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Macrophage Migration Inhibitory Factor as a Potential Plasma Biomarker of Cognitive Impairment in Cerebral Small Vessel Disease

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