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ARTICLE

Association of Bone Mineral Density to Cerebral Small Vessel Disease Burden

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Abstract

Objective
To test the hypothesis that bone mineral loss is mechanistically related to cerebral small vessel disease (SVD), we investigated the relationship between bone mineral density and the prevalence and intensity of SVD among patients with stroke.

Methods
We analyzed data of 1,190 consecutive patients with stroke who were >50 years of age and underwent both brain MRI and dual-energy x-ray absorptiometry from the stroke registry of Chung-Ang University Hospital in Seoul, Korea. The patients were categorized into 3 groups according to their bone mineral density (normal, osteopenia, and osteoporosis). White matter hyperintensities, silent lacunes, cerebral microbleeds, and extensive perivascular space were assessed from brain MRI. Multinomial logistic regression model was used to examine the association between osteoporosis and total SVD score. We also recruited 70 patients with stroke to study serum bone turnover markers and microRNAs related to both cerebral atherosclerosis and bone metabolism to understand bone and brain interaction.

Results
Osteoporosis was determined among 284 patients (23.9%), and 450 patients (37.8%) had osteopenia. As bone mineral density decreased, total SVD score and the incidence of every SVD phenotype increased except strictly lobar cerebral microbleeds. Multinomial logistic regression analysis showed that osteoporosis was independently associated with severe SVD burden. The levels of microRNA-378f were significantly increased among the patients with osteoporosis and maximal total SVD score and positively correlated with parathyroid hormone and osteocalcin.

Conclusions
These findings suggest a pathophysiologic link between bone mineral loss and hypertensive cerebral arteriolar degeneration, possibly mediated by circulating microRNA.

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Glossary

ANOVA = analysis of variance; **BG** = basal ganglia; **BMD** = bone mineral density; **CARPET** = Cerebral Atherosclerosis Research With Positron Emission Tomography; **CI** = confidence interval; **CMB** = cerebral microbleed; **CS** = centrum semiovale; **eGFR** = estimated glomerular filtration rate; **FLAIR** = fluid-attenuated inversion recovery; **miR-378f** = microRNA-378f; **mRS** = modified Rankin Scale; **OR** = odds ratio; **PTH** = parathyroid hormone; **PVS** = perivascular space; **SVD** = small vessel disease; **WMH** = white matter hyperintensities.

Cerebral small vessel disease (SVD) is associated with dementia, stroke, and vascular death.[1,2] Although conventional vascular risk factors such as hypertension and dyslipidemia are related to the presence and progression of cerebral SVD, uncertainties about its exact pathophysiology exist, which hampers effective therapeutic strategy.[1,3] The histologic features of white matter hyperintensities (WMH) of presumed vascular origin, one of the most common cerebral SVD phenotypes, include arteriolar wall thickening caused by hyalinosis and fibrosis, a large perivascular space (PVS) with inflammatory cell deposition, and blood-brain barrier disruption leading to the loss of vascular integrity.[4–6] Accordingly, several studies have found that increased arterial stiffness is positively associated with cerebral SVD burden.[6,7]

Reduced bone mineral density (BMD) is another prevalent and critical medical condition among the elderly because it is associated with increased fracture risk and mortality.[8] A recent study has indicated that osteoporosis and vascular dysfunction are closely related beyond the aging factor.[9,10] Bone density loss has been associated with increased vascular calcification and arteriosclerosis, which can aggravate systemic arterial stiffness and end-organ damage.[9,11] Studies involving elderly people without stroke history have revealed that cerebral WMH and osteoporosis are closely associated with each other.[12,13] However, studies focusing on the relationship between BMD and diverse cerebral SVD phenotypes are rare. Moreover, a pathophysiologic link between bone and cerebral arteries is still illusive. Therefore, we investigated the impact of bone density on cerebral arteriosclerosis among a stroke population.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was reviewed and approved by the Institutional Review Board of Chung-Ang University Hospital (C20131101070). Informed consent was exempted due to the retrospective study design and minimal use of personal information.

Patient Inclusion

Between January 2014 and November 2018, patients admitted to Chung-Ang University Hospital, a tertiary hospital located in the center of Seoul, Korea, due to cerebral infarction or TIA were eligible to be included. Among 1963 patients >50 years of age, consecutive patients who had undergone both brain MRI and bone mineral densitometry were selected from a prospectively registered stroke database, which included clinical, laboratory, and brain imaging information. Hypertension is defined as the use of antihypertensive medications or blood pressure levels of >140/90 mm Hg on repeated measurements after patients had been stabilized. Diabetes is defined as the use of diabetic medications or glycated hemoglobin levels exceeding 6.5% at admission. Combined medical history such as atrial fibrillation, coronary artery disease, and smoking status and laboratory data were obtained from the stroke registry. Stroke mechanism was determined according to Trial of Org 10172 in Acute Stroke Treatment classification criteria.[14] Premorbid neurologic functional status before the index stroke was categorized with the modified Rankin Scale (mRS), which consists of 7 grades from 0 (no functional deficit) to 6 (death), and patients were dichotomized at 2 for statistical analysis.

