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Retrospective Study

Association of bone turnover biomarkers with severe intracranial and extracranial artery stenosis in type 2 diabetes mellitus patients

INTRODUCTION

Intracranial and extracranial artery stenosis is an established predictor of cerebral infarction[1]. Therefore, a clear understanding of risk factors for intracranial and extracranial artery stenosis is important for the prevention of cerebrovascular events. Atherosclerosis is a critical stage of arterial stenosis, and abnormal bone metabolism is associated with the development of atherosclerosis[2]. Vascular calcification is one of the pathologic mechanisms of atherogenesis, and it is a programmed form of osteogenesis that is induced by inflammatory cytokines in blood vessels. The pathological process involves the trans-differentiation of vascular smooth muscle cells into osteoblasts, which are then associated with the activation of vascular osteogenesis, enhanced bone turnover, and abnormal mineral metabolism.

Atherosclerotic calcification is an independent predictor of the mortality and morbidity of cardiovascular and cerebrovascular diseases[3]. The extent and severity of vascular calcification are a reflection of the atherosclerotic plaque burden, and as an outcome of atherosclerosis, vascular calcification causes vascular sclerosis and dysfunction and is accepted as the basic pathological process in many cardiovascular diseases[4]. Vascular calcification is an active and regulated process with similarities to bone formation and is mediated by many of the same processes that promote bone formation[5]. Calcification of atherosclerotic plaques is considered a complex physiological process mediated by both inhibitor and promoter interactions, including

between osteoclast- and osteoblast-like arterial cells. Plaque calcification is an important factor in plaque instability, which is conducive to plaque rupture and thrombosis. Accordingly, it is a predictor of future cardiovascular events.

Research shows that patients with type 2 diabetes mellitus (T2DM) who experience a transient ischemic attack or an anterior circulation ischemic stroke are more likely to have a lipid-rich necrotic core and vascular plaques with calcification, and the biological processes of bone formation and calcified atherosclerotic plaque share many common features in patients with T2DM[6]. Additionally, vascular calcification is considered a major risk factor for T2DM and is always associated with cardiovascular complications.

Bone turnover biomarkers (BTMs) are indicators of bone metabolism and may be related to the progression of vascular calcification. Bone metabolism is associated with dyslipidemia and diabetes, which are both important risk factors for atherosclerosis. BTM levels are also related to glucose and lipid metabolism[7]. Thus, BTM levels may be useful biomarkers for assessing the risk of cardiovascular events in patients with T2DM.

Osteocalcin (OC) is a biochemical marker of bone formation that specifically mediates bone mineralization and is expressed mainly by osteoblasts. Osteoblasts are also known to secrete endocrine factors that regulate insulin production and adipose tissue metabolism. OC can extend the metabolic endocrine function of bone with obvious extraosseous effects and is vital for not only bone metabolism but also lipid and glucose metabolism. OC can directly regulate energy metabolism, including glucose and lipids, and is involved in the process of vascular calcification affecting the progression of atherosclerosis[8]. OC is also associated with atherosclerotic disease specifically in patients with T2DM[9]. Another BTM, the C-terminal cross-linked telopeptide of type I collagen (CTX), is a marker of bone resorption. Recent clinical research identified CTX as an independent predictor of increased common carotid artery wall intima - media thickness in the elderly population[10]. The BTM procollagen type I N-peptide (PINP) was shown to predict the risk of myocardial infarction in older male patients[11].

Clinical studies to date have mainly studied the associations between different BTMs and atherosclerosis, whereas few studies have explored the correlations between BTM levels and severe intracranial and extracranial artery stenosis in patients with T2DM. Thus, we aimed to determine whether BTMs are associated with intracranial and extracranial atherosclerosis and investigate the value of BTMs as potential indicators for risk assessment and intervention targets for severe intracranial and extracranial artery stenosis in patients with T2DM.

MATERIALS AND METHODS

Study sample and data collection

This cross-sectional study included 257 consecutive patients with T2DM who were hospitalized in our facility between January 2018 and December 2019 and underwent evaluation of intracranial and extracranial arteries. Data were recorded by trained physicians who used a standardized questionnaire to collect medical history information, including age, sex, and history of stroke, coronary artery disease (CAD), hypertension, dyslipidemia, smoking, and alcohol consumption. Diseases were recorded based on codes from the International Classification, Ninth Revision. Physical parameters, including height, weight, and body mass index (BMI), were measured. T2DM was diagnosed according to criteria recommended by the American Diabetes Association in 1997[12]. T2DM diagnosis was based on fasting plasma glucose $\geq$ 7.0 mmol/L or plasma glucose $\geq$ 11.1 mmol/L or hemoglobin (HbA1c) $\geq$ 6.5%. Patients were excluded if they were taking any medication that might influence bone metabolism, such as steroids, thiazides, vitamin D or calcium supplements, antiresorptive or hormone therapy, or were known to have any of the following conditions: acute diabetic complications, malignant tumor, infection, hepatic failure, chronic kidney disease, heart failure, thyroid disease, parathyroid disease, or gastrointestinal disease. The ethics committee of Xuanwu Hospital of Capital Medical University approved this study. All participants provided informed consent.

