

Association between serum homocysteine and cerebral microbleeds in patients with hypertension: a cross-sectional study

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Abstract

Background

Cerebral microbleeds (CMBs) are a common MRI marker of cerebral small vessel disease in patients with hypertension. This study investigated the association of serum homocysteine (Hcy) with the presence, burden, and topographic distribution of CMBs in hypertensive adults.

Methods

This cross-sectional study enrolled hospitalized hypertensive patients aged ≥ 50 years. CMBs were assessed on brain MRI and classified by location (lobar, deep, mixed) and burden (absent, mild, moderate-to-severe). Logistic regression and restricted cubic spline analyses were used to examine the association between serum Hcy and CMBs. Stratified analyses were performed with interaction testing.

Results

Among 364 hypertensive patients, 88 had CMBs and 276 did not. Higher serum Hcy was independently associated with CMB presence, both as a continuous variable (OR 1.10, 95% CI 1.03–1.17) and as a categorical variable (≥ 15 vs. <15 $\mu\text{mol/L}$: OR 2.15, 95% CI 1.11–4.16). Restricted cubic spline analysis suggested an approximately linear association (P for nonlinearity = 0.526). Higher Hcy levels were also related to greater CMB burden and were significantly higher in patients with deep or mixed CMBs than in those without CMBs. Hcy was modestly correlated with total CMB count (Spearman ρ = 0.249, P = 0.0001). A significant interaction was observed between Hcy and female sex (P for interaction = 0.003).

Conclusions

In hypertensive adults, elevated serum Hcy was independently associated with the presence of CMBs, with evidence of a stronger association in women. Higher Hcy levels were also related to greater CMB burden and deep or mixed CMBs.

1. Background

Cerebral microbleeds (CMBs) are an important hemorrhagic neuroimaging marker of cerebral small vessel disease (CSVD). Histopathologically, CMBs correspond to discrete perivascular hemosiderin deposits that appear as rounded, hypointense foci on T2*-weighted gradient-recalled echo or susceptibility-weighted imaging (SWI)[1, 2]. CMBs are believed to result from erythrocytes leakage through structurally compromised small cerebral vessels, most commonly in the context of hypertensive arteriopathy or cerebral amyloid angiopathy (CAA)[3]. Increasing evidence indicates that the presence of CMBs is associated with a higher risk of intracerebral hemorrhage, ischemic stroke, cognitive impairment, and mortality[1, 3, 4]. Nevertheless, the biological mechanisms underlying CMB formation remain incompletely understood and likely involve a complex interplay of endothelial dysfunction, blood-brain barrier disruption, inflammation, and other forms of microvascular injury[5, 6]. Therefore, a deeper understanding of the determinants of CMBs holds substantial clinical and pathophysiological significance.

Hypertension is one of the most well-established risk factors for CMBs, and previous studies have shown a close relationship between blood pressure levels and CMB burden[7, 8]. From an imaging perspective, CMB topography may provide clues regarding the underlying vascular pathology: deep CMBs are more commonly linked to hypertensive arteriopathy, whereas strictly lobar CMBs are more frequently associated with CAA[2, 3]. However, the radiological heterogeneity of CMBs is not fully captured by anatomical location alone, as lobar and deep lesions often coexist within the same individual. Accordingly, quantitative assessment of lesion burden combined with topographic classification may provide a more comprehensive characterization of the mechanisms contributing to CMB development in hypertensive populations[9].

Homocysteine (Hcy) is a sulfur-containing amino acid intermediate in methionine metabolism. Circulating Hcy levels are influenced by genetic background, nutritional status, lifestyle factors, renal function, and medication use[10, 11]. Hyperhomocysteinemia (HHcy) is common in China, with a notably high prevalence among individuals with hypertension[12]. Accumulating evidence suggests that elevated Hcy contributes to vascular endothelial injury, oxidative stress, inflammation, and atherosclerotic disease, and has been implicated in both cardiovascular and cerebrovascular disorders[10, 11, 13, 14]. Within the field of CSVD, previous studies have linked HHcy to white matter hyperintensities (WMH), lacunar infarction, and overall small-vessel disease burden[15–17]. These findings support the hypothesis that Hcy may also play a role in the pathogenesis of CMBs via microvascular endothelial dysfunction and blood-brain barrier damage[3, 18]. However, direct evidence regarding the relationship between Hcy and CMBs remains limited and inconclusive. Although one prior study reported an association between Hcy levels and CMBs in cognitively impaired individuals[19], several critical questions remain unanswered. First, whether serum Hcy is independently associated with the presence, burden, and anatomical distribution of CMBs in hypertensive patients has not been systematically evaluated. Second, the dose-response nature of this relationship has yet to be characterized. Third, it remains unknown whether the Hcy-CMB association differs by sex or other clinically relevant factors, given reported sex differences in Hcy-related metabolic profiles and in the manifestation of CSVD[12, 20, 21].

The present study therefore aimed to address these gaps by investigating the association between serum Hcy levels and the presence of CMBs in adults with hypertension, and to further assess whether serum Hcy is related to CMB burden and anatomical distribution. We also explored whether this association exhibits a linear dose-response pattern and whether it differs across clinically relevant subgroups, including sex and the presence of WMH.

2. Methods

2.1 Study participants

A total of 1,177 consecutive individuals were admitted to Ward 3, Department of Neurology, the Second Affiliated Hospital of Guangzhou Medical University between December 2021 and August 2023. Among them, 708 patients had a diagnosis of hypertension. After applying the predefined inclusion and exclusion criteria, 364 hypertension patients were ultimately included in the present study (Fig. 1).

This retrospective, single-center study was conducted in accordance with the Declaration of Helsinki and was approved by the Clinical Research and Application Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University (approval number: 2022-YJS-ks-05). The requirement for written informed consent was waived by the ethics committee.

Participants were eligible if they met all of the following criteria: (1) age 50 years or older; (2) diagnosed with hypertension according to the 2018 Chinese Guidelines for the Management of Hypertension; and (3) underwent brain magnetic resonance imaging during hospitalization. Participants were excluded if: (1) their brain MRI images were of poor quality; or (2) intracranial hemorrhage was attributable to head trauma.

2.2 Clinical data collection and laboratory measurements

Demographic, clinical, and laboratory data were retrospectively collected from the electronic medical records. These included age, sex, body weight, height, body mass index (BMI), history of ischemic stroke, coronary artery disease, diabetes mellitus, and hypertension, as well as smoking and alcohol consumption status. Admission systolic blood pressure (SBP) and diastolic blood pressure (DBP), use of statins or antiplatelet agents, and routine laboratory parameters were also recorded.