Cerebral SVD Burden Analysis

Brain MRI was performed with a 3.0T scanner (Avanto; Philips, Eindhoven, the Netherlands), which included at least 5 sequences: diffusion-weighted imaging, fluid-attenuated inversion recovery (FLAIR), susceptibility-weighted imaging, time-of-flight magnetic resonance angiography, and T2-weighted imaging. Cerebral SVD burden analysis included WMH, deep cerebral microbleeds (CMBs), extensive PVS, and silent old lacunes, and the definition of each cerebral SVD was based on recent experts' consensus.[15] In brief, asymptomatic lacunes were defined as round or ovoid lesions with a diameter of 3 to 20 mm in the territory of a perforating arteriole such as the basal ganglia (BG), internal capsule, corona radiata, thalamus, or brainstem, with CSF signal density on FLAIR images and no increased signal on diffusion-weighted images.[15] Multiple lacunes were designated to examine a dose-dependent relationship when there were ≥3 asymptomatic lacunes. PVS was defined as small (<3 mm) punctuate or linear hyperintensities on T2-weighted images, and its presence and extent were measured in 2 different brain axial planes, centrum semiovale (CS_PVS) and BG_PVS levels, with a previously validated scale (grade 0–4).[16] CMBs were small ovoid dark signal voids (<5 mm) with associated blooming observed on susceptibility-weighted sequences,[15] which were further categorized into 2 groups: (1) deep CMBs, located in deep brain structures supplied by perforating arterioles, and (2) strictly lobar CMBs, those CMBs located in cerebral cortices and cerebellum. The

severity of WMH was analyzed at the periventricular level from FLAIR images, which was categorized into 4 groups according to the Fazekas grading system as follows: 0 (no lesion), 1 (punctate lesions), 2 (beginning confluent), or 3 (confluent, completely surrounding lateral ventricles).[17] Fazekas grade 3 WMH was defined as severe WMH. Total SVD score was derived from the summation of following SVD phenotype (from 0–4 points): any lacune, severe WMH with Fazekas grade 3, any CMB, and moderate to severe BG_PVS (grade 2–4).[18]

BMD Measurement

Bone mineralization status was measured by dual energy x-ray absorptiometry (Lunar Prodigy; GE Medical System, Madison, WI) at the lumbar spine (first–fourth vertebrae) and total hip joints following standardized procedures recommended by the International Society for Clinical Densitometry for the diagnosis of osteoporosis in clinical practice.[19] It was performed as a part of routine patient care to prevent fracture after stroke. Patients were categorized into 3 groups according to their hip joint T-score: normal (T1), T ≥ −1.0; osteopenia (T2), T = between −1.0 and −2.5; and osteoporosis (T3), T ≤ −2.5.

Biomarker Cohort

We prospectively enrolled patients with ischemic stroke or TIA to investigate the potential mechanistic link between cerebral SVD and BMD by analyzing serum microRNAs that are known to involve cerebral artery and bone metabolism. The Cerebral Atherosclerosis Research With Positron Emission Tomography (CARPET) study is a prospective registry to understand the pathophysiology of atherosclerosis by applying whole-body ^{18}F-fluorodeoxyglucose PET CT scan among patients with stroke. It had been approved by the Institutional Review Board of Chung-Ang University Hospital (C2015061) and was conducted in accordance with the Declaration of Helsinki. After informed consent, a 5-mL venous blood sample was taken in the morning when the patient had been stabilized after stroke before taking ^{18}F-fluorodeoxyglucose PET CT. Four microRNAs related to bone metabolism and vascular pathology had been selected after extensive literature review. The primer sequence, potential targets, and previous literature of the selected microRNAs are illustrated in table 1. MicroRNA was extracted by using commercial kit (Qiagen, GmbH, Hilden, Germany) after centrifuging serum at 1,000g for 10 minutes, as previously reported.[22] All samples were examined 3 times, and the relative amount of the target microRNA was determined by calculating $2^{-\Delta CT}$ with the ratio between the microRNA and RNA, U6 small nuclear 2 as a reference concentration derived from the standard curve. Osteogenesis-related serum markers such as alkaline phosphatase, osteocalcin, parathyroid hormone (PTH), C-telopeptide of collagen type 1, and 25-hydroxyvitamin D were also examined.

Statistical Analysis

Continuous variables were expressed as mean ± SD and compared by analysis of variance (ANOVA) with post hoc Bonferroni correction. Categorical variables were expressed as the number of patients and percentage and analyzed by linear-by-linear association test. First, we compared clinical and laboratory variables according to BMD status to evaluate the clinical characteristics of patients with stroke with osteoporosis. We found that the patients with stroke with osteoporosis also had increased burden of every SVD phenotype except strictly lobar CMB. Next, we compared clinical and laboratory variables according to total SVD burden, after categorizing included patients into 4 groups according to total SVD burden score (0 = none, 1 = mild, 2 = moderate, 3–4 = severe group). Multinomial logistic regression model was constructed to determine whether osteoporosis is independently related to total SVD burden by including variables derived from bivariate analysis, with a group of no SVD burden as reference. For sensitivity analysis, multivariable logistic regression analysis was conducted to assess whether BMD at the hip joint is independently associated with each subtype of cerebral SVD. The odds ratios (ORs) and confidence intervals (CIs) of the osteopenia and osteoporosis groups were determined to evaluate the dose-response relationship with the normal BMD group used as a reference. We also constructed multivariable logistic regression model with the BMD at the lumbar spine to examine whether BMD loss at a different site would have a similar effect on cerebral SVD.

From the biomarker cohort, we compared the expression levels of microRNAs and bone turnover markers according to BMD status by ANOVA. Correlation analysis was performed among microRNAs and bone turnover markers by Pearson correlation to understand the possible role of microRNA on bone metabolism. Next, the microRNA associated with osteoporosis was examined according to the total SVD burden and each cerebral SVD subtypes. All statistical analyses were performed with SPSS version 22.0 (SPSS, Inc, Chicago, IL) at a 5% significance level.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

A total of 1,190 consecutive patients with stroke were finally included in the stroke registry cohort (mean age 70.5 ± 9.9 years). There were 543 female patients after the exclusion of 773 patients without adequate brain MRI or BMD (figure 1). The comparison of clinical characteristics and laboratory variables according to BMD is shown in table 2. Osteoporosis was determined among 284 patients (23.9%), while 450 patients (37.8%) had osteopenia. The prevalence of osteoporosis was higher among female patients with stroke (238 of 543 patients, 43.8%) compared to male patients with stroke (64 of 647 patients, 7.1%). The patient groups with reduced BMD were significantly older with more hypertension and