Biochemical measurements

After overnight fasting for 12 h, peripheral venous blood samples were collected early the next morning for all patients. Fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (Scr), blood urea nitrogen (Bun), and uric acid (UA) levels were measured by an automatic analyzer. The glycated HbA1c level was measured by high-pressure liquid chromatography. Insulin (INS), C-peptide (CP), OC, CTX, and PINP levels were determined using an electrical chemiluminescent immunoassay.

Ultrasonography

Sonographers performed color Doppler ultrasound to obtain images of the common carotid arteries, external carotid arteries, internal carotid arteries, and subclavian arteries. Severe extracranial stenosis was defined as the narrowing of vessel diameter by ≥ 70% in any of these arteries. Sonographers also performed transcranial Doppler ultrasound to obtain images of the anterior cerebral arteries, middle cerebral arteries, posterior cerebral arteries, basilar arteries, and vertebral arteries. Severe intracranial artery stenosis was defined as the narrowing of vessel diameter by ≥ 70% in any of these arteries. According to the results of these examinations, patients were assigned to four groups: No severe intracranial and extracranial artery stenosis (NOCS) group, only severe intracranial artery stenosis (ICAS) group, only severe extracranial artery stenosis (ECAS) group, and combined severe intracranial and extracranial artery stenosis (COAS) group. For analysis, the AS group included all patients with severe intracranial and/or extracranial artery stenosis (ICAS + ECAS + COAS groups).

Statistical analysis

Normally distributed data are presented as the mean ± SD, and data with a skewed distribution are presented as the median (interquartile range). Categorical variables are shown as frequencies (percentages). We used the Mann–Whitney U test to compare

mean values between the NOCS and AS groups. The Kruskal–Wallis test was used to compare mean values among the NOCS, ICAS, ECAS, and COAS groups. When no subgroup had an expected count below five, we used the Pearson χ^2 test. If any subgroup had an expected count below five, we used Fisher's exact test. We performed Spearman correlation analysis to calculate correlation coefficients between BTM levels and glucose and lipid parameters. We used multivariate logistic regression models to estimate the association of artery stenosis with BTM levels. Receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) values were used to examine the ability of BTM levels to predict the incidence of artery stenosis. All statistical analyses were performed with the SPSS Statistical Package (version 26.0). The results were considered statistically significant if the corresponding P value was less than 0.05.

RESULTS

Patient characteristics

Among the 257 T2DM patients included in this study, 136 were female and 121 were male. The mean age of all participants was 66 ± 11 years (range, 36–93 years). The average duration of T2DM among all patients was 12.0 ± 13.0 years. Overall, 87.2% (n = 224) had no stenosis (NOCS group), and 12.8% (n = 33) had some form of stenosis (AS group). The clinical and laboratory parameters for all patients and the groups with and without artery stenosis are presented in Table 1. Compared with the NOCS group, the AS group had a higher percentage of patients with a history of stroke (9.8% vs 27.3%, P = 0.004) and higher levels of HbA1c, OC, CTX, and PINP (all P < 0.05).

The clinical and laboratory parameters of patients categorized further based on the location of artery stenosis are presented in Table 2. Among the 33 patients with T2DM with artery stenosis, 7.4% (n = 19) had only severe intracranial artery stenosis (ICAS group), 3.1% (n = 8) had only severe extracranial artery stenosis (ECAS group), and 2.3% (n = 6) had both severe intracranial and extracranial artery stenosis (COAS group). Compared with that in the NOCS group, the frequency of previous

strokes was higher in all three groups (21.1% in ICAS group *vs* 25.0% in ECAS group *vs* 50.0% in COAS group *vs* 9.8% in NOCS, *P* = 0.009). Compared with the NOCS group, the ICAS group had a lower frequency of hyperlipidemia, and the ECAS group had a higher frequency of hyperlipidemia (21.1% in the ICAS group *vs* 75.0% in the ECAS group *vs* 50% in the NOCS group, *P* = 0.029). The OC levels differed significantly among the four groups (*P* = 0.012). Further statistical analyses determined that the OC levels in the COAS and ICAS groups were higher than those in the NOCS group (*P* = 0.034 and 0.039, respectively). While the ECAS group showed a trend toward higher OC levels compared with the NOCS group, the difference was not significant (*P* = 0.077). Similarly, the CTX levels differed among the four groups (*P* = 0.027), and further statistical analysis showed that the CTX level in the COAS group was higher than that in the NOCS group (*P* = 0.017). The ICAS group also showed a trend toward higher CTX levels than the NOCS group, but this difference did not reach statistical significance (*P* = 0.062). The PINP concentrations were comparable among the four groups, although a weak trend toward a reduced PINP was seen in the NOCS group (*P* = 0.059).

Associations between BTM levels and artery stenosis

We applied four multivariate logistic regression models to identify independent predictors of artery stenosis (Table 3). With the unadjusted Model 1, the OC level was found to be significantly associated with an increased risk of artery stenosis [odds ratio (OR) = 1.123; 95%CI: 1.049–1.203; *P* = 0.001]. A similar association was observed with the model further adjusted for age and sex (Model 2, OR = 1.117; 95%CI: 1.041–1.199; *P* = 0.002). With further adjustment of the model for hypertension, hyperlipidemia, CAD, duration of T2DM, smoking status, and drinking status, the OC level remained a predictor of high risk for artery stenosis (Model 3, OR = 1.117; 95%CI: 1.039–1.201; *P* = 0.003). A significant association between the OC level and artery stenosis persisted after further adjustment of the model for BMI, Scr, Bun, UA, and HbA1c (Model 4, OR = 1.109; 95%CI: 1.029–1.196; *P* = 0.007). CTX and PINP levels were also significantly

associated with artery stenosis in all models with adjustment for different potential confounding factors (all $P < 0.05$).