Serum homocysteine levels were measured using a chemiluminescent immunoassay on an ARCHITECT i2000SR automated analyzer (Abbott, USA) with the dedicated ARCHITECT/Alinity i homocysteine reagent kit. In the primary analyses, serum Hcy was treated as a continuous variable. HHcy was defined as a serum Hcy level $\geq 15 \mu\text{mol/L}$ [12]. Participants were accordingly categorized into a low-Hcy group ($< 15 \mu\text{mol/L}$) and a high-Hcy group ($\geq 15 \mu\text{mol/L}$).

Other laboratory measurements included serum potassium, creatinine, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose, hemoglobin A1C (HbA1c), uric acid (UA) and fibrinogen. All biochemical parameters were measured using standard laboratory procedures at the Second Affiliated Hospital of Guangzhou Medical University.

2.3 MRI evaluation

All participants underwent brain magnetic resonance imaging on either a 1.5-T scanner (Achieva 1.5 T, Philips, Eindhoven, Netherlands) or a 3.0-T scanner (MAGNETOM Verio 3.0 T, Siemens, Erlangen, Germany). The MRI protocol included axial T1-weighted imaging (T1WI), axial T2-weighted imaging (T2WI), coronal T2 fluid-attenuated inversion recovery sequence (FLAIR) imaging, and axial susceptibility weighted imaging (SWI).

CMBs were defined according to established neuroimaging criteria as round or ovoid foci of low signal intensity on SWI, measuring 2–10 mm in diameter, without corresponding signal abnormalities on T1WI or T2WI [2]. Based on their anatomical distribution, CMBs were classified as strictly lobar, strictly deep/infratentorial or mixed. Strictly lobar CMBs were located in the cortical-subcortical regions, whereas strictly deep/infratentorial CMBs were located in the basal ganglia, thalamus, internal or external capsule, brainstem, or cerebellum. Mixed CMBs were defined as the coexistence of lobar and deep/infratentorial CMBs. According to the quantitative criteria described by Lee et al. [22], CMB burden was categorized as mild (1–5 lesions), moderate (6–15 lesions), or severe (> 15 lesions). Because of the limited number of participants with moderate or severe CMB burden, the moderate and severe categories were combined as moderate-to-severe (≥ 6 CMBs) for subsequent analyses.

White matter hyperintensities (WMH) of presumed vascular origin were defined according to established neuroimaging standards as hyperintense lesions on T2WI and FLAIR images that appeared isointense or hypointense on T1WI, without a central cerebrospinal fluid-like cavity [2]. WMH was considered present when the Fazekas score was ≥ 1 in either the periventricular or deep white matter region [23].

All brain MRI scan were independently reviewed by two experienced physicians who were blinded to the clinical data. The two readers had 16 and 8 years of experience, respectively, and both completed standardized training before image assessment. Discrepancies between the two readings were resolved by consensus.

2.4 Statistical analysis

Categorical variables were expressed as frequencies and percentages. Continuous variables were reported as mean $\pm$ standard deviation (SD) for normally distributed data, or as median (interquartile range, IQR) for non-normally distributed data. Group comparisons were conducted using the

chi-square test or Fisher's exact test for categorical variables, and the Student's t-test or Mann-Whitney U test for continuous variables, as appropriate. For comparisons among three or more groups, one-way analysis of variance (ANOVA) or the Kruskal–Wallis test was applied accordingly.

Logistic regression models were used to evaluate the association between serum Hcy and the presence of CMBs, with results reported as odds ratios (ORs) and 95% confidence intervals (CIs). Serum Hcy was analyzed both as a continuous variable and as a categorical variable (< 15 vs. ≥ 15 μmol/L). A crude model and three multivariable-adjusted models were constructed. The crude model included no covariate adjustment. Model 1 was adjusted for sex, age, and BMI. Model 2 was further adjusted for SBP, DBP, smoking status, alcohol consumption, and antiplatelet medication. Model 3 was additionally adjusted for creatinine, uric acid, LDL-C and the presence of WMH. Covariates included in the multivariable models were selected on the basis of univariable analyses ($P < 0.10$) and clinical relevance.

To explore the dose-response relationship between serum Hcy and the presence of CMBs, restricted cubic spline analysis was performed with four knots placed at the 5th, 35th, 65th, and 95th percentiles, adjustment for covariates included in Model 3. Odds ratios were calculated relative to the median Hcy level. Additionally, threshold analysis was performed to identify any potential breakpoint in the association between serum Hcy and CMBs.

Stratified analyses were conducted according to sex (male vs. female), age (< 75 vs. ≥ 75 years), BMI (< 24 vs. ≥ 24 kg/m²), smoking status (yes vs. no), history of ischemic stroke (yes vs. no), coronary heart disease (yes vs. no), diabetes mellitus (yes vs. no), and the presence of WMH (yes vs. no). Interaction P values were derived from logistic regression models that included interaction terms between serum Hcy and each stratification variable.

As exploratory imaging phenotype analyses, serum Hcy levels were compared across CMB location and burden categories using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction for pairwise comparisons. Given the skewed distribution of CMB counts, Spearman's rank correlation analysis was used to assess the correlation between serum Hcy levels and the number of CMBs.

All statistical analyses were performed using R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria) and Free Statistics software (version 2.1.1; Beijing, China). All tests were two-sided, and a P value < 0.05 was considered statistically significant.

3. Result

3.1 Baseline characteristics of study participants with and without CMBs

As shown in Figure 1, a total of 1,177 patients were screened during the study period. After applying the predefined inclusion and exclusion criteria, 364 patients with hypertension were included in the final analysis. The baseline characteristics of participants with and without CMBs are summarized in Table 1.

Among the 364 participants, 276 (75.8%) were in the non-CMB group and 88 (24.2%) in the CMB group. Compared with the non-CMB group, individuals with CMBs were older, more likely to be male and smokers, and exhibited higher SBP, creatinine, uric acid, and Hcy levels, as well as a greater prevalence of WMH. Specifically, the mean serum Hcy level was significantly higher in the CMB group (14.2 ± 4.9 μmol/l) than in the non-CMB group (11.6 ± 4.2 μmol/l).

Regarding the distribution and severity of CMBs among the 88 affected patients, mixed-location CMBs were associated with the highest lesion burden: 62.1% of mixed CMBs were classified as moderate-to-severe (≥6 lesions), whereas the vast majority of strictly lobar CMBs (96.8%) and strictly deep CMBs (96.4%) were mild (1-5 lesions). The difference in severity distribution across CMB subtypes was highly significant ($P < 0.001$) (Table 2).