Table 1 Primer Sequences and Potential Role of Selected MicroRNAs in Bone and Vascular System

Name	Primer	Bone system	Vascular and Nervous System
miR-17-5p	5′CAAAGUGCUUACAGUGCAGGUAG	miR-17 suppressed bone formation by targeting Smad5 and Smad7[20,21]	miR-17-5p elevated among patients with stroke with unstable plaque and is related to stroke recurrence[22]
miR-93-5p	5′CAAAGUGCUGUUCGUGCAGGUAG	miR-93-5p overexpression inhibited osteoblast mineralization by targeting Sp7[23]	miR-93 regulated vascular endothelial growth factor expression.[24]
Let7i	5′UGAGGUAGUAGUUUGUGCUGUU	Let7i enhanced osteogenesis and repressed adipogenesis by regulating HMGA2[25]	Let7i decreased after coronary artery disease and inhibited TLR4 expression[26] Let7i decreased among patients with stroke and is predicted to regulate leukocyte response[27]
miR-378f	5′ACUGGACUUGGAGCCAGAAG	miR-378 overexpression increased the expression of osteogeneis and angiogenesis-related gene in human bone marrow mesenchymal stem cells[28]	miR-378 inhibited caspase-3 expression and attenuated ischemic injury in cardiomyocytes[29]
			miR-378 controls cardiac hypertrophy targeting mitogen-activated protein kinase pathway factors[30]
RNU6-2	5′CGCAAGGATGACACGCAAATTC	Reference gene	Reference gene

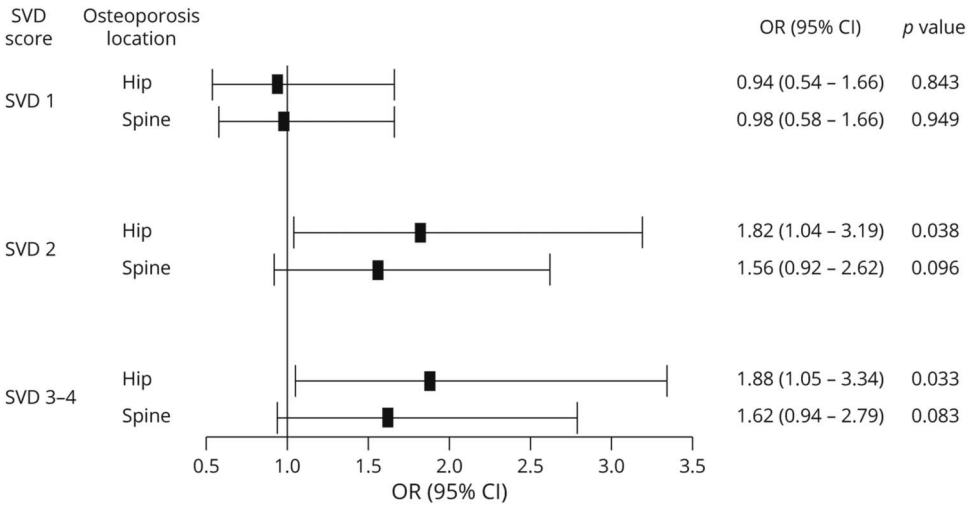
Abbreviations: miR = microRNA; RNU6-2 = RNA, U6 small nuclear 2.

previous stroke. However, the prevalence of diabetes mellitus or atrial fibrillation was not different among the 3 groups. As BMD decreased, body mass index, hemoglobin, and estimated glomerular filtration rate (eGFR) showed decreasing tendency, while high-sensitivity C-reactive protein showed increasing tendency.

Cerebral SVD burden analysis identified the presence of WMH in 956 patients (80.3%), with severe WMH in 131 patients (11.0%). Asymptomatic old lacunes were detected in 772 patients (64.9%), and CMB was noted in 591 patients (49.7%). Deep CMBs were detected in 358 patients (30.1%), and 233 patients (19.6%) had strictly lobar CMBs. Moderate to severe BG_PVS was detected in 358 patients (30.1%), and a similar degree of CS_PVS was present in 623 patients (52.3%). All cerebral SVD phenotypes showed an increasing tendency with decreasing BMD except strictly lobar-type CMB (table 2). Severe SVD burden with total SVD score of 3 to 4 was detected in 264 patients (22.2%), while the zero SVD score group included 240 patients (20.1%). When the patients were compared in terms of SVD severity, mean age, female sex, the presence of hypertension, and previous stroke, the number of patients with premorbid functional deficit (mRS score ≥2) increased as total SVD burden increased (table 3). Total cholesterol, hemoglobin, and eGFR showed decreasing tendency while serum homocysteine showed increasing trend as total SVD burden increased (table 3). The T scores measured at both hip joints and lumbar spines were decreased according to total SVD score increase (table 3). The proportion of patients with osteoporosis was significantly increased with greater SVD burden, and more than one-third of patients with stroke with severe total SVD burden (38.6%) also had osteoporosis at hip joint (table 3). Multinomial logistic regression analysis adjusted for age, sex, hypertension, premorbid mRS score ≥2, history of stroke, eGFR, hemoglobin, total cholesterol, and homocysteine showed that osteoporosis at the hip joint was independently related to increased total SVD burden on the reference group of patients with zero SVD score (SVD 1 group: OR 0.94, 95% CI 0.54–1.66, p = 0.843; SVD 2 group: OR 1.82, 95% CI 1.04–3.19, p = 0.038; SVD 3–4 group: OR 1.88, 95% CI 1.05–3.34, p = 0.033, figure 1). Multinomial adjusted analysis showed that osteoporosis at the spine had a trend of a positive relationship with increased SVD score (SVD 1 group: OR 0.98, 95% CI 0.58–1.66, p = 0.949; SVD 2 group: OR 1.56, 95% CI 0.92–2.62, p = 0.096; SVD 3–4 group: OR 1.62, 95% CI 0.94–2.79, p = 0.083, figure 1).