Correlation of BTM levels and parameters of glucose and lipid metabolism

The results of the Spearman correlation analysis of associations between BTM levels and parameters of glucose and lipid metabolism are presented in Table 4. This analysis showed that the OC level was associated with the levels of TC ($r = 0.185$; $P = 0.003$) and LDL-C ($r = 0.213$; $P = 0.001$). Additionally, the CTX level was associated with the levels of FPG ($r = –0.134$; $P = 0.031$), TC ($r = 0.147$; $P = 0.018$), and LDL-C ($r = 0.197$; $P = 0.003$). The PINP level was associated with the levels of FPG ($r = –0.141$; $P = 0.024$) and LDL-C ($r = 0.129$; $P = 0.039$).

Predictive ability of BTM levels for artery stenosis

The ROC curves for the abilities of BTM levels to predict the incidence of artery stenosis in patients with T2DM are shown in Figure 1. The AUC (95%CI) values were 0.673 (0.580, 0.766) for OC, 0.644 (0.538, 0.751) for CTX, and 0.639 (0.536, 0.742) for PINP. All three BTMs were found to be significant predictors of artery stenosis risk among the patients with T2DM included in this study ($P < 0.001$).

DISCUSSION

In this study, we explored the associations among BTM levels, previous stroke, and the burden and location of intracranial and extracranial artery stenosis in patients with T2DM. We also investigated the correlations among BTM levels and parameters of glucose and lipid metabolism in patients with T2DM. From the cross-sectional data analyzed in this study, we observed a considerable incidence of artery stenosis among patients with T2DM. Moreover, T2DM patients with artery stenosis were more likely to have a history of stroke than those without artery stenosis, regardless of the location of artery stenosis, and the highest incidence of previous stroke was observed among patients with both severe intracranial and extracranial artery stenosis (COAS group).

We also found that BTM levels showed significant correlations with the risk of severe intracranial and extracranial artery stenosis. In this study, circulating OC, CTX, and PINP concentrations were significantly higher in T2DM patients with artery stenosis (AS group) than in those without artery stenosis (NOCS group, Table 1). Some differences were observed among the trends in BTMs according to the location of artery stenosis. OC levels were higher in all subgroups with artery stenosis, whereas CTX levels were only higher in the COAS and ICAS groups than in the NOCS group. Our analyses indicated that all three BTMs were independent risk factors for severe intracranial and extracranial artery stenosis in patients with T2DM, in support of our hypothesis, and these associations were independent of possible confounders. Based on their correlation with indicators of glucose and lipid metabolism, the elevated BTM levels identified a particularly unfavorable metabolic profile, mostly related to dyslipidemia. Higher BTM levels were associated with elevated LDL-C, and the OC level was positively correlated with the TC level. However, the CTX level was negatively correlated with FPG and positively correlated with TC. Furthermore, the PINP level was negatively correlated with FPG. These findings suggest that BTM levels can reflect altered glucose and lipid metabolism as well as atherosclerosis. The role of BTMs in bone metabolism along with their influence on glucose and lipid homeostasis and stroke risk confirms the tight interaction of the cardiovascular–bone metabolism axis. Overall, the present study suggests that BTMs may represent novel biomarkers of accelerated atherosclerosis in patients with T2DM, offering promising tools for cardiovascular risk stratification among patients with T2DM.

T2DM is a common metabolic disease, and its incidence continues to increase worldwide. The incidence of atherosclerosis in patients with T2DM is high, and large vessel disease due to atherosclerosis seriously threatens the life and health of patients with T2DM. Thus, effective strategies for the prevention and treatment of large vessel disease in the context of T2DM are needed to improve public health globally. Even with reductions in traditional risk factors for cardiovascular disease (CVD), achieved *via* better blood lipid control and smoking cessation, the incidence of

CVD remains high. Moreover, blood glucose control does not completely reduce the mortality attributed to atherosclerotic and vascular calcification-related CVD among patients with T2DM. Therefore, other pathological mechanisms of atherosclerosis need to be characterized to support the discovery of therapeutic targets that can be used to identify, diagnose, evaluate, and treat patients with T2DM at high risk of atherosclerosis as early as possible to improve the prognosis of patients with T2DM.

With research advances related to bone health and vascular lesions, the significance and key role of the bone–vascular axis in vascular lesions are increasingly recognized. The instability of atherosclerotic plaques is associated with a higher level of calcification, and severe vascular calcification can be regarded as a nonspecific marker of atherosclerosis. The calcified atherosclerosis burden is considered the main predictor of risk for CVD events and death[13]. T2DM is associated with greater intraplaque calcification volume and a greater proportion of calcification within a plaque. Vascular calcification was also shown to be a powerful risk factor for cardiovascular death in patients with T2DM and results in the development of severe atherosclerosis[14]. Likely, several important mediators of bone mineral homeostasis are also involved in the development of arterial calcification.