Table 1 Clinical characteristics of CMB and non-CMB groups.

variables	Total(n=364)	Non-CMB(n=276)	CMBs(n=88)	P value
Male sex, n (%)	195 (53.6)	136 (49.3)	59 (67.0)	0.004
Age (years)	70.1 ± 9.2	69.2 ± 9.3	72.6 ± 8.7	0.002
BMI (kg/m ²)	24.3 ± 3.3	24.4 ± 3.2	24.1 ± 3.5	0.57
Smoking, n (%)	84 (23.1)	55 (19.9)	29 (33)	0.01
Alcohol consumption, n (%)	48 (13.2)	33 (12.0)	15 (17.0)	0.22
Antiplatelet use, n (%)	108 (29.7)	76 (27.5)	32 (36.4)	0.11
Ischemic stroke, n (%)	105 (28.8)	77 (27.9)	28 (31.8)	0.48
Coronary heart disease, n (%)	59 (16.2)	45 (16.3)	14 (15.9)	0.93
Diabetes mellitus, n (%)	101 (27.7)	71 (25.7)	30 (34.1)	0.13
SBP (mmHg)	136.4 ± 16.3	135.0 ± 15.7	140.7 ± 17.4	0.005
DBP (mmHg)	81.2 ± 10.9	80.6 ± 10.8	83.0 ± 11.1	0.08
Total cholesterol (mmol/L)	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	0.96
Triglycerides (mmol/L)	1.3 (0.9, 1.8)	1.3 (0.9, 1.7)	1.3 (1.0, 1.9)	0.82
LDL-C (mmol/L)	2.8 ± 1.0	2.8 ± 1.0	2.8 ± 1.0	0.99
Hcy (μmol/ L)	12.2 ± 4.6	11.6 ± 4.2	14.2 ± 4.9	<0.001
Creatinine (μmol/ L)	84.4 ± 38.0	81.2 ± 39.3	94.3 ± 32.0	0.005
Uric acid (μmol/ L)	351.1 ± 109.9	343.4 ± 109.9	375.3 ± 107.1	0.02
Fibrinogen (g/ L)	3.3 ± 1.0	3.3 ± 0.9	3.5 ± 1.1	0.19
HbA1c (%)	6.4 ± 1.4	6.4 ± 1.5	6.4 ± 1.2	0.91
WMH, n (%)	239 (65.7)	167 (60.5)	72 (81.8)	<0.001

Abbreviations: CMB, cerebral microbleed; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol, Hcy, homocysteine; WMH, white matter hyperintensity. Data are presented as mean ± standard deviation, median (interquartile range), or n (%).

Table 2 Association between CMB location and CMB burden.

CMBs grade	Total (n=364)	Non-CMB (n=276)	Lobar CMBs (n=31)	Deep CMBs (n=28)	Mix CMBs (n=29)	P-value*	Data are
Non-CMBs	276 (75.8)	276 (100)	0 (0)	0 (0)	0 (0)	< 0.001	
Mild	68 (18.7)	0 (0)	30 (96.8)	27 (96.4)	11 (37.9)		
Moderate-to-severe	20 (5.5)	0 (0)	1 (3.2)	1 (3.6)	18 (62.1)		

presented as n (%). Mild CMB burden was defined as 1–5 lesions, and moderate-to-severe burden as ≥6 lesions. *P value was calculated using the chi-square test to compare the distribution of CMB burden across the three CMB subtypes (lobar, deep, and mixed).

3.2 Higher serum Hcy was independently associated with the presence of CMBs

Table 3 presents the results of the multivariable logistic regression analysis according to Hcy levels. In the unadjusted model, higher serum Hcy levels were significantly associated with an increased risk of CMBs (OR 1.13, 95% CI 1.07-1.19). This association remained significant after adjusting for sex, age, and BMI in Model 1 (OR 1.10, 95% CI 1.04-1.16). The association persisted after further adjustment for SBP, DBP, smoking, alcohol consumption, and antiplatelet medication uses in Model 2 (OR 1.11, 95% CI 1.05-1.18, $P < 0.001$). Moreover, the positive association remained significant in Model 3 after additional adjustment for creatinine, uric acid, LDL-C and WMH (OR 1.10, 95% CI 1.03-1.17, $P = 0.005$).

To further assess the robustness of this association, Hcy was also analyzed as a categorical variable using a cutoff of 15 μmol/L. Participants were classified into a low-Hcy group (Hcy < 15 μmol/L) and a high-Hcy group (Hcy ≥ 15 μmol/L). In the crude model, patients in the high-Hcy group had a significantly higher likelihood of CMBs than those in the low-Hcy group (OR 3.05, 95% CI 1.75-5.31, $P < 0.001$). After adjustment for confounding factors, this association remained significant in Model 2 (OR 2.58, 95% CI 1.42-4.67, $P = 0.002$) and Model 3 (OR 2.16, 95% CI 1.12-4.18, $P = 0.022$) (Table 3).

Restricted cubic spline analysis further demonstrated a significant dose-response association between serum Hcy levels and the presence of CMBs after multivariable adjustment (P for overall association = 0.018), with no evidence of nonlinearity (P for nonlinearity = 0.526). Using 11.4 $\mu\text{mol/L}$ as the reference value ($\text{OR} = 1.0$), the odds of CMBs increased progressively with increasing Hcy levels (Fig. 2). Threshold analysis estimated a potential breakpoint at 9.98 $\mu\text{mol/L}$; however, the likelihood ratio test did not support a significant threshold effect ($P = 0.12$; Supplementary Table S1).

Table 3 Association between Hcy and the presence of CMBs in patients with hypertension.

	Crude Model		Model 1		Model 2		Model 3	
	OR (95%CI)	<i>P</i> value	OR (95%CI)	<i>P</i> value	OR (95%CI)	<i>P</i> value	OR (95%CI)	<i>P</i> value
Hcy levels (per 1 $\mu\text{mol/L}$)	1.13 (1.07-1.19)	<0.001	1.10 (1.04-1.16)	0.001	1.11 (1.05-1.18)	<0.001	1.10 (1.03-1.17)	0.005
Hcy category								
<15 $\mu\text{mol/L}$	Reference	-	Reference	-	Reference	-	Reference	-
$\geq 15 \mu\text{mol/L}$	3.05 (1.75-5.31)	<0.001	2.36 (1.33-4.20)	0.003	2.58 (1.42-4.67)	0.002	2.15 (1.11-4.16)	0.022

Abbreviations: CI, confidence interval; OR, odds ratio; Hcy, homocysteine; HHcy, hyperhomocysteinemia. Model definitions: Crude model: no covariate adjustment. Model 1: adjusted for age (years), sex, and body mass index (BMI, kg/m^2). Model 2: additionally adjusted for systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), smoking status (current/ever vs never), alcohol consumption, and antiplatelet agent uses. Model 3: additionally adjusted for serum creatinine, uric acid, LDL-C and white matter hyperintensity (WMH). Values of $P < 0.05$ were considered statistically significant. Analyses included 364 patients with hypertension; CMBs were present in 88/364 (24.2%).

3.3 Subgroup and interaction analyses of the Hcy-CMB association

Stratified analyses were performed according to sex, age, BMI, smoking status, ischemic stroke, diabetes mellitus, coronary heart disease, and WMH (Fig. 3, Supplementary Table S2). A significant interaction was found for sex (P for interaction = 0.003), with a stronger positive association between serum Hcy and CMBs in female participants (adjusted OR 1.29, 95% CI 1.09-1.53) than in male participants (adjusted OR 1.06, 95% CI 0.98-1.14).

