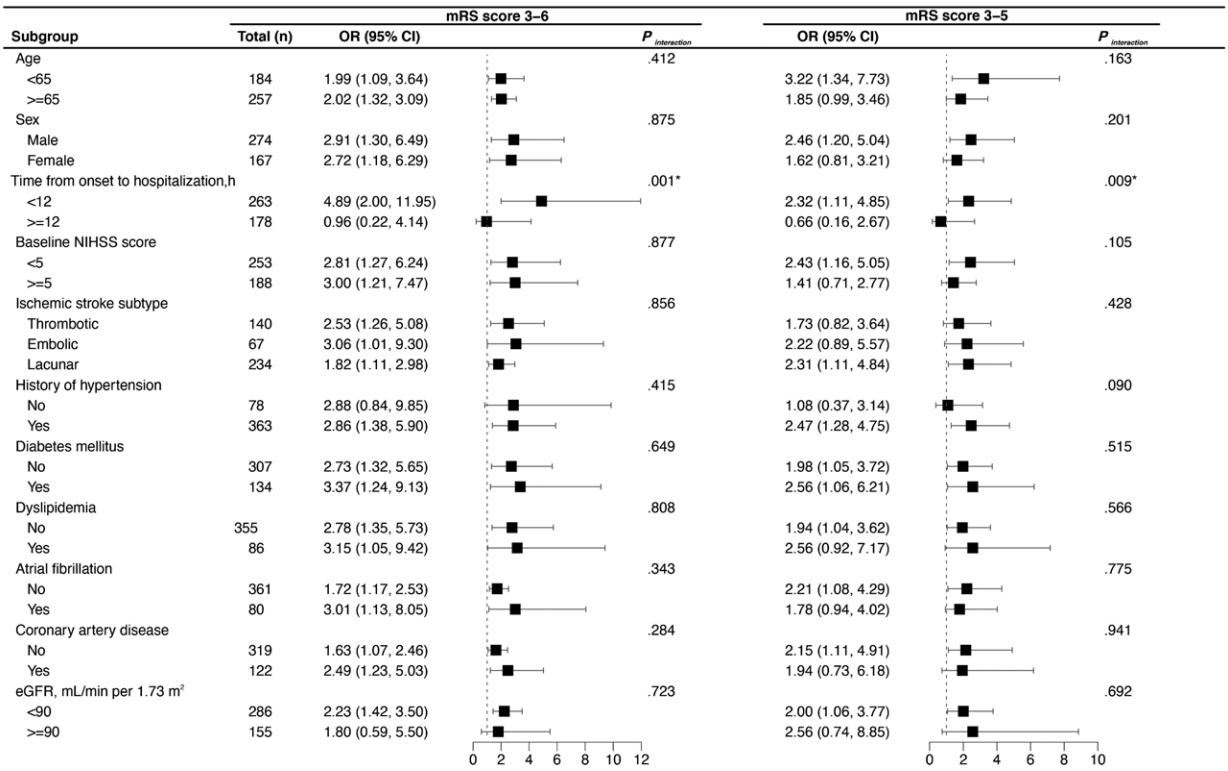

Figure 3. Subgroup analysis for determining the factors affecting the association between serum β2M levels and poor functional outcomes. Adjusted for age, sex, ischemic stroke subtype, baseline National Institutes of Health Stroke Scale score, history of atrial fibrillation and coronary artery disease, high-density lipoprotein cholesterol, cystatin C, creatinine, uric acid, and C-reactive protein. β2M = β2-microglobulin; CI = confidence interval; eGFR = estimated glomerular filtration rate; mRS = modified Rankin Scale; NIHSS = The National Institutes of Health Stroke Scale; OR = odds ratio.

serum β2M levels were associated with an increased risk of ischemic stroke among females, especially in patients who had experienced thrombotic stroke.[18] Hu et al studied 135 patients with ischemic stroke and reported that high β2M levels in serum upon admission increased the risk of recurrence approximately 3 years after the initial event.[29] Finally, a large prospective study revealed that β2M was significantly associated with the occurrence of stroke in patients with asymptomatic carotid atherosclerosis at the 3-year follow-up.[30] Collectively, these studies suggest that serum β2M is a risk factor for stroke and a poor prognosis; however, these studies failed to properly adjust for several important AIS prognostic factors, such as the NIHSS score and kidney function.