Several mineralization markers have been identified in atheromatic plaques[15]. Currently, the effects of BTM levels on atherosclerosis or plaque calcification remain unclear, with conflicting data reported in the literature. Previous studies have mainly examined the relationship between BTM levels and the prevalence of atherosclerotic diseases and found that BTM levels may be predictors of cardiovascular risk in T2DM patients[16]. In patients with T2DM, OC was identified as an independent risk factor for carotid atherosclerosis[17] and shown to play a role in CVD[18]. OC was also found to be deposited at sites of vascular calcification[19]. Another study found that progressive calcification of atherosclerotic plaques was accompanied by a significant increase in OC[20]. Type I collagen is a major collagen component of the intima, media, and adventitia of blood vessel walls and is significantly increased in

atherosclerosis. PINP is a precursor of type I collagen, and CTX is the carboxyl-terminal degradation product of type I collagen. Thus, the expression levels of both BTMs are increased in atherosclerosis accordingly. Abnormal matrix collagen turnover in vessel walls eventually causes arterial stiffness, which is associated with CVD risk, and increased arterial stiffness is associated with both collagen degradation (CTX) and synthesis[21]. Increased osteoclast activity has been observed in T2DM[22], and CTX expression was found in areas of intimal hyperplasia and late-calcified plaques[23]. Gafane *et al*[24] found a positive association between large artery stiffness and the CTX level. Another study found that OC and CTX levels are related to an increased risk of cardiac and carotid calcified plaque development[25]. However, research on the correlation between BTM levels and severe intracranial and extracranial stenosis in patients with T2DM is lacking. The present study found that BTM levels were significantly associated with the risk of artery stenosis in unadjusted and fully adjusted models accounting for potential confounders. These results suggest for the first time that BTMs are independent risk factors for severe intracranial and extracranial stenosis in patients with T2DM. Notably, these associations were independent of other known cardiovascular risk factors in T2DM. Additionally, our results indicate that BTMs may participate in and contribute to the pathogenesis and progression of atherosclerosis.

The mechanisms by which BTMs influence atherosclerosis remain unclear. One possibility is that OC plays an important role in glucose metabolism[26,27]. Consistently, in this study, OC was significantly associated with glucose regulation. We also observed that PINP and CTX levels were negatively correlated with FPG. A previous study found that PINP is negatively correlated with FPG and HbA1c[28]. We also found that all tested BTMs were associated with indicators of dyslipidemia. Barchetta *et al*[29] also reported an association between OC and TC. We also observed differences in glucose and lipid metabolism about artery stenosis in patients with T2DM. Patients with artery stenosis had higher levels of HbA1c than those without artery stenosis. However, indicators of dyslipidemia differed between

patients with intracranial *vs* extracranial artery stenosis in our study. Jin *et al*[30] found that hyperlipidemia was an independent predictor of extracranial artery stenosis but not intracranial artery stenosis, and in the present study, a higher frequency of hyperlipidemia was observed in the ECAS group.

Metabolic variables may represent the underlying mechanism for the association between BTMs and atherosclerosis, as increased BTM levels correlated with several metabolic risk factors and increased atherosclerosis. BTMs may influence glucose and lipid homeostasis, while cardiovascular risk factors can regulate the expression of mineralization markers and promote the calcification process.

The strengths of the present study include overall profiling of BTM levels in patients with T2DM, which has not been reported previously, and extensive correlation analyses between BTM levels and indicators of glucose metabolism, lipid metabolism, and vascular disease, which ultimately allowed us to identify independent associations between BTM levels and atherosclerosis in patients with T2DM. However, this study also has several limitations. Due to its cross-sectional design, we could not establish causal relationships among the correlating factors. Further prospective studies with relevant clinical endpoints are needed to clarify whether BTMs play a causal role in the development of atherosclerosis and to assess the effect of BTM-lowering therapies on the development of atherosclerosis. We plan to carry out large-scale longitudinal studies in the future. Finally, in vivo animal studies are required to elucidate the role of BTMs in the pathogenic mechanisms of atherosclerosis and plaque calcification.

Based on our findings that BTM levels are strongly correlated with artery stenosis risk and disturbance of glucose and lipid metabolism, BTMs should be investigated as indicators for predicting the risk of CVD and as potential therapeutic targets for artery stenosis in patients with T2DM. Multimodal imaging technologies can identify intracranial and extracranial atherosclerosis, which indicates an increased risk of ischemic events or artery stenosis. The combination of such imaging analyses and measurement of serum BTM levels offers a promising strategy

for evaluating the diagnosis, pathogenesis, and progression of intracranial and extracranial stenosis. Therefore, we should test BTMs in patients with T2DM, complete vascular examinations in patients with elevated BTMs, and conduct active monitoring, follow-up, and treatment in this part of the population.

CONCLUSION

Elevated BTM levels correlated with an increased risk of artery stenosis in patients with T2DM as well as with several indicators of metabolic syndrome. Accordingly, BTM levels may serve as circulating endocrine markers that reflect the regulation of glucose and lipid metabolism, thereby reflecting the risk of vascular disease in patients with T2DM. BTMs may also represent potential therapeutic targets for atherosclerosis, and additional research is warranted to explore the underlying mechanism linking BTMs and atherosclerosis. Patients with high BTM levels should be considered a high-risk group in efforts to prevent cardiovascular events.