3.4 Serum Hcy levels were significantly elevated in deep and mixed CMB subtypes

CMBs were anatomically classified into four groups: non-CMBs, strictly lobar CMBs, strictly deep CMBs, and mixed CMBs. As illustrated in Fig. 4, serum Hcy levels were significantly elevated in patients with deep CMBs (median 13.6 $\mu\text{mol/L}$; $P = 0.009$), and mixed CMBs (median 14.4 $\mu\text{mol/L}$; $P = 0.0003$) compared with those without CMBs (median 10.8 $\mu\text{mol/L}$). In contrast, no significant difference was observed between patients with lobar CMBs (median 11.7 $\mu\text{mol/L}$; $P = 0.33$) and those without CMBs. Among the three CMB subtypes, the highest median Hcy level was found in the mixed CMB group, followed by the deep CMB group. However, pairwise comparisons revealed no statistically significant differences among the three subtypes (lobar vs. deep, $P = 1.00$; lobar vs. mixed, $P = 0.58$; deep vs. mixed, $P = 1.00$), despite numerically higher levels in the deep and mixed groups (Supplementary Table S3).

3.5 Higher Hcy levels were associated with greater CMB burden and positively correlated with CMB count

As shown in Fig. 5, serum Hcy levels varied significantly according to CMB burden. Compared with patients without CMBs, those with mild CMB burden (1-5 lesions) exhibited significantly higher Hcy levels ($P = 0.005$), as did those with moderate-to-severe CMB burden (≥ 6 lesions; $P = 0.0002$). However, no significant difference was observed between the mild and moderate-to-severe groups ($P = 1.00$).

In addition to categorical analyses, serum Hcy level showed a modest yet statistically significant positive correlation with the number of CMBs (Spearman $\rho = 0.249$, $P < 0.0001$; Fig. 6). Although the data points were widely scattered, higher Hcy levels tended to be associated with a greater number of CMBs. This finding further supports a potential link between elevated Hcy and increased hemorrhagic small-vessel injury.

4. Discussion

In this cross-sectional study of adults aged 50 years or older with hypertension, we found that higher serum Hcy levels were independently associated with the presence of CMBs. This association remained significant after adjustment for conventional vascular risk factors, creatinine, uric acid, and WMH. Restricted cubic spline analysis further confirmed a linear dose-response relationship between serum Hcy levels and CMB risk. Subgroup analyses revealed a significant interaction by sex, with a stronger association observed in women, whereas no significant interactions were found for other covariates, including WMH. Beyond the presence of CMBs, higher Hcy levels were also associated with greater CMB burden and were significantly elevated in deep and mixed CMB subtypes. Moreover, a modest but significant positive correlation was observed between Hcy levels and the total number of CMBs. Collectively, these findings suggest that Hcy may contribute to hemorrhagic small-vessel injury across a spectrum of severity and anatomical distribution in hypertensive individuals, and that sex-specific pathways may modulate this relationship.

The baseline characteristics of our cohort are largely consistent with the established risk profile of CMBs. Compared with patients without CMBs, those with CMBs were older, more likely to be male, and had higher systolic blood pressure, creatinine, uric acid, and Hcy levels, as well as a greater prevalence of WMH. These differences align with previous reports identifying age, hypertension, renal dysfunction, and elevated Hcy as key determinants of CMBs [1, 7, 19, 24]. Importantly, the higher Hcy levels observed in the CMB group persisted after multivariable adjustment, suggesting an independent contribution beyond traditional vascular risk factors.

Homocysteine has long been recognized as a risk factor for vascular disease, with accumulating evidence linking elevated Hcy to cardiovascular events, ischemic stroke, and atherosclerosis [10, 13, 25]. Within the cerebrovascular system, Hcy has been implicated in the pathogenesis of CSVD, with studies reporting associations between elevated Hcy and lacunar infarction, WMH, and overall CSVD burden [26–28]. However, evidence specifically addressing CMBs has remained relatively limited, and the available data derive mainly from selected clinical populations [19, 29]. Against this background, the present study extends the existing literature by focusing on a hypertensive population that is at particularly high risk for small-vessel injury and CMB formation [3, 7, 30].

Our findings demonstrate that elevated serum Hcy is independently associated with the presence of CMBs in hypertensive adults, even after comprehensive adjustment for demographic factors, vascular risk factors, renal function, and presence of WMH. This association exhibited a linear dose-response pattern, with the odds of CMBs increasing progressively across the range of Hcy concentrations. These results are consistent with prior studies linking Hcy to other CSVD phenotypes [16, 26, 28] and extend the literature by demonstrating a similar relationship specifically for CMBs in a hypertensive population. The persistence of the association after adjustment for renal function and WMH suggests that Hcy may exert direct effects on the cerebral microvasculature beyond being merely a marker of global vascular injury.

Interestingly, we observed a significant interaction between Hcy and sex, with a stronger association between Hcy and CMBs in women than in men. This sex-specific effect persisted after adjustment for multiple covariates and was not explained by differences in age, blood pressure, or other vascular risk factors. Several mechanisms may account for this finding. First, estrogen has been shown to modulate Hcy metabolism, and postmenopausal women typically exhibit higher Hcy levels than premenopausal women [31, 32]. Second, sex differences in CSVD pathology have been increasingly recognized, with some studies reporting a greater burden of CSVD in women after menopause [20, 21, 33]. Third, women may be more susceptible to Hcy-induced endothelial dysfunction due to differences in nitric oxide bioavailability and oxidative stress responses [34–36]. These observations suggest that sex-specific pathways may modulate the relationship between Hcy and hemorrhagic small-vessel injury, and that future studies should consider sex as a potential effect modifier when evaluating Hcy-lowering strategies.

Beyond the presence of CMBs, we found that higher Hcy levels were associated with greater CMB burden, as reflected by both categorical severity grading and continuous CMB count. The positive correlation between serum Hcy and CMB count was modest (Spearman $\rho = 0.249$), indicating that higher Hcy levels were associated with a greater number of CMBs. Notably, elevated Hcy levels were observed in deep and mixed CMB subtypes, but not in the lobar subtype. This preferential involvement of deep CMBs may be explained by the known effects of Hcy on hypertensive arteriopathy. Specifically, Hcy promotes endothelial dysfunction, oxidative stress, and smooth muscle cell proliferation, processes that may compromise the integrity of cerebral small vessels, particularly in the setting of hypertensive deep perforator arteriopathy. [25, 37–39]. In contrast, lobar CMBs are more closely linked to CAA, a pathology primarily characterized by β -amyloid deposition in cortical and leptomeningeal vessels and therefore less directly influenced by Hcy metabolism [39–41]. Furthermore, the significant elevation in mixed CMBs likely reflects the fact that patients with mixed CMBs invariably have deep lesions and, by definition, a higher total CMB count, representing a greater overall disease burden. Thus, the observed Hcy elevation in mixed CMBs is consistent with the dose-response relationship between Hcy and CMB severity, rather than indicating a distinct anatomical susceptibility. Taken together, these findings suggest that Hcy contributes to hemorrhagic small-vessel injury primarily through exacerbation of hypertensive microangiopathy, with the degree of elevation paralleling the severity of CMB burden.

