A cohort study of relationship between serum calcium levels and cerebral microbleeds (CMBs) in ischemic stroke patients with AF and/or RHD

Junfeng Liu (MD)b, Deren Wang (PhD)b, Yao Xiong (MD)a, Bian Liu (MD)a, Chenchen Wei (MD)a, Zhenxing Ma (PhD)a, Bo Wu (PhD)a, Ruozhen Yuan (MD)a, Hehan Tang (MD)b, Ming Liu (PhD)a,*

Abstract
Calcium is an essential element for life and has cerebroprotective property in stroke patients. Low serum calcium levels were found to be related to large hematoma volumes in intracerebral hemorrhagic patients and hemorrhagic transformation in ischemic stroke patients after thrombolysis. However, their impact on hemorrhage-prone small vessel disease represented by cerebral microbleeds (CMBs) is uncertain. We aim to investigate whether low serum calcium levels are associated with presence and location of CMBs.

Ischemic stroke patients with atrial fibrillation (AF) and/or rheumatic heart disease admitted to our hospital were consecutively and prospectively enrolled. Demographic and clinical information were collected and analyzed according to the occurrence and location of CMBs, and levels of serum calcium. We used logistic regression analysis to estimate the multivariable adjusted relationship between serum calcium levels and the presence or location of CMBs.

Among the 67 patients (28 males; mean age, 67.3 years) in the final analysis, 39 (58.2%) were found to have CMBs. After adjustment for age, sex, smoking habits, drinking habits, and renal impairment, the presence of CMBs and deep CMBs was, respectively, 4.96- and 4.83-fold higher in patients with lower serum calcium levels (<2.15 mmol/L) than in patients with higher serum calcium levels.

Lower serum calcium levels (<2.15 mmol/L) are independently associated with the presence of CMBs and deep CMBs in ischemic stroke patients with AF and/or rheumatic heart disease, which should be verified and extended in large cohorts, with other types of stroke patients and the general population.

Abbreviations: AF = Atrial fibrillation, CMBs = Cerebral microbleeds, HDL = high-density lipoprotein, ICH = Intracerebral hemorrhage, IS = Ischemic stroke, LDL = low-density lipoprotein, NIHSS = National Institutes of Health Stroke scale, RHD = Rheumatic heart disease, SWI = Susceptibility-weighted imaging, TG = triglycerides.

Keywords: atrial fibrillation, cerebral microbleeds, ischemic stroke, rheumatic heart disease, serum calcium levels

1. Introduction
Cerebral microbleeds (CMBs) are detected as small, rounded hypointense lesions on T2* or susceptibility-weighted imaging (SWI), and are focal hemosiderin deposit resulting from minimal blood leakage from damaged small vessels, which are regarded as markers of pathological vascular changes.[1,2] CMBs are frequently observed in patients with intracerebral hemorrhage (ICH),[3,11] ischemic stroke (IS),[3,4] vascular cognitive impairment,[5] and even in healthy elderly individuals.[6] Considering those, CMBs can predict risk of subsequent stroke, including ICH and IS,[3,4] and are also a predictor of cognitive impairment and neurodegeneration,[6,8] which has evoked great attention for its adverse effect on individuals.

The pathophysiologic features of CMBs can differ according to their location, with lobar (strictly in the lobar region) CMBs attributable to cerebral amyloid angiopathy, whereas deep (basal ganglia, thalamus, and brainstem) CMBs are indicative of hypertensive vasculopathy.[1,9,10] Despite those, the molecular mechanisms involved in CMBs are still argued and remain to be clarified.

Calcium is an essential element for life and has cerebroprotective property in stroke through neurovascular mechanism. According to the previous studies, low serum calcium levels attribute to poor outcome, extensive infarction in patients with IS, and large hematoma volumes in patients with ICH.[11–14] Recently, a study[15] in China indicated that low serum calcium
was independently associated with hemorrhagic transformation in acute IS patients after thrombolysis. However, little attention has been paid to its roles in hemorrhage-prone small vessel disease represented by CMBs. The Kashima Scan Study,[16] a population-based cohort study, demonstrated that hypertension and lower calcium intake had joint effects on the risk of CMBs in healthy individuals.

Considering all these aforementioned studies about calcium, we studied their relation to CMBs in IS patients with atrial fibrillation (AF) and/or rheumatic heart disease (RHD), testing the hypothesis that low level of serum calcium is correlated with presence and the location of CMBs.