Next, we analyzed the impact of decreased BMD on each cerebral SVD phenotype and its severity/location by adjusting the forementioned clinical and laboratory variables. Multivariable logistic regression analysis showed that hip joint osteopenia and osteoporosis were an independent predictor of the presence of WMH (osteopenia: OR 1.58, 95% CI 1.09–2.29; osteoporosis: OR 1.61, 95% CI 0.89–2.93) and severe WMH (osteopenia: OR 2.03, 95% CI 1.09–3.78; osteoporosis: OR 4.12, 95% CI 2.03–8.36, table 4). BMD decrease at the spine also independently predicted severe WMH (osteopenia: OR 1.87, 95% CI 1.10–3.19; osteoporosis: OR 2.54, 95% CI 1.43–4.51, table 4). Regarding CMB, osteoporosis at both sites was independently associated with the presence of CMB (osteoporosis at hip: OR 1.57, 95% CI 1.05–2.35; osteoporosis at spine: OR 2.54, 95% CI 1.43–4.51), multiple CMBs (osteoporosis at hip: OR 1.76, 95% CI 1.10–2.81; osteoporosis at spine: OR 1.69, 95% CI 1.12–2.54), and deep CMB (osteoporosis at hip: OR 1.61,

Figure 1 Association Between Osteoporosis and Total SVD Score



Multinomial logistic regression analysis disclosed that osteoporosis at the hip joint was independently associated with maximum total small vessel disease (SVD) group after adjustment for age, sex, hypertension, premorbid neurologic status, stroke history, serum hemoglobin, total cholesterol, estimated glomerular filtration rate, and homocysteine (SVD 1 group: odds ratio [OR] 0.94, 95% confidence interval [CI] 0.54–1.66, $p = 0.843$; SVD 2 group: OR 1.82, 95% CI 1.04–3.19, $p = 0.038$; SVD 3–4 group: OR 1.88, 95% CI 1.05–3.34, $p = 0.033$). Presence of osteoporosis at the spine showed a trend of positive association with the maximum total SVD group (SVD 1 group: OR 0.98, 95% CI 0.58–1.66, $p = 0.949$; SVD 2 group: OR 1.56, 95% CI 0.92–2.62, $p = 0.096$; SVD 3–4 group, OR 1.62, 95% CI 0.94–2.79, $p = 0.083$).

95% CI 1.04–2.48; osteoporosis at spine: OR 1.52, 95% CI 1.04–2.23), but the relationship with strictly lobar CMB was neutral (table 4). Only BMD decrease at the hip joint was independently related to the presence of lacune (osteopenia: OR 1.59, 95% CI 1.16–2.17; osteoporosis: OR 1.92, 95% CI 1.22–3.02) and multiple lacunes (osteopenia: OR 1.69, 95% CI 1.19–2.40; osteoporosis: OR 1.74, 95% CI 1.09–2.40, table 4). Hip joint BMD decrease was also independently related to BG_PVS (osteopenia: OR 1.51, 95% CI 1.06–2.14; osteoporosis: OR 1.90, 95% CI 1.21–2.98), but its relationship with CS_PVS was not significant (osteopenia: OR 1.23, 95% CI 0.92–1.65; osteoporosis: OR 1.00, 95% CI 0.66–1.49, table 4). The impact of spine BMD on lacune or PVS did not reach statistical significance after adjustment for clinical and laboratory variables (table 4).

This prospective cohort study registered 103 patients after informed consent, but 70 patients were finally included after the exclusion of 33 patients without a blood sample. Serum biomarker study disclosed that microRNA-378f (miR-378f) and osteocalcin were markedly elevated among the patients with stroke with osteoporosis (table 5 and figure 2A). The expression of miR-378f was negatively correlated with BMD ($r = -0.253$, $p = 0.063$, figure 2B) and positively correlated with serum PTH ($r = 0.366$, $p = 0.007$, figure 2C) and osteocalcin ($r = 0.422$, $p = 0.002$, figure 2D). When the level of miR-378f was examined according to total SVD burden, patients with increased SVD burden had a significantly higher level of miR-378f compared to those with no SVD burden (0 SVD score 1.3 ± 1.2, 1 SVD score 3.6 ± 4.1, 2 SVD score 6.2 ± 6.1, 3 SVD score 2.4 ± 1.8, 4 SVD score 15.1 ± 20.1, $p = 0.008$, figure 2E). The expression of miR-378f was significantly elevated among the patients with severe WMH (no WMH 2.4 ± 2.2, grade 1 WMH 3.9 ± 4.9, grade 2 WMH 3.2 ± 2.1, grade 3 WMH 11.4 ± 15.6, $p = 0.030$, figure 2F), but it was not significantly altered according to the presence of CMB (no CMB 3.3 ± 3.3, 1 CMB 3.9 ± 3.4, ≤4 or multiple CMBs 4.0 ± 6.3, ≥5 CMBs 9.1 ± 14.0, $p = 0.146$, figure 2G) and lacune (no lacune 4.0 ± 5.7, 1 lacune = 3.7 ± 4.0, ≥2 lacunes 5.2 ± 8.5, $p = 0.791$, figure 2H).

Discussion

This study demonstrated that BMD loss was closely related to the increased burden of overall cerebral SVD and its diverse phenotypes among patients with stroke. The association remained significant after adjustment for clinical and laboratory variables, and a dose-response relationship was suspected between hip joint BMD and WMH. However, its relation with nonarteriosclerotic SVD phenotype was neutral. Circulating miR-378f level was associated with both osteoporosis and severe SVD burden and was correlated with serum PTH and osteocalcin. These findings suggest a pathophysiologic link between bone degeneration and cerebral small arteriolar dysfunction.