In this study, β2M levels were higher in patients who had poor outcomes after 3 months than in those who had good outcomes; however, they were not associated with the NIHSS score at admission. This is consistent with the findings of Hu et al[31] The baseline NIHSS score is particularly important, as it represents the severity of neurological deficits and is a strong predictor of recovery after stroke.[32] Moreover, growing evidence suggests that renal dysfunction and cerebrovascular diseases are associated in several complex ways, indicating that indicators of renal function can also serve as biomarkers of cerebrovascular diseases.[33–35] In the present study, we excluded patients with severe renal impairment from our analysis, and after adjusting for the baseline NIHSS score, renal function,

and other traditional risk factors in the multivariate models, the association between serum β2M levels and poor functional outcomes remained significant and increased compared to that of the unadjusted model. These adjusted factors may explain some, but not all, of the association between serum β2M levels and AIS prognosis. Serum β2M level may affect the prognosis of AIS via other unknown etiological pathways in a way that is independent of the changes driven by traditional mechanisms.

A unique finding of the present study is that the interaction between the time from onset to hospitalization and serum β2M levels had a significant effect on the risk of poor functional outcomes. The exact mechanisms driving this phenomenon are currently unknown. Most physicians will be familiar with the saying "time is brain"; a loss of 1.9 million neurons per minute is observed in acute cerebral ischemia. This loss emphasizes the urgency of seeking treatment as soon as possible after an ischemic event.[36] Indeed, initiating therapy as soon as possible after symptom onset is significantly associated with improved outcomes after AIS.[37,38] If these research findings are further substantiated, it would considerably augment the clinical applicability of serum β2M levels in the acute phase of AIS patients, thus enhancing its value in clinical decision-making and patient management.

Therefore, the findings of the present study appear to be contradictory. Chen et al[28] investigated the time course of changes in β2M expression in a rat model of stroke, demonstrating a discrepancy between the time taken to reach a peak in expression levels in different tissues, wherein the expression peaked at 3 hours in the serum and cerebrospinal fluid but peaked much later at 24 hours in brain tissues. This discrepancy suggested that the sources of β2M release differed and were time-dependent in the acute stage of cerebral ischemia. Within a few hours of AIS occurrence, the massive inflammatory reactions that occur in the early stage can further aggravate brain damage and lead to varying degrees of bodily dysfunction.[39] We hypothesize that these inflammatory responses can upregulate β2M expression in the serum, thereby further affecting the prognosis of AIS. However, with the change in time after ischemic stroke, elevated serum β2M levels may influence functional recovery via the peripheral immune response and changes in neural plasticity in the CNS. Of course, we must consider that the location of the disease and the severity of clinical symptoms are important factors affecting the time of admission. Larger sample sizes and more accurate temporal stratification studies are warranted to test this hypothesis. Overall, the findings of the present study suggest that elevated serum β2M levels can affect the neurological deterioration of patients, thus resulting in poor prognosis. Further, this role may be affected by the time course of events occurring after cerebral ischemia injury.

To the best of our knowledge, this is the first clinical study to perform subgroup and sensitivity analyses to investigate factors mediating the association between β2M levels and AIS prognosis, although the sample size was small. To this end, we made comprehensive adjustments to the models to account for a set of important confounders, which proved to be very stable. The models led to variables being identified that were independent of coexisting factors affecting cardiovascular risk and renal function. However, some limitations should be considered when interpreting this study's results. First, this was a single-center study with a relatively small sample size, and future validation is needed through multicenter studies with greater numbers of participants to ensure that the findings are robust. Second, we only quantified the baseline β2M levels, and the length of the follow-up period was relatively short at just 3 months; therefore, whether the β2M levels would continue to change over longer periods and what role such changes would have on the long-term prognosis remain unknown. Finally, the time from onset to hospitalization affected the association between serum β2M levels and the risk of poor functional outcomes following AIS; however, this observed association might stem from several potential pathways, including neuroinflammation, oxidative stress, or renal dysfunction, all of which can influence outcomes of AIS patients, and the results should be interpreted with caution.

5. Conclusion

In conclusion, this study demonstrated that elevated serum β2M levels, a commonly used blood marker in clinical practice, were related to poor functional outcomes 3 months after AIS. This association was affected by the time from onset to hospitalization and independent of conventional cerebrovascular risk factors and those related to renal function. However, the mechanisms involved remain poorly understood. Therefore, further studies are warranted to better characterize the role of temporal changes in serum β2M levels in the pathogenesis of AIS and their effects on prognosis, to determine whether intervention on serum β2M levels can improve the prognosis of AIS patients.

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