Several limitations of this study should be acknowledged. First, the sample size was modest, which may have resulted in wider confidence intervals for some subgroup estimates. Nevertheless, the consistency between the continuous and categorical analyses of Hcy supports the robustness of our primary findings. Second, because of the cross-sectional design, temporal sequence and causality cannot be determined. Thus, we cannot conclude whether elevated Hcy directly contributes to CMB development, whether CMBs and Hcy share common upstream determinants, or whether the association merely reflects overall vascular injury burden. Third, residual confounding cannot be entirely excluded. In particular, we did not collect detailed information on folate status, vitamin B12 levels, dietary habits, or prior Hcy-lowering treatment, all of which may influence Hcy metabolism [11, 42]. Fourth, Hcy was measured at a single time point and may not capture long-term exposure, potentially leading to regression dilution bias. Finally, this was a hospital-based Chinese hypertensive cohort aged 50 years or older, and the generalizability of our findings to other ethnic groups, younger populations, or non-hypertensive settings remains to be established. Despite these limitations, the consistency of the findings across multivariable models, together with the observed linear dose-response relationship, strengthens the credibility of our results and provides a foundation for future investigations.

5. Conclusions

This study demonstrates that elevated serum Hcy is independently associated with CMBs in hypertensive adults, with a linear dose-response relationship and a stronger effect in women. These findings suggest that Hcy may represent a potential modifiable risk factor for hemorrhagic cerebral small-vessel injury in hypertension, particularly in postmenopausal women. Higher Hcy levels were also associated with greater CMB

burden and deep or mixed CMB distributions. Prospective longitudinal studies are needed to clarify temporality and determine whether serum Hcy predicts CMB development or progression.

Abbreviations

BMI
body mass index
CAA
cerebral amyloid angiopathy
CHD
coronary heart disease
CI
confidence interval
CMB
cerebral microbleed
CSVD
cerebral small vessel disease
DBP
diastolic blood pressure
Hcy
homocysteine
HHcy
hyperhomocysteinemia
IS
ischemic stroke
LDL-C
low-density lipoprotein cholesterol
MRI
magnetic resonance imaging
OR
odds ratio
RCS
restricted cubic spline
SBP
systolic blood pressure
SWI
susceptibility-weighted imaging
WMH
white matter hyperintensity.

Declarations

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Authors’ contributions

Lixuan Zhan and Li Huang conceived and designed the study, analyzed the data and drafted the manuscript. Wenhao Zhang and Jinrong Li contributed to data collection. Wen Li interpreted the results and critically revised the manuscript. Jiaqi Jiang contributed to data interpretation and refinement of the study findings. All authors reviewed and approved the final version of the manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Clinical Research and Application Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University approved this study (approval no. 2022-YJS-ks-05). The requirement for written informed consent was waived by the ethics committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

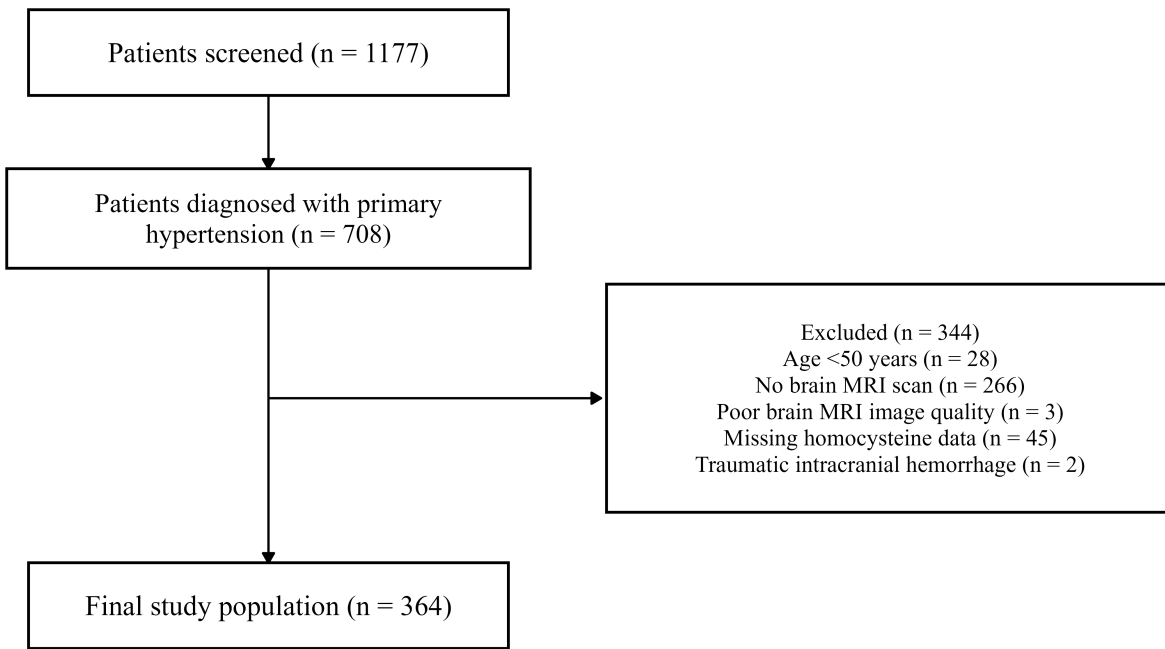


Figure 1

Flowchart of study population selection.