Both cerebral SVD and reduced BMD were prevalent among patients with stroke. The systemic derangement of mineral homeostasis is known to deplete bone mineral storage and aggravate ectopic calcification of vessel wall.[31] Several studies have shown that patients with osteoporosis frequently also have arteriosclerosis and vascular calcification.[31,32] Observational studies of postmenopausal women revealed that the presence of carotid atherosclerosis is closely associated with osteoporosis and vertebral fracture.[33,34] Bone metabolism markers such as PTH or vitamin D are related to increased cerebral WMH burden.[35,36] Therefore, it is possible that bone loss may aggravate systemic arterial stiffness through arteriosclerotic degeneration, which deteriorates cerebral small arteriolar dysfunction. Several observational studies reported that osteoporosis is related to profound neurologic sequelae

Table 2 Comparison of Clinical Variables According to Bone Mineral Density

	Normal (n = 456)	Osteopenia (n = 450)	Osteoporosis (n = 284)	*p* Value
Age , mean (SD), y	65.7 (8.7)	70.7 (9.1)	78.1 (7.8)	<0.001
Sex, female, n (%)	85 (18.6)	220 (48.9)	238 (83.8)	<0.001
Hypertension, n (%)	259 (56.7)	291 (64.7)	202 (71.1)	0.001
Diabetes mellitus, n (%)	141 (30.9)	147 (32.7)	102 (35.9)	0.167
Previous stroke, n (%)	70 (15.4)	81 (18.0)	74 (26.1)	0.001
Atrial fibrillation, n (%)	89 (19.5)	73 (16.2)	67 (23.6)	0.293
Previous mRS score >2, n (%)	17 (3.7)	35 (7.8)	61 (21.5)	<0.001
BMI, mean (SD), kg/m^2	24.8 (2.9)	24.1 (3.1)	22.7 (3.6)	<0.001
Waist circumference, mean (SD), cm	88.9 (8.7)	86.6 (10.6)	83.1 (10.0)	<0.001
WBC, mean (SD), ×10^9/L	8.6 (7.1)	7.8 (2.7)	8.1 (3.7)	0.061
Hemoglobin, mean (SD), g/dL	14.3 (1.8)	13.5 (1.8)	12.7 (2.0)	<0.001
Platelet, mean (SD), ×10^9/L	226 (68)	231 (70)	238 (85)	0.114
LDL cholesterol, mean (SD), mg/dL	104.1 (32.7)	105.1 (31.9)	100.8 (34.9)	0.225
FBS, mean (SD), mg/dL	145.5 (63.6)	144.6 (67.1)	138.0 (55.1)	0.255
hsCRP, mean (SD), mg/dL	6.0 (21.8)	6.4 (16.0)	12.0 (34.1)	0.001
eGFR, mean (SD), mL/min/1.73 m^2	84.7 (22.2)	80.0 (27.0)	74.2 (30.2)	<0.001
Stroke etiology by TOAST, n (%)				0.246
Large artery atherosclerosis	128 (28.1)	149 (33.1)	80 (28.2)	
Cardioembolism	93 (20.4)	79 (17.6)	67 (23.6)	
Small vessel occlusion	83 (18.2)	78 (17.3)	37 (13.0)	
Any WMH, n (%)	318 (69.7)	376 (83.6)	262 (92.3)	<0.001
Severe WMH, n (%)	18 (3.9)	44 (9.8)	69 (24.3)	<0.001
Any lacune, n (%)	250 (54.8)	311 (69.1)	211 (74.3)	<0.001
Multiple lacune, n (%)	88 (19.3)	132 (29.3)	92 (32.4)	<0.001
Any CMB, n (%)	193 (42.3)	221 (49.1)	177 (62.3)	<0.001
Multiple CMB, n (%)	79 (17.3)	105 (23.3)	94 (33.1)	<0.001
Deep CMB, n (%)	111 (24.3)	134 (29.8)	113 (39.8)	<0.001
Strictly lobar CMB, n (%)	82 (18.0)	87 (19.3)	64 (22.5)	0.140
BG_PVS, n (%)	87 (19.1)	143 (31.8)	128 (45.1)	<0.001
CS_PVS, n (%)	213 (46.7)	250 (55.6)	160 (56.3)	0.006

Abbreviations: BMI = body mass index; BG_PVS = extensive perivascular space at basal ganglia; CMB = cerebral microbleeds; CS_PVS = extensive perivascular space at centrum semiovale; eGFR = estimated glomerular filtration rate; FBS = fasting blood sugar; hsCRP = high-sensitivity C-reactive protein; LDL = low density lipoprotein; mRS = modified Rankin Scale; TOAST = Trial of. Org 10172 in Acute Stroke Treatment; WBC = white blood cell; and WMH = white matter hyperintensities.
The *p* values were driven by analysis of variance.

and cognitive dysfunction among patients with stroke.[37,38] Our study found that reduced BMD is independently and consistently associated with the presence of hypertensive cerebral SVD burdens such as WMH, PVS, and CMB. However, the effect of reduced BMD on SVD burden was not homogeneous for every SVD phenotype. The relationship between BMD and severe WMH seemed to be most pronounced and dose responsive. Although a significant positive relationship was observed between BMD and overall CMB, there was a neutral relationship between BMD decrement and strictly lobar-type CMB, which is a marker of cerebral amyloid angiopathy.[39] The association between BMD and CS_PVS,

Table 3 Comparison of Clinical Variables According to Total Small Vessel Disease Score