REFERENCES

1 **Jiang C**, Zhang J, Zhu J, Wang X, Wen Z, Zhao X, Yuan C; CARE-II Investigators. Association between coexisting intracranial artery and extracranial carotid artery atherosclerotic diseases and ipsilateral cerebral infarction: a Chinese Atherosclerosis Risk Evaluation (CARE-II) study. *Stroke Vasc Neurol* 2021; 6: 595-602 [PMID: 33903178 DOI: 10.1136/svn-2020-000538]

2 **Thompson B**, Towler DA. Arterial calcification and bone physiology: role of the bone-vascular axis. *Nat Rev Endocrinol* 2012; 8: 529-543 [PMID: 22473330 DOI: 10.1038/nrendo.2012.36]

3 **Bos D**, Leening MJ, Kavousi M, Hofman A, Franco OH, van der Lugt A, Vernooij MW, Ikram MA. Comparison of Atherosclerotic Calcification in Major Vessel Beds on

the Risk of All-Cause and Cause-Specific Mortality: The Rotterdam Study. *Circ Cardiovasc Imaging* 2015; **8** [PMID: 26659376 DOI: 10.1161/CIRCIMAGING.115.003843]

4 **Demer LL**, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation* 2008; **117**: 2938-2948 [PMID: 18519861 DOI: 10.1161/CIRCULATIONAHA.107.743161]

5 **Towler DA**. Commonalities Between Vasculature and Bone: An Osseocentric View of Arteriosclerosis. *Circulation* 2017; **135**: 320-322 [PMID: 28115412 DOI: 10.1161/circulationaha.116.022562]

6 **Wagenknecht LE**, Divers J, Register TC, Russell GB, Bowden DW, Xu J, Langefeld CD, Lenchik L, Hruska KA, Carr JJ, Freedman BI. Bone Mineral Density and Progression of Subclinical Atherosclerosis in African-Americans With Type 2 Diabetes. *J Clin Endocrinol Metab* 2016; **101**: 4135-4141 [PMID: 27552541 DOI: 10.1210/jc.2016-1934]

7 **Bellissimo MP**, Roberts JL, Jones DP, Liu KH, Taibl KR, Uppal K, Weitzmann MN, Pacifici R, Drissi H, Ziegler TR, Alvarez JA. Metabolomic Associations with Serum Bone Turnover Markers. *Nutrients* 2020; **12** [PMID: 33081124 DOI: 10.3390/nu12103161]

8 **Yang SW**, Hennessy RR, Khosla S, Lennon R, Loeffler D, Sun T, Liu Z, Park KH, Wang FL, Lerman LO, Lerman A. Circulating osteogenic endothelial progenitor cell counts: new biomarker for the severity of coronary artery disease. *Int J Cardiol* 2017; **227**: 833-839 [PMID: 27836295 DOI: 10.1016/j.ijcard.2016.10.036]

9 **Reyes-Garcia R**, Rozas-Moreno P, Jimenez-Moleon JJ, Villoslada MJ, Garcia-Salcedo JA, Santana-Morales S, Muñoz-Torres M. Relationship between serum levels of osteocalcin and atherosclerotic disease in type 2 diabetes. *Diabetes Metab* 2012; **38**: 76-81 [PMID: 21996253 DOI: 10.1016/j.diabet.2011.07.008]

10 **Leli C**, Pasqualini L, Vaudo G, Gaggioli S, Scarponi AM, Mannarino E. Carotid intima-media thickness and bone turnover: the role of C-terminal telopeptide of type I collagen. *Intern Emerg Med* 2010; **5**: 127-134 [PMID: 20182821 DOI: 10.1007/s11739-010-0356-y]

11 **Yeap BB**, Alfonso H, Chubb SA, Byrnes E, Beilby JP, Ebeling PR, Allan CA, Schultz C, Hankey GJ, Golledge J, Flicker L, Norman PE. Proportion of Undercarboxylated

Osteocalcin and Serum P1NP Predict Incidence of Myocardial Infarction in Older Men. *J Clin Endocrinol Metab* 2015; **100**: 3934-3942 [PMID: 26308289 DOI: 10.1210/jc.2015-1899]

12 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693 DOI: 10.1002/ (SICI)1096-9136 (199807)15:7<539::AID-DIA668>3.0.CO;2-S]

13 **Allison MA**, Hsi S, Wassel CL, Morgan C, Ix JH, Wright CM, Criqui MH. Calcified atherosclerosis in different vascular beds and the risk of mortality. *Arterioscler Thromb Vasc Biol* 2012; **32**: 140-146 [PMID: 22034514 DOI: 10.1161/ATVBAHA.111.235234]

14 **Lei MH**, Wu YL, Chung SL, Chen CC, Chen WC, Hsu YC. Coronary Artery Calcium Score Predicts Long-Term Cardiovascular Outcomes in Asymptomatic Patients with Type 2 Diabetes. *J Atheroscler Thromb* 2021; **28**: 1052-1062 [PMID: 33162430 DOI: 10.5551/jat.59386]

15 **Scimeca M**, Anemona L, Granaglia A, Bonfiglio R, Urbano N, Toschi N, Santeusanio G, Schiaroli S, Mauriello S, Tancredi V, Schillaci O, Bonanno E, Mauriello A. Plaque calcification is driven by different mechanisms of mineralization associated with specific cardiovascular risk factors. *Nutr Metab Cardiovasc Dis* 2019; **29**: 1330-1336 [PMID: 31653516 DOI: 10.1016/j.numecd.2019.08.009]