2. Methods

This research project was carried out under the auspices of the National Natural Science Foundation of China “Study on small vessel pathological mechanism of cerebral hemorrhage after cardioembolic stroke using SWI markers.” The study protocol was approved by the biomedical ethics committee of West China Hospital. Written informed consent was obtained from participants or their guardians.

IS patients with AF and/or RHD were prospectively and consecutively enrolled after admission to West China Hospital, Sichuan University, Chengdu, China, between January 2014 and September 2015. To be enrolled, patients had to have a diagnosis of stroke according to World Health Organization criteria,[17] further confirmed by computed tomography scanning or magnetic resonance imaging. AF was defined as a history of persistent AF or paroxysmal AF, supported by past ECG or diagnosed by the attending physicians based on ECG and/or 24-h ECG monitoring during admission.[18] RHD was diagnosed according to International Classification of Diseases, 10th ed., criteria and further confirmed by echocardiography.[19] Patients were excluded from the study if they were reluctant to participate in the registration; if they did not undergo SWI or serum calcium analysis or if serum calcium levels were not obtained within 48 h after admission.

A standardized form was used to collect data on patient baseline information, including demographic characteristics, stroke severity on admission, risk factors (including hypertension, diabetes mellitus, hyperlipidemia, previous transient ischemic attacks, history of stroke, and current smoking and alcohol consumption), renal impairment (medical history or eGFR <60 mL/min/1.73 m²), hypertension (all criteria), further confirmed by echocardiography.[19] Patients were excluded from the study if they were less than 18 years of age or had a history of previous IS. A total of 140 consecutive IS patients with AF and/or RHD were enrolled in the study, but 48 (34.3%) were excluded because they did not complete SWI and another 25 (17.9%) were excluded because serum calcium levels within 48 h after admission were unavailable. Of 67 patients included in the final analysis, 28 (41.8%) were men, and mean age at stroke onset was 67.3 ± 11.98 years.

CMBs were detected in 39 patients (58.2%). Of these, 14 had single CMBs, and 25 had multiple CMBs (≥2). CMBs were most commonly present as deep or infratentorial bleeding (26/39, 67%), followed by strictly lobar bleeding (13/39, 33%). Baseline characteristics of IS patients with AF and/or RHD are shown in Table 1 according to whether they had CMBs. Patients with CMBs were more likely to be older and have history of hypertension (all P < 0.05, Table 1).

In the study, the serum calcium levels ranged from 1.89 to 2.57 mmol/L (mean value, 2.22 mmol/L). The distributions of serum calcium levels according to CMBs presence and location are shown in Fig. 1. Patients were subgrouped into tertiles based on serum levels of calcium within 48 h after admission (Table 2): tertile1, <2.15 mmol/L; tertile2, 2.15–2.26 mmol/L; and tertile3, >2.26 mmol/L.

As shown by Table 2, patients in the lowest tertile of serum calcium were more likely to be older and have renal impairment compared with patients in the highest tertile. Considering the association between CMBs and the tertiles of serum calcium, subjects in lowest tertile of serum calcium were associated with higher presence of CMBs (P = 0.01) and deep CMBs (P = 0.02), but not with strictly lobar CMBs (Table 2). However, relationships between the tertiles of serum calcium and CMBs or deep CMBs were no significant after adjusting for age, sex, smoking habits, drinking habits, and renal impairment (P > 0.05).

When serum calcium levels were dichotomized comparing the lowest tertile to all other categories, patients with serum calcium levels ≤2.15 mmol/L had higher presence of CMBs (P = 0.005, OR = 4.84, 95% CI 1.53–15.34) and higher risk of deep CMBs.
Table 1
Baseline characteristics of ischemic stroke patients with AF/RHD with and without CMBs.