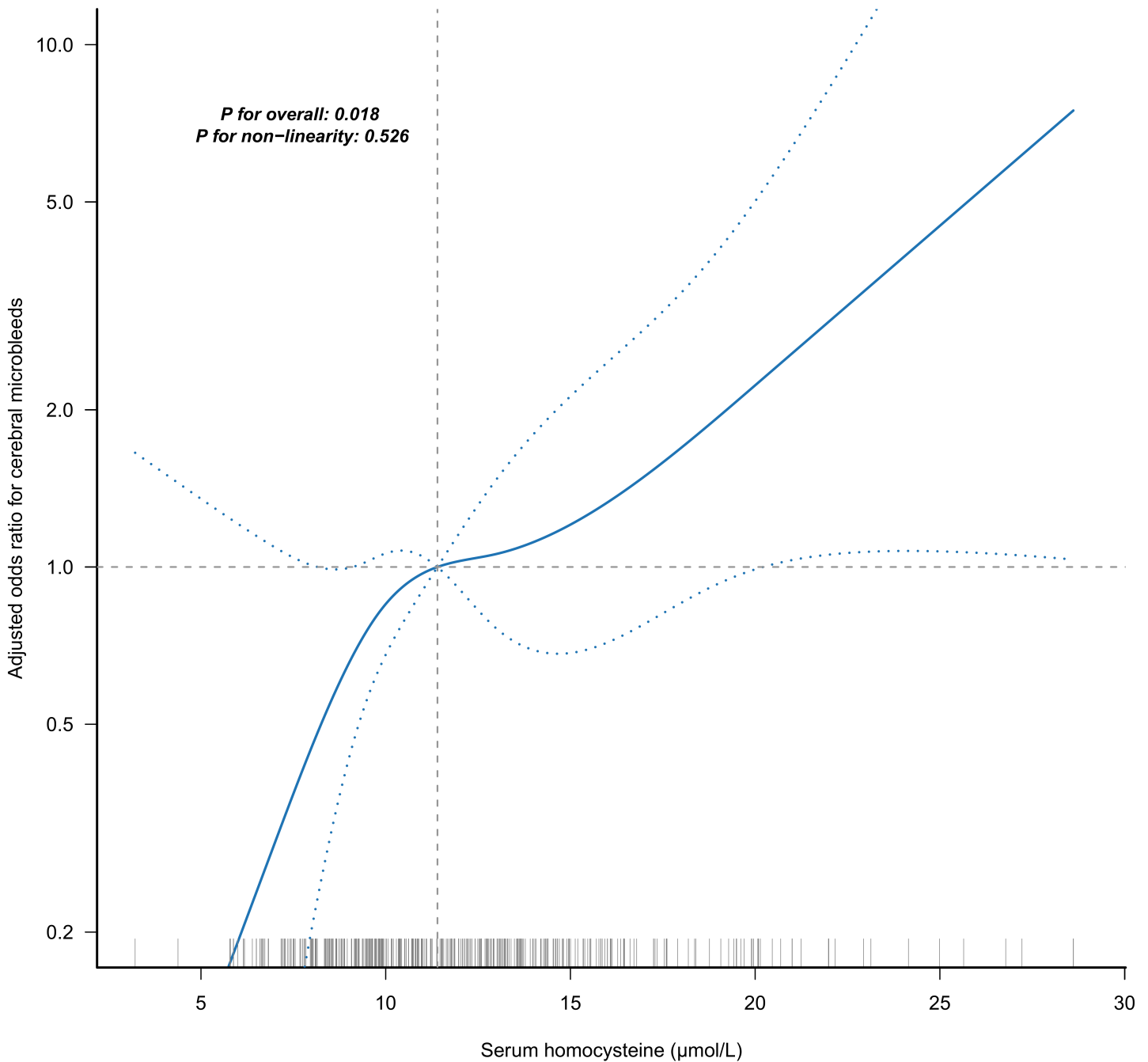


Figure 2

Restricted cubic spline (RCS) showing the dose-response association between serum Hcy and the odds of CMBs.

A multivariable logistic regression model was fitted with Hcy modeled as an RCS function using four knots at the 5th, 35th, 65th, and 95th percentiles of the Hcy distribution. The solid line represents the estimated odds ratio (OR) for CMBs, and the dashed lines denote the 95% confidence interval. The reference point (OR = 1.0) was anchored at 11.4 $\mu\text{mol/L}$ (the 50th percentile of Hcy). The model was adjusted for sex, age, BMI, SBP, DBP, smoking status, alcohol consumption, antiplatelet medication use, creatinine, uric acid, LDL-C and WMH presence. The overall association was significant (P for overall = 0.018), with no evidence of nonlinearity (P for nonlinearity = 0.526).

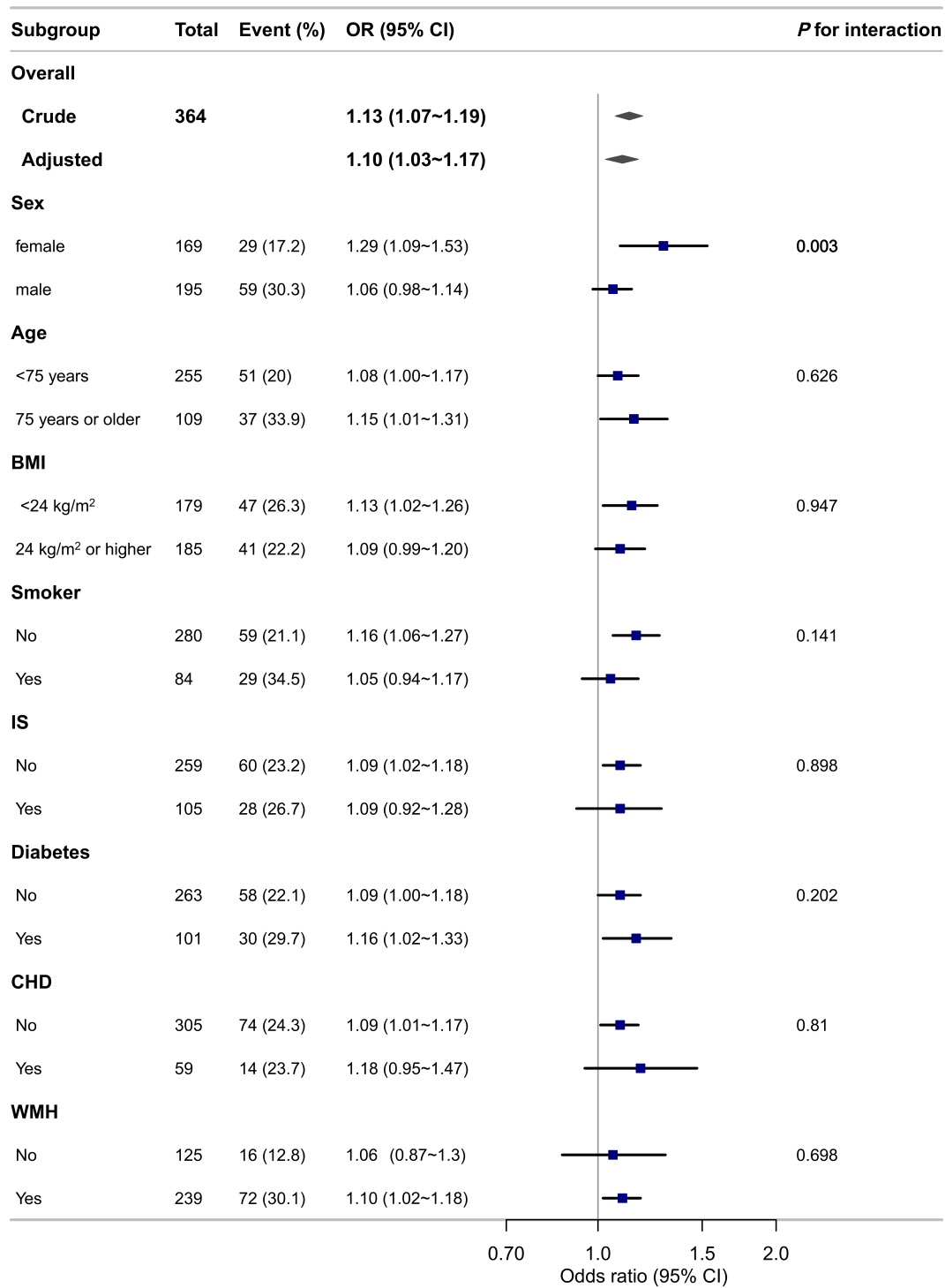


Figure 3

Forest plot of the association between serum Hcy level and CMBs across prespecified subgroups. Abbreviations: IS, ischemic stroke; CHD, coronary heart disease; WMH, white matter hyperintensity. Adjusted for all covariates in Model 3 except the stratification variable itself. The vertical dashed line indicates an OR of 1.0 (no effect).