16 **Zwakenberg SR**, van der Schouw YT, Schalkwijk CG, Spijkerman AMW, Beulens JWJ. Bone markers and cardiovascular risk in type 2 diabetes patients. *Cardiovasc Diabetol* 2018; **17**: 45 [PMID: 29571288 DOI: 10.1186/s12933-018-0691-2]

17 **Sheng L**, Cao W, Cha B, Chen Z, Wang F, Liu J. Serum osteocalcin level and its association with carotid atherosclerosis in patients with type 2 diabetes. *Cardiovasc Diabetol* 2013; **12**: 22 [PMID: 23342952 DOI: 10.1186/1475-2840-12-22]

18 **Levinger I**, Brennan-Speranza TC, Zulli A, Parker L, Lin X, Lewis JR, Yeap BB. Multifaceted interaction of bone, muscle, lifestyle interventions and metabolic and cardiovascular disease: role of osteocalcin. *Osteoporos Int* 2017; **28**: 2265-2273 [PMID: 28289780 DOI: 10.1007/s00198-017-3994-3]

19 **Tyson KL**, Reynolds JL, McNair R, Zhang Q, Weissberg PL, Shanahan CM. Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arterioscler Thromb Vasc Biol* 2003; **23**: 489-494 [PMID: 12615658 DOI: 10.1161/01.ATV.0000059406.92165.31]

20 **Polonskaya YV**, Kashtanova EV, Murashov IS, Volkov AM, Kurguzov AV, Chernyavsky AM, Ragino YI. Associations of Osteocalcin, Osteoprotegerin, and Calcitonin with Inflammation Biomarkers in Atherosclerotic Plaques of Coronary Arteries. *Bull Exp Biol Med* 2017; **162**: 726-729 [PMID: 28429221 DOI: 10.1007/s10517-017-3698-x]

21 **Cotie LM**, Currie KD, McGill GM, Cameron AJ, McFadden AS, Phillips SM, MacDonald MJ. Associations between measures of vascular structure and function and systemic circulating blood markers in humans. *Physiol Rep* 2016; **4** [PMID: 27670408 DOI: 10.14814/phy2.12982]

22 **Gilbert MP**, Pratley RE. The impact of diabetes and diabetes medications on bone health. *Endocr Rev* 2015; **36**: 194-213 [PMID: 25738213 DOI: 10.1210/er.2012-1042]

23 **Barascuk N**, Skjøt-Arkil H, Register TC, Larsen L, Byrjalsen I, Christiansen C, Karsdal MA. Human macrophage foam cells degrade atherosclerotic plaques through cathepsin K mediated processes. *BMC Cardiovasc Disord* 2010; **10**: 19 [PMID: 20409295 DOI: 10.1186/1471-2261-10-19]

24 **Gafane LF**, Schutte R, Kruger IM, Schutte AE. Large artery stiffness and carotid intima-media thickness in relation to markers of calcium and bone mineral metabolism in African women older than 46 years. *J Hum Hypertens* 2015; **29**: 152-158 [PMID: 25119885 DOI: 10.1038/jhh.2014.71]

25 **Liu D**, Chen L, Dong S, Peng Z, Yang H, Chen Y, Li L, Zhou H, Zhou R. Bone mass density and bone metabolism marker are associated with progression of carotid and cardiac calcified plaque in Chinese elderly population. *Osteoporos Int* 2019; **30**: 1807-1815 [PMID: 31190121 DOI: 10.1007/s00198-019-05031-5]

26 **Cipriani C**, Colangelo L, Santori R, Renella M, Mastrantonio M, Minisola S, Pepe J. The Interplay Between Bone and Glucose Metabolism. *Front Endocrinol (Lausanne)* 2020; **11**: 122 [PMID: 32265831 DOI: 10.3389/fendo.2020.00122]

27 **Ma H**, Lin H, Hu Y, Li X, He W, Jin X, Gao J, Zhao N, Gao X. Serum levels of osteocalcin in relation to glucose metabolism and carotid atherosclerosis in Chinese middle-aged and elderly male adults: the Shanghai Changfeng Study. *Eur J Intern Med* 2014; **25**: 259-264 [PMID: 24521696 DOI: 10.1016/j.ejim.2014.01.017]

28 **Li W**, Liu X, Liu L, Zhang L, Li M, Liu R, Li T, Chen E, Liu S. Relationships of Serum Bone Turnover Markers With Metabolic Syndrome Components and Carotid Atherosclerosis in Patients With Type 2 Diabetes Mellitus. *Front Cardiovasc Med* 2022; **9**: 824561 [PMID: 35548441 DOI: 10.3389/fcvm.2022.824561]

29 **Barchetta I**, Ceccarelli V, Cimini FA, Bertoccini L, Fraioli A, Alessandri C, Lenzi A, Baroni MG, Cavallo MG. Impaired bone matrix glycoprotein pattern is associated with increased cardio-metabolic risk profile in patients with type 2 diabetes mellitus. *J Endocrinol Invest* 2019; **42**: 513-520 [PMID: 30132286 DOI: 10.1007/s40618-018-0941-x]

30 **Jin H**, Peng Q, Nan D, Lv P, Liu R, Sun W, Teng Y, Liu Y, Fan C, Xing H, Xu K, Huang Y. Prevalence and risk factors of intracranial and extracranial artery stenosis in asymptomatic rural residents of 13 villages in China. *BMC Neurol* 2017; **17**: 136 [PMID: 28720076 DOI: 10.1186/s12883-017-0924-0]