	0 (n = 240)	1 (n = 370)	2 (n = 316)	3–4 (n = 264)	*p* Value
Age, mean (SD), y	65.4 (9.4)	68.7 (9.5)	72.6 (9.4)	75.3 (8.5)	<0.001
Sex, female, n (%)	98 (40.8)	161 (43.5)	141 (44.6)	143 (54.2)	0.003
Hypertension, n (%)	117 (48.8)	225 (60.8)	207 (65.5)	203 (76.9)	<0.001
Diabetes mellitus, n (%)	71 (29.6)	115 (31.1)	115 (36.4)	89 (33.7)	0.156
Smoking, n (%)	67 (27.9)	99 (26.8)	85 (26.9)	40 (15.2)	0.001
Atrial fibrillation, n (%)	47 (19.6)	75 (20.3)	60 (19.0)	47 (17.8)	0.517
Previous stroke, n (%)	15 (6.3)	56 (15.1)	77 (24.4)	77 (29.2)	<0.001
Premorbid mRS score >2, n (%)	3 (1.3)	19 (5.1)	38 (12.0)	53 (20.1)	<0.001
BMI, mean (SD), kg/m^2	24.3 (3.1)	24.1 (3.2)	23.8 (3.3)	23.9 (3.5)	0.259
Waist circumference, mean (SD), cm	87.0 (8.8)	87.2 (9.5)	86.4 (11.6)	85.8 (9.7)	0.322
WBCs, mean (SD), ×10^9/L	8.7 (8.4)	8.2 (4.6)	7.9 (2.8)	8.1 (3.5)	0.273
Hemoglobin, mean (SD), g/dL	13.9 (1.7)	13.8 (1.8)	13.6 (2.0)	13.2 (2.2)	<0.001
Total cholesterol , mean (SD), mg/dL	181.9 (46.6)	174.5 (44.5)	174.3 (48.5)	168.3 (47.5)	0.014
Non-HDL cholesterol, mean (SD), mg/dL	136.9 (44.2)	102.6 (51.8)	130.1 (45.5)	123.6 (42.4)	0.475
Total cholesterol/HDL cholesterol, mean (SD)	4.7 (7.5)	4.0 (1.2)	4.2 (1.4)	4.0 (1.2)	0.086
FBS, mean (SD), mg/dL	145.8 (69.4)	141.5 (60.1)	144.5 (62.1)	143.5 (62.5)	0.845
hsCRP, mean (SD), mg/dL	4.6 (12.9)	7.6 (24.9)	7.9 (22.9)	9.8 (29.6)	0.099
eGFR, mean (SD), mL/min/1.73 m^2	87.9 (24.9)	80.5 (24.8)	78.9 (26.6)	75.3 (28.0)	<0.001
Homocysteine, mean (SD), μmol/L	13.1 (5.5)	13.9 (7.0)	15.1 (8.0)	15.1 (7.4)	0.002
BMD T score at hip, mean (SD)	−1.05 (1.10)	−1.24 (1.06)	−1.69 (1.24)	−2.10 (1.38)	<0.001
BMD T score at spine, mean (SD)	−0.48 (1.88)	−0.62 (1.81)	−1.13 (1.77)	−1.47 (1.91)	<0.001
Osteoporosis at hip, n (%)	27 (11.3)	57 (15.4)	98 (31.0)	102 (38.6)	<0.001
Osteoporosis at spine, n (%)	32 (13.3)	54 (14.6)	81 (25.6)	79 (29.9)	<0.001

Abbreviations: BMD = bone mineral density; BMI = body mass index; eGFR = estimated glomerular filtration rate; FBS = fasting blood sugar; HDL = high density lipoprotein; hsCRP = high-sensitivity C-reactive protein; mRS = modified Rankin Scale; WBC = white blood cell.
p values were driven by analysis of variance.

which is regarded as another phenotype of amyloid angiopathy,[40] was not maintained in adjusted analysis. This selective pattern of relationship advocates the mechanistic link between BMD decrease and hypertensive cerebral arteriolar dysfunction.

A causal relationship between reduced BMD and cerebral SVD burden cannot be ascertained from this study, and the reverse might also be plausible. Observational studies showed that increased cerebral SVD can result in gait disturbance, depression, or reduced overall daily activity,[41,42] which may limit sun exposure and weight-bearing stress on the skeletal system, accelerating BMD loss. Although the proportion of patients with mRS score ≥2 before index stroke became more prevalent as the total SVD score increased, the association between BMD and diverse cerebral SVD phenotypes remained significant after adjustment for premorbid neurologic status and stroke history. It is also possible that degenerated bony microenvironment may lead to reduced endothelial progenitor cell capacity and brain vasculature dysfunction. Hematopoietic stem cells maintain close contact with osteoblasts within the bone marrow niche to preserve their pluripotency.[43] The disruption of the bone marrow microenvironment in the diabetic condition could undermine hematopoietic/endothelial progenitor cell activity and endothelial repair function.[44] Our study population showed a pattern of decreasing hemoglobin and increasing cerebral SVD burden according to BMD loss, suggesting a link between bony degeneration and cerebral arteriolar dysfunction via senescent hematopoietic/endothelial activity. Studies focusing on endothelial regenerative capacity among patients with osteoporosis might be necessary to fully understand the impact of reduced BMD on arteriosclerotic degeneration.

Table 4 Multivariable Logistic Regression Model to Predict Cerebral Small Vessel Disease