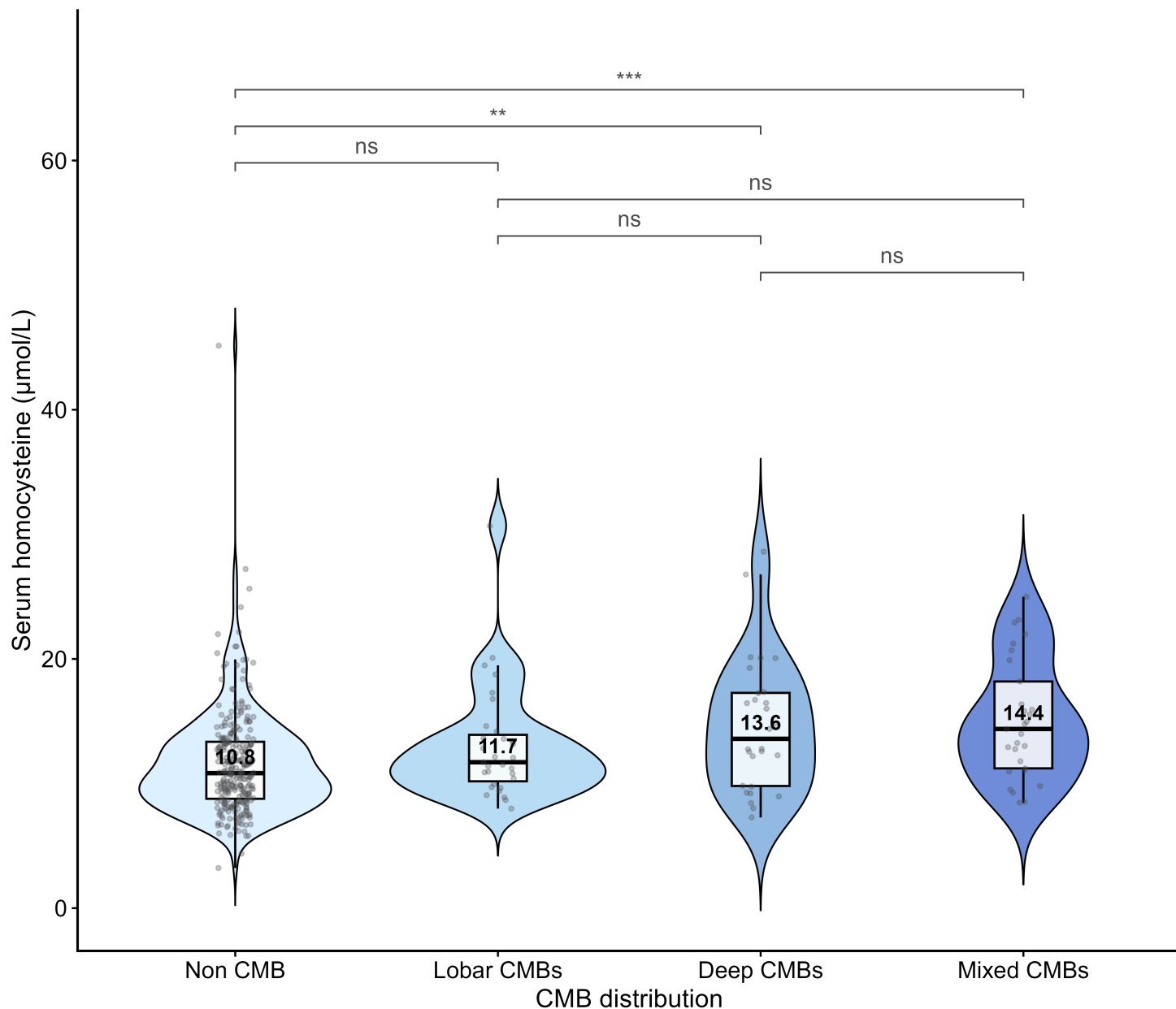


Figure 4

Serum Hcy levels according to CMB distribution.

Violin plots display Hcy levels across four groups: non-CMB, lobar, deep, and mixed CMBs. Within each violin, the thick horizontal line represents the median Hcy level, and the box denotes interquartile range (IQR); the median value is annotated above each plot. Overall group differences were evaluated using the Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction for pairwise comparisons. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. No significant differences were found among the three CMB subtypes.