Figure 1 Receiver operating characteristic curve analysis of the diagnostic value of bone turnover biomarker levels for artery stenosis in type 2 diabetes mellitus patients. OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

Table 1 Basic and clinical characteristics of all participants and those in the no severe intracranial and extracranial artery stenosis and artery stenosis groups

Characteristics	All patients (n = 257)	NOCS group (n = 224)	AS group (n = 33)	P value
Age (yr)	66.0 (11.0)	66.0 (11.0)	68.0 (12.0)	0.236
Male, n (%)	121 (47.1)	103 (46.0)	18 (54.5)	0.358
Duration of T2DM (yr)	12.0 (13.0)	12.0 (14.0)	13.0 (11.0)	0.438
Risk factors, n (%)				
Hypertension	189 (73.5)	165 (73.7)	24 (72.7)	0.910
Hyperlipidemia	124 (48.2)	112 (50.0)	12 (36.4)	0.143
CAD	74 (28.8)	63 (28.3)	11 (33.3)	0.548
Previous stroke	31 (12.1)	22 (9.8)	9 (27.3)	0.004
Current smoker	74 (28.8)	67 (29.9)	7 (21.2)	0.303
Current drinker	64 (24.9)	59 (26.3)	5 (15.2)	0.165
BMI (kg/m^2)	25.76 (3.57)	25.78 (3.59)	25.76 (3.45)	0.597
Glucose (mmol/L)	7.48 (3.58)	7.49 (3.53)	6.72 (4.25)	0.563
HbA1c (%)	7.60 (2.50)	7.60 (2.60)	8.40 (2.90)	0.037
INS (μIU/mL)	12.21 (12.71)	12.00 (10.71)	16.82 (18.93)	0.159
CP (ng/mL)	2.33 (1.52)	2.33 (1.53)	2.40 (1.89)	0.754
TC (mmol/L)	4.26 (1.50)	4.27 (1.41)	4.23 (2.25)	0.721
TG (mmol/L)	1.48 (1.38)	1.50 (1.39)	1.41 (1.36)	0.433
LDL-C (mmol/L)	2.38 (1.27)	2.39 (1.20)	2.27 (1.79)	0.781
HDL-C (mmol/L)	1.12 (0.47)	1.13 (0.47)	1.04 (0.38)	0.320
Scr (μmol/L)	62.0 (24.0)	62.0 (22.8)	60.0 (43.0)	0.144
Bun (mmol/L)	5.98 (2.30)	5.92 (2.12)	6.82 (3.60)	0.137
UA (μmol/L)	341.0 (126.5)	342.0 (127.3)	339.0 (1520)	0.823
OC (ng/mL)	10.60 (5.87)	10.27 (5.87)	12.63 (7.07)	0.001
CTX (pg/mL)	0.31 (0.21)	0.29 (0.19)	0.39 (0.30)	0.007
PINP (pg/mL)	34.40 (20.96)	33.39 (20.37)	43.92 (25.50)	0.010

Data are presented as the mean ± SD unless otherwise indicated. NOCS: No severe intracranial and extracranial artery stenosis; AS: Artery stenosis; CAD: Coronary artery disease; BMI: Body mass index; HbA1c: Hemoglobin; INS: Insulin; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Scr: Serum creatinine; Bun: Blood urea nitrogen; UA: Uric acid; OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

Table 2 Basic and clinical characteristics of patients in the no severe intracranial and extracranial artery stenosis, intracranial artery stenosis, severe extracranial artery stenosis and combined severe intracranial and extracranial artery stenosis groups

Characteristics	NOCS group (n = 224)	ICAS group (n = 19)	ECAS group (n = 8)	COAS group (n = 6)	P value
Age (yr)	66.0 (11.0)	65.0 (16.0)	67.5 (13.0)	71.0 (10.0)	0.373
Male, n (%)	103 (46.0)	10 (52.6)	5 (62.5)	3 (50)	0.780
Duration of T2DM (yr)	12.0 (14.0)	15.0 (11.0)	18.0 (20.8)	11.5 (7.0)	0.635
Risk factors, n (%)					
Hypertension	165 (73.7)	13 (68.4)	5 (62.5)	6 (100)	0.527
Hyperlipidemia	112 (50.0)	4 (21.1)	7 (75.0)	2 (33.3)	0.029
CAD	63 (28.3)	7 (36.8)	2 (25.0)	2 (33.3)	0.826
Previous stroke	22 (9.8)	4 (21.1)	2 (25.0)	3 (50.0)	0.009
Current smoker	67 (29.9)	4 (21.1)	1 (12.5)	2 (33.3)	0.682
Current drinker	59 (26.3)	4 (21.1)	1 (12.5)	0 (0)	0.554
BMI (kg/m²)	25.78 (3.59)	26.06 (3.90)	25.32 (2.33)	23.96 (12.48)	0.821
FPG (mmol/L)	7.49 (3.53)	7.83 (4.13)	7.13 (6.89)	5.76 (2.48)	0.446
HbA1c (%)	7.60 (2.60)	7.90 (3.10)	9.15 (1.70)	7.90 (1.80)	0.067