|                        | with CMBs n=39 | without CMBs n=28 | P     |
|------------------------|----------------|-------------------|-------|
| Male, n (%)            | 19 (48.7)      | 9 (32.1)          | 0.18  |
| Age, mean±SD           | 69.85±11.33    | 63.71±12.14       | 0.04  |
| Current smoking, n (%) | 6 (15.4)       | 5 (17.9)          | 0.79  |
| Alcohol intake, n (%)  | 4 (10.3)       | 6 (21.4)          | 0.21  |
| Hypertension, n (%)    | 21 (53.8)      | 8 (28.6)          | 0.04  |
| Diabetes mellitus, n (%)| 11 (28.2)    | 11 (39.3)         | 0.34  |
| Renal impairment, n (%)| 7 (17.9)       | 3 (10.7)          | 0.41  |
| Previous TIA/stroke, n (%)| 8 (20.5)   | 10 (35.7)         | 0.17  |
| NIHSS on admission, mean±SD | 7.62±5.86   | 10.82±7.57        | 0.06  |
| Total cholesterol (mmol/L), mean±SD | 4.08±0.98   | 4.05±0.98         | 0.90  |
| TG (mmol/L), mean±SD   | 1.56±1.57      | 1.37±0.80         | 0.56  |
| HDL (mmol/L), mean±SD  | 1.40±0.36      | 1.34±0.32         | 0.49  |
| LDL (mmol/L), mean±SD  | 2.29±0.86      | 2.26±0.80         | 0.86  |
| Serum calcium (mmol/L), mean±SD | 2.20±1.45   | 2.23±0.73         | 0.31  |

AF=atrial fibrillation, CMBs=cerebral microbleeds, HDL=high-density lipoprotein, LDL=low-density lipoprotein, NIHSS=National Institutes of Health Stroke scale, RHD=rheumatic heart disease, TG=triglycerides.

Figure 1. Distributions of Serum Ca levels according to CMB status.

Table 2
Distribution of demographic and clinical characteristics across different serum calcium tertiles.

|                        | Tertile 1 (n=25) (<2.15) | Tertile 2 (n=21) (2.15–2.26) | Tertile 3 (n=21) (>2.26) | P     |
|------------------------|---------------------------|-------------------------------|--------------------------|-------|
| Male, n (%)            | 13 (52.0)                 | 8 (38.1)                      | 7 (33.3)                 | 0.41  |
| Age, mean±SD           | 72.32±12.11               | 65.43±11.45                  | 63.14±10.64              | 0.02  |
| Current smoking, n (%) | 6 (24.0)                  | 4 (19.0)                      | 1 (4.8)                  | 0.20  |
| Alcohol intake, n (%)  | 3 (12.0)                  | 2 (9.5)                       | 5 (23.8)                 | 0.38  |
| Hypertension, n (%)    | 15 (60.0)                 | 6 (26.8)                      | 138 (38.1)               | 0.09  |
| Diabetes mellitus, n (%)| 10 (40.0)                | 7 (33.3)                      | 5 (23.8)                 | 0.51  |
| Renal impairment, n (%)| 7 (28.0)                  | 0 (0.0)                       | 3 (14.3)                 | 0.03  |
| Previous TIA/stroke, n (%)| 4 (16.0)                | 9 (42.9)                      | 5 (23.8)                 | 0.11  |
| NIHSS on admission, mean±SD | 9.16±6.23               | 6.62±5.95                    | 9.05±8.30                | 0.96  |
| Total cholesterol (mmol/L), mean±SD | 3.80±1.07               | 4.27±0.99                    | 4.17±0.79                | 0.22  |
| TG (mmol/L), mean±SD   | 1.17±0.75                 | 1.69±1.69                    | 1.64±1.37                | 0.32  |
| HDL (mmol/L), mean±SD  | 1.30±0.39                 | 1.47±0.26                    | 1.37±0.35                | 0.25  |
| LDL (mmol/L), mean±SD  | 2.12±0.85                 | 2.33±0.89                    | 2.39±0.76                | 0.52  |
| Presence of CMBs, n (%)| 20 (80.0)                 | 8 (38.1)                      | 11 (52.4)                | 0.01  |
| Strictly lobar CMBs, n (%)| 5 (20.0)                 | 4 (19.0)                      | 4 (19.0)                 | 1.00  |
| Deep CMBs, n (%)       | 15 (60.0)                 | 4 (19.0)                      | 7 (33.3)                 | 0.02  |

CMBs=cerebral microbleeds, HDL=high-density lipoprotein, LDL=low-density lipoprotein, NIHSS=National Institutes of Health Stroke scale, TG=triglycerides.

(P=0.006, OR =4.23, 95% CI 1.47–12.14) compared with those >2.15 mmol/L in the univariate analysis. When we adjusted for age, sex, smoking habits, drinking habits, and renal impairment, the presence of CMBs and deep CMBs was, respectively, 4.96- and 4.83-fold higher in patients with lower serum calcium levels (<2.15 mmol/L) than in patients with higher serum calcium levels (Table 3).