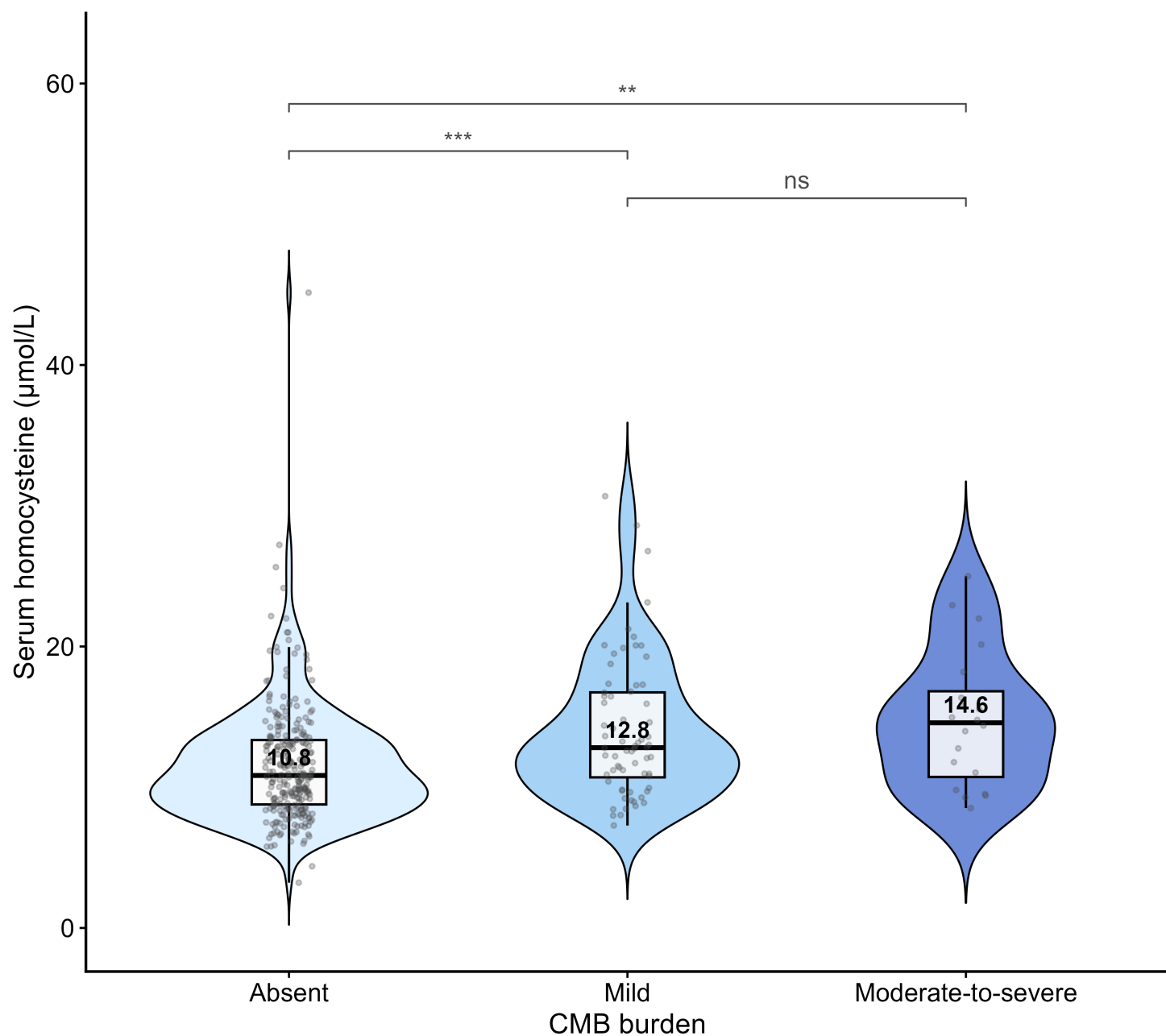


Figure 5

Serum Hcy levels according to CMB burden.

CMB burden was categorized as absent, mild (1-5 lesions), and moderate-to-severe (≥ 6 lesions). Violin plots illustrate the distribution of Hcy levels, with embedded box plots showing the median (thick horizontal line) and IQR. Overall group differences were evaluated using the Kruskal–Wallis test, followed by Dunn’s post hoc test with Bonferroni correction for pairwise comparisons. ns, not significant; ** $P < 0.01$; *** $P < 0.001$.

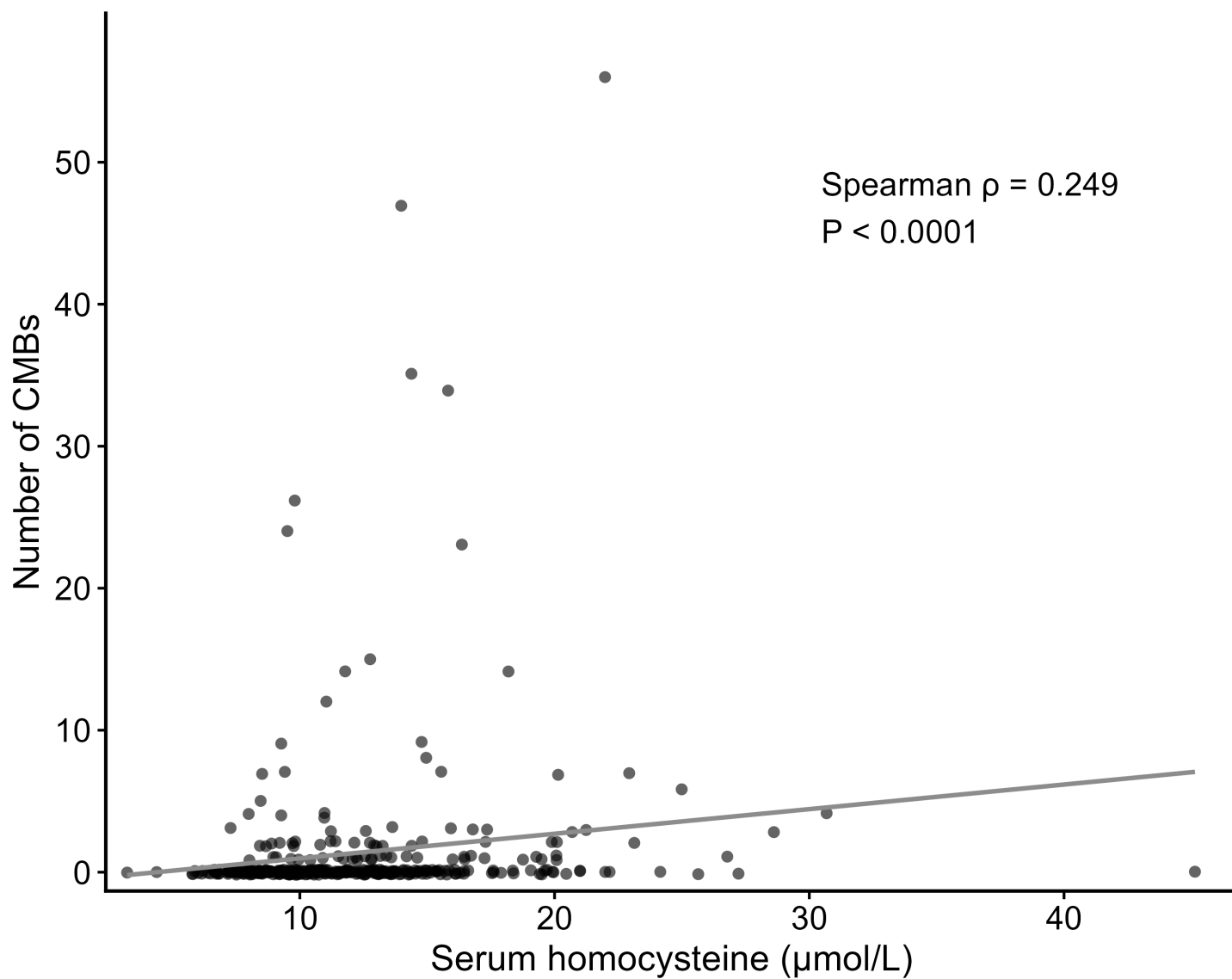


Figure 6

Correlation between serum Hcy level and the number of CMBs.

Black dots represent individual participants and were slightly jittered to improve visualization. The grey line indicates the fitted linear trend. Correlation was assessed using Spearman's rank-order correlation coefficient.

Supplementary Files

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