	Hip BMD		Spine BMD	
	Osteopenia OR (CI)	Osteoporosis OR (CI)	Osteopenia OR (CI)	Osteoporosis OR (CI)
Any WMH	1.58 (1.09–2.29)	1.61 (0.89–2.93)	0.92 (0.64–1.34)	1.02 (0.60–1.71)
Severe WMH	2.03 (1.09–3.78)	4.12 (2.03–8.36)	1.87 (1.10–3.19)	2.54 (1.43–4.51)
Any CMB	1.14 (0.85–1.52)	1.57 (1.05–2.35)	1.41 (1.06–1.88)	1.61 (1.13–2.31)
Multiple CMB	1.26 (0.87–1.81)	1.76 (1.10–2.81)	1.41 (1.00–1.99)	1.69 (1.12–2.54)
Deep CMB	1.17 (0.85–1.63)	1.61 (1.04–2.48)	1.32 (0.96–1.81)	1.52 (1.04–2.23)
Lobar CMB	1.01 (0.70–1.46)	1.06 (0.64–1.73)	1.21 (0.84–1.73)	1.20 (0.77–1.86)
Any Lacune	1.59 (1.16–2.17)	1.92 (1.22–3.02)	1.30 (0.95–1.77)	1.48 (0.99–2.22)
Multiple lacune	1.69 (1.19–2.40)	1.74 (1.09–2.77)	1.19 (0.85–1.66)	1.13 (0.75–1.70)
BG_PVS	1.51 (1.06–2.14)	1.90 (1.21–2.98)	1.07 (0.76–1.49)	1.21 (0.82–1.80)
CS_PVS	1.23 (0.92–1.65)	1.00 (0.66–1.49)	0.94 (0.70–1.25)	0.84 (0.58–1.20)

Abbreviations: BG_PVS = extensive perivascular space at basal ganglia level; BMD = bone mineral density; CI = confidence interval; CMB = cerebral microbleed; CS_PVS = extensive perivascular space at centrum semiovale; OR = odds ratio; WMH = white matter hyperintensities of presumed vascular origin.
The model was adjusted for age, sex, hypertension, premorbid modified Rankin Scale score ≥2, stroke history, estimated glomerular filtration rate, hemoglobin, total cholesterol, and homocysteine. The OR was derived from the reference of the group with normal bone mineral density.

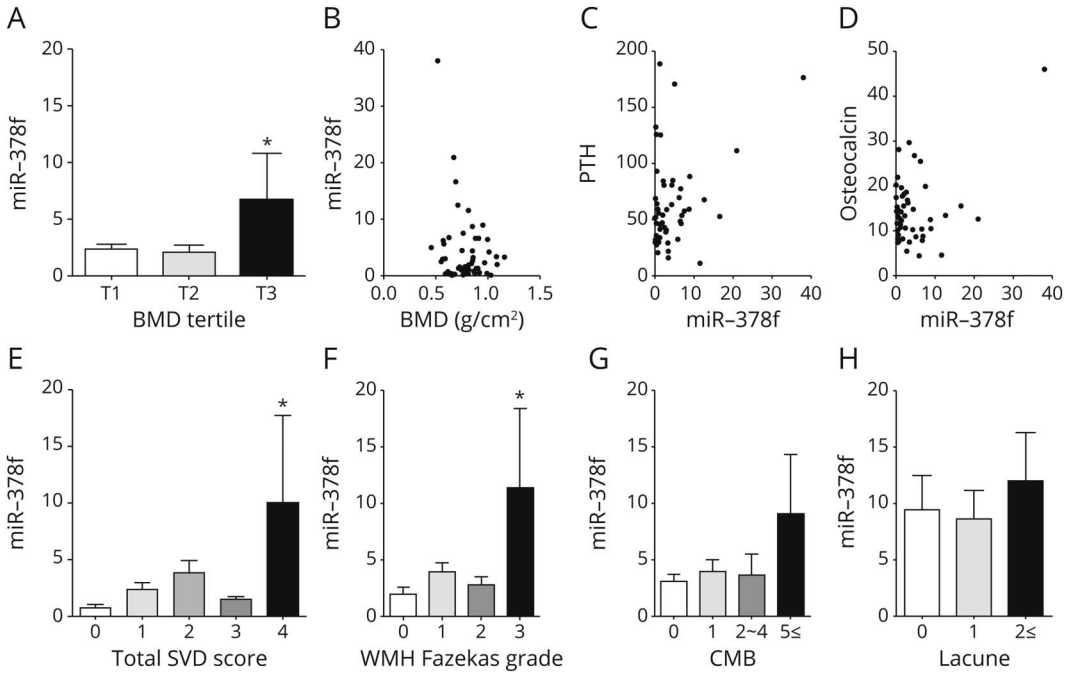
MicroRNAs are small noncoding RNAs that can regulate gene expression by interfering with the posttranscriptional protein coding process, and their expression signature can reflect diverse cardiovascular disorders.[45] The expression of miR-378f was significantly increased among patients with osteoporosis and positively correlated with serum PTH and osteocalcin levels. MiR-378f was upregulated in the group with most severe SVD burden and those with severe WMH. An in vitro study disclosed that miR-378 stimulates gene expression related to both osteogenesis and angiogenesis in human bone marrow mesenchymal stem cells.[28] In vitro and in vivo overexpression of miR-378f attenuates neuronal ischemic injury by regulating apoptosis executioner caspase-3.[29] In the heart, miR-378f prevented cardiac hypertrophy by targeting mitogen-activated protein kinase pathway factors.[30] On the basis of previous study results reporting osteogenic and vascular protective effects of miR-378f, its elevation among patients with osteoporosis and extensive SVD burden may reflect an intrinsic protective mechanism against degenerative processes of bone and brain. Whether the miR-378f level could be a biomarker reflecting SVD burden needs further longitudinal studies.