4. Discussion

We found that lower levels of serum calcium (<2.15 mmol/L) were independently associated with the presence of CMBs and deep CMBs in IS patients with AF and/or RHD, but not with strictly lobar CMBs.

In the current study, we found the presence of CMBs was 58.2% in IS patients with AF and/or RHD, which is higher than the other studies reported (15–35%). These differences may be due in part to ethnic and clinical differences among the patients in the study. Furthermore, we were careful to use only SWI, which is more sensitive at detecting CMBs than...
conventional T2*‐weighted gradient‐recalled echo imaging used in many previous studies.[23–25]

Although one cohort study[16] has shown that lower calcium intake and hypertension had joint effects on the risk of CMBs in healthy individuals, we provide preliminary evidence in a small cohort of Chinese IS patients with AF and/or RHD that lower serum calcium levels (≤2.15 mmol/L) were associated with presence of CMBs.

Three possible mechanisms may explain why low serum calcium levels are related to the presence of CMBs. First, low serum calcium levels might contribute to blood pressure elevation through inducing relaxation of isolated arteries by activating calcium receptors in perivascular nerves.[26] Second, calcium intake can reduce platelet aggregation and total cholesterol levels,[9] and hemostasis,[29] it is provable that the low serum calcium levels may have a synergistic effect on CMBs risk. Our results indicate that lower serum calcium levels (≤2.15 mmol/L) are associated with presence of deep CMBs, but not with strictly lobar CMBs, providing insight into the potential role of calcium in CMBs. It is consistent with the previous study,[9] showing that classic markers of cardio or cerebrovascular disease had relationships with deep or infratentorial CMBs (hypertensive type) but not with strictly lobar CMBs (cerebral amyloid angiopathy type).

The present study has several limitations. Firstly, the study is a single‐center, hospital‐based study with a small, highly specific stroke patient population, which limited the extension of our conclusions. Larger, multicenter, and population‐based studies are required to confirm our findings. Secondly, our study only focused on total serum calcium, whereas ionized calcium, the physiologically active compartment, was not measured. Furthermore, only a single serum calcium level admitted within 48 h was measured. The levels of serum calcium were impossible to remain unchanged after stroke, so the fluctuation of calcium levels during acute period might be better for clarifying whether there is an independent association between serum calcium levels and CMBs. Thirdly, The statistical power of our study was limited because of the fact that about 20% eligible patients were excluded for lacking serum calcium levels within 48 h after admission, so a prospective study in about 20% eligible patients were excluded for lacking serum calcium levels within 48 h after admission, so a prospective study in which serum calcium levels are measured systematically and repeatedly is needed. The study was performed in stroke patients with AF and/or RHD, so future study should determine whether the association between serum calcium levels and CMBs is involved with other types of stroke patients and the general population.

5. Conclusions

In conclusion, our results demonstrated that lower serum levels of calcium (≤2.15 mmol/L) are independently and significantly related to higher presence of CMBs in IS patients with AF and/or RHD, especially among those with deep CMBs. Considering the small cohort included in the study, further studies are warranted to elucidate which accurate mechanism underlies the associations we found between the serum calcium levels and CMBs.