INS (µIU/mL)	12.00 (10.71)	16.99 (19.11)	12.78 (19.72)	15.90 (25.32)	0.334
CP (ng/mL)	2.33 (1.53)	2.52 (1.45)	1.56 (2.95)	2.03 (2.31)	0.599
TC (mmol/L)	4.27 (1.41)	3.85 (1.98)	4.92 (2.35)	4.35 (2.25)	0.535
TG (mmol/L)	1.50 (1.39)	1.59 (1.31)	1.29 (1.31)	1.16 (1.85)	0.585
LDL-C (mmol/L)	2.39 (1.20)	2.27 (1.76)	2.62 (1.88)	2.01 (2.27)	0.943
HDL-C (mmol/L)	1.13 (0.47)	0.98 (0.29)	1.25 (1.21)	1.04 (0.43)	0.170
Scr (µmol/L)	62.0 (22.8)	59.0 (48.0)	78.5 (44.8)	60.0 (40.3)	0.499
Bun (mmol/L)	5.92 (2.12)	6.36 (2.74)	7.92 (6.76)	6.57 (5.00)	0.419
UA (µmol/L)	342.0 (127.3)	358.0 (174.0)	322.5 (153.3)	303.0 (136.3)	0.746
OC (ng/mL)	10.27 (5.87)	12.76 (8.14)	11.89 (4.02)	15.15 (11.43)	0.012
CTX (pg/mL)	0.29 (0.19)	0.41 (0.29)	0.35 (0.23)	0.65 (0.51)	0.027
PINP (pg/mL)	33.39 (20.37)	41.25 (24.71)	47.22 (39.85)	51.28 (37.96)	0.059

Data are presented as the mean ± SD unless otherwise indicated. NOCS: No severe intracranial and extracranial artery stenosis; ICAS: Severe intracranial artery stenosis; ECAS: Severe extracranial artery stenosis; COAS: Combined severe intracranial and extracranial artery stenosis; CAD: Coronary artery disease; BMI: Body mass index; HbA1c: Hemoglobin; INS: Insulin; FPG: Fasting plasma glucose; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Scr: Serum creatinine; Bun: Blood urea nitrogen; UA: Uric acid; OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

Table 3 Association of bone turnover biomarker levels with artery stenosis

Variable	Model	OR (95%CI)	*P* value
OC	Model 1	1.123 (1.049-1.203)	0.001
	Model 2	1.117 (1.041-1.199)	0.002
	Model 3	1.117 (1.039-1.201)	0.003
	Model 4	1.109 (1.029-1.196)	0.007
CTX	Model 1	9.750 (1.759-54.059)	0.009
	Model 2	8.674 (1.529-49.211)	0.015
	Model 3	10.833 (1.725-68.037)	0.011
	Model 4	8.526 (1.189-61.119)	0.033
PINP	Model 1	1.024 (1.006-1.042)	0.010
	Model 2	1.022 (1.003-1.041)	0.025
	Model 3	1.022 (1.003-1.042)	0.021
	Model 4	1.020 (1.001-1.040)	0.044

Model 1: Unadjusted, crude model; Model 2: Adjusted for age and gender; Model 3: Further adjusted for hypertension, hyperlipidemia, coronary artery disease, duration of type 2 diabetes mellitus, current smoker, current drinker; Model 4: Further adjusted for body mass index, serum creatinine, blood urea nitrogen, uric acid, and hemoglobin. OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide.

Table 4 Spearman correlation coefficients for the associations between bone turnover biomarker levels and indicators of glucose and lipid metabolism

Variable	OC		CTX		PINP	
	r	P value	r	P value	r	P value
FPG	-0.102	0.103	-0.134	0.031	-0.141	0.024
HbA1c	-0.012	0.846	-0.022	0.724	-0.012	0.845
INS	-0.050	0.425	-0.086	0.172	-0.031	0.628
CP	0.021	0.741	0.045	0.473	0.067	0.286
TC	0.185	0.003	0.147	0.018	0.112	0.074
TG	0.020	0.753	-0.058	0.351	-0.041	0.514
LDL-C	0.213	0.001	0.187	0.003	0.129	0.039
HDL-C	0.020	0.745	0.062	0.319	0.015	0.811

OC: Osteocalcin; CTX: C-terminal cross-linked telopeptide of type I collagen; PINP: Procollagen type I N-peptide; FPG: Fasting plasma glucose; HbA1c: Hemoglobin; INS: Insulin; CP: C-peptide; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

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1 Yuan Wang, Gang Liu, Haiqing Song, Catherine Cao, Xunming Ji, Guodong Cao. "Elevated Lipoprotein-Associated Phospholipase A2 Is Associated With Intracranial Atherosclerosis", Frontiers in Neurology, 2022
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5 "Abstracts", International Journal of Stroke, 2010
Crossref
25 words — 1%

6 assets.researchsquare.com
Internet
25 words — 1%

7 Manuel Scimeca, Lucia Anemona, Annarita Granaglia, Rita Bonfiglio et al. "Plaque calcification is driven by different mechanisms of mineralization associated with specific cardiovascular risk factors", Nutrition, Metabolism and Cardiovascular Diseases, 2019
Crossref
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8 D. Liu, L. Chen, S. Dong, Z. Peng, H. Yang, Y. Chen, L. Li, H. Zhou, R. Zhou. "Bone mass density and bone metabolism marker are associated with progression of carotid and cardiac calcified plaque in Chinese elderly population", Osteoporosis International, 2019
Crossref

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