Table 5 Bone Turnover Markers and MicroRNA Expression Levels According to Bone Mineral Density

	Normal (n = 36)	Osteopenia (n = 19)	Osteoporosis (n = 15)	*p* Value
miR-17-5p relative expression, mean (SD)	24.8 (45.6)	24.1 (43.8)	12.9 (13.3)	0.85
miR-93-5p relative expression, mean (SD)	37.7 (82.7)	29.5 (50.2)	12.7 (14.8)	0.69
let7i relative expression, mean (SD)	106.8 (123.1)	93.0 (106.2)	55.6 (44.2)	0.59
miR-378f relative expression, mean (SD)	3.7 (3.2)	3.3 (4.7)	12.1 (15.9)	0.01
Alkaline phosphatase, mean (SD), U/L	95.7 (97.2)	85.4 (29.3)	75.8 (23.0)	0.66
Calcium, mean (SD), mg/dL	8.9 (0.5)	8.8 (0.5)	8.9 (0.5)	0.48
Osteocalcin, mean (SD), ng/mL	11.9 (4.0)	15.6 (6.6)	18.9 (11.9)	0.02
PTH, mean (SD), pg/mL	49.2 (26.9)	60.3 (38.8)	79.5 (63.7)	0.11
CTX, mean (SD), ng/mL	0.40 (0.19)	1.13 (2.14)	1.76 (3.23)	0.10
25-Hydroxyvitamin D, mean (SD), nmol/L	15.5 (7.7)	15.9 (7.5)	11.8 (6.7)	0.39

Abbreviations: CTX = C-terminal telopeptide of collagen type 1; miR = microRNA; PTH = parathyroid hormone.
Relative expression of the microRNA was determined by calculating $2^{-\Delta Ct}$ with the ratio between the microRNA and RNA, U6 small nuclear 2 as a reference from the standard curve.

Figure 2 Relationship Between miR-378f and BMD-SVD Axis



(A) Patients with stroke with osteoporosis were associated with increased circulating microRNA-378f (miR-378f) levels (relative expression levels of miR-378f: normal 3.7 ± 3.2, osteopenia 3.3 ± 4.7, osteoporosis 12.1 ± 15.9, p = 0.01 by analysis of variance [ANOVA]), and (B) circulating miR-378f levels tended to inversely correlate with the bone density (r = −0.250, p = 0.063. (C) Expression of miR-378f was significantly correlated with (C) serum parathyroid hormone (PTH) (r = 0.366, p = 0.007 by Pearson correlation) and (D) osteocalcin (r = 0.422, p = 0.002 by Pearson correlation). (E) Circulating serum miR-378f was markedly increased among patients with advanced cerebral small vessel disease (SVD) burden (relative expression levels of miR-378f: 0 SVD score 1.3 ± 1.2, 1 SVD score 3.6 ± 4.1, 2 SVD score 6.2 ± 6.1, 3 SVD score 2.4 ± 1.8, 4 SVD score 15.1 ± 20.1, p = 0.008 by ANOVA). (F) Expression of miR-378f was significantly increased among patients with increased cerebral white matter hyperintensity (WMH) (no WMH = 2.4 ± 2.2, grade 1 = 3.9 ± 4.9, grade 2 = 3.2 ± 2.1, grade 3 = 11.4 ± 15.6, p = 0.030 by ANOVA), (G) but its expression was not significantly altered according to the presence of cerebral microbleed (CMB) (no CMB 3.3 ± 3.3, 1 CMB 3.9 ± 3.4, ≤4 multiple CMBs 4.0 ± 6.3, ≥5 CMBs 9.1 ± 14.0, p = 0.146 by ANOVAG) or (H) lacune (no lacune 4.0 ± 5.7, 1 lacune 3.7 ± 4.0, ≥2 lacunes 5.2 ± 8.5, p = 0.791 by ANOVA). BMD = bone mineral density.

Although the pathophysiologic link between cerebral SVD and BMD warrants further investigation, this study demonstrated that patients with lower BMD had higher burden of cerebral SVD, which can synergistically increase the risk of falls and fracture. This study found that ≈38% of patients with stroke with total SVD burden score 3 to 4 also had osteoporosis. Previous studies have shown the high prevalence of low BMD among patients with stroke and the rapid progression of bone mineral depletion after stroke.[46] A recent study demonstrated that the incidence of fracture after stroke is considerable, with its cumulative incidence functions of ≈4.4% the first year, 8.1% at 2 years, and 13.0% at 4 years after stroke.[47] Screening and early intervention for low BMD among patients with stroke may be an efficient strategy to prevent stroke-associated fracture. Moreover, several studies have shown the vascular protective potential of vitamin D supplementation and bisphosphonate.[48,49] Our group reported that vitamin D deficiency is related to chronic cerebral SVD burdens among patients with stroke, suggesting that vitamin D deficiency could be an effective therapeutic target for both reduced BMD and increased cerebral SVD.[36] Future studies may be necessary to investigate whether intervention for low BMD in the stroke population could also attenuate the progression of cerebral SVD.

Several limitations exist in this study. First, not every admitted patient with stroke underwent BMD evaluation, which may result in selection bias. The patients who did not undergo BMD evaluation tended to be older with more severe neurologic deficit than the patients with BMD. Exclusion of these patients might have attenuated the strength of relationship between cerebral SVD burden and BMD loss. The time delay from stroke onset to BMD evaluation was not homogeneous because the patients underwent bone density evaluation when their neurologic status had been stabilized. Because bone loss starts immediately after vascular injury and aggravates until several months after stroke, the impact of stroke on BMD could be variable among patients.[46] Second, in vitro and in vivo mechanistic study of the role of miR-378f on the bone-brain axis has not been performed. However, we performed in-depth analyses with variable SVD phenotype from brain MRI and blood markers on bone metabolism to understand its functional implication. Longitudinal studies investigating the miR-378f level in relation to osteoporosis treatment and SVD progression may disclose the impact of miR-378f on SVD pathogenesis and its biomarker potential. Last, this study was conducted from a single center. The prevalence of CMB (49.7%) seems to be higher than previous reports, but recent studies from Asian populations show similar prevalence of

CMB between 40% and 51%.[36,50] The prevalence of CMB could be variable according to the demographic characteristics of included patients and brain MRI protocols. Therefore, more studies involving multiple ethnic groups and institutions are required to generalize our findings.

Decreased BMD was accompanied by increased cerebral SVD burden among patients with stroke, especially phenotypes related to hypertensive arteriopathy. The intimate association between the 2 pathologic conditions suggests a mechanistic link between the 2 distinct organs, possibly mediated by circulating microRNAs.

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