References

[1] Greenberg SM, Vermeer MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol 2009;8:145–74.
[2] Fazekas F, Kleinert R, Roob G, et al. Histopathologic analysis of foci of spontaneous intracerebral hemorrhage: evidence of microangiopathy‐related microbleeds. AJNR Am J Neuroradiol 1999;20:637–42.
[3] Bokura H, Saika R, Yamaguchi T, et al. Microbleeds are associated with subsequent hemorrhagic and ischemic stroke in healthy elderly individuals. Stroke 2011;42:1867–71.
[4] Chairudinou A, Kakar P, Fox Z, Werring DJ. Cerebral microbleeds and recurrent stroke risk: systematic review and meta‐analysis of prospective ischemic stroke and transient ischemic attack cohorts. Stroke 2013;44:995–1001.
[5] Greigouere S, Smith K, Jager H, et al. Cerebral microbleed and long‐term cognitive outcome: longitudinal cohort study of stroke clinic patients. Cerebrovasc Dis 2012;33:430–4.
[6] Altmann‐Schneider I, Trompet S, de Craen AJ, et al. Cerebral microbleeds are predictive of mortality in the elderly. Stroke 2011;42:638–44.
[7] Choi‐Kwon S, Han K, Choi S, et al. Poststroke depression and emotional incontinence: factors related to acute and subacute stages. Neurology 2012;78:1130–7.
[8] WardlawJM, Smith EE, Rixels GI, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2015;12:822–38.
[9] Vermeer MW, van der Luijg A, Ikram MA, et al. Prevalence and risk factors of cerebral microbleeds: the Rotterdam scan study. Neurology 2008;70:1208–14.
[10] Poels MM, Vermeer MW, Ikram MA, et al. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. Stroke 2010;41:S103–4.
[11] Ovbiagele B, Liebeskind DS, Starkman S, et al. Are elevated admission calcium levels associated with better outcomes after ischemic stroke? Neurology 2006;67:170–3.
[12] Buck BH, Liebeskind DS, Saver JL, et al. Association of higher serum calcium levels with smaller infarct volumes in acute ischemic stroke. Arch Neurol 2007;64:1287–91.
[13] Inoue Y, Miyashita F, Toyoda K, Minematsu K. Low serum calcium levels contribute to larger hematoma volume in acute intracerebral hemorrhage. Stroke 2013;44:2004–6.
[14] Ovbiagele B, Starkman S, Teal P, et al. VISTA Investigators, Serum calcium as prognosticator in ischemic stroke. Stroke 2008;39:2231–6.
[15] Guo Y, Yan S, Zhang S, et al. Lower serum calcium level is associated with hemorhagic transformation after thrombolysis. Stroke 2015;46:1359–64.
[16] Hara M, Yashikishii Y, Nannin H, et al. Joint effect of hypertension and lifestyle‐related risk factors on the risk of brain microbleeds in healthy individuals. Hypertens Res 2013;36:789–94.
[17] Hatano S. Experience from a multicentre stroke registry: a preliminary report. Bull World Health Organ 1976;54:541–53.
[18] Wang D, Liu M, Hao Z, et al. Features of acute ischemic stroke with rheumatic heart disease in a hospitalized Chinese population. Stroke 2012;43:2833–7.
[19] Brott T, Adams HPJr, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–70.
[20] Lei C, Lin S, Wu B, Li H, Liu M, You C. Lipid levels are regionally associated with cerebral microbleeds in patients with intracerebral hemorrhage. J Stroke Cerebrovasc Dis 2014;23:1195–8.
[21] Poels MMF, Ikram MA, Van Der Lugt A, et al. Incidence of cerebral microbleeds in the general population. Stroke 2011;42:656–61.
[22] Xiao L, Sun W, Lan W, et al. Correlation between cerebral microbleeds and S100B/RAGE in acute lacunar stroke patients. J Neurol Sci 2014;340:208–12.
[23] Fiehler J, Albers GW, Boulanger JM, et al. MR STROKE Group. Bleeding risk analysis in stroke imaging before thrombolysis (BRASIL): pooled analysis of T2*-weighted magnetic resonance imaging data from 570 patients. Stroke 2007;38:4738–44.
[24] Gratz PP, El-Koussy M, Hsieh K, et al. Preexisting cerebral microbleeds on susceptibility-weighted magnetic resonance imaging and post-thrombolysis bleeding risk in 392 patients. Stroke 2014;45:1684–8.
[25] Dannenberg S, Scheitz JF, Rozanski M, et al. Number of cerebral microbleeds and risk of intracerebral hemorrhage after intravenous thrombolysis. Stroke 2014;45:2900–5.
[26] Bukoski RD, Bian K, Wang Y, Mupanomunda M. Perivascular sensory nerve Ca2+ receptor and Ca2+-induced relaxation of isolated arteries. Hypertension 1997;30:1431–9.
[27] Umesawa M, Iso H, Ishihara J, et al. Dietary calcium intake and risks of stroke, its subtypes, and coronary heart disease in Japanese: the JPHC study cohort I. Stroke 2008;39:2449–56.
[28] Fukuoka T, Nakashima Y, Harada M, et al. Effect of whole blood clotting time in rats with ionized hypocalcemia induced by rapid intravenous citrate infusion. J Toxicol Sci 2006;31:229–34.
[29] Prodan CI, Stoner JA, Gordon DL, Dale GL. Cerebral microbleeds in nonlacunar brain infarction are associated with lower coated-platelet levels. J Stroke Cerebrovasc Dis 2014;23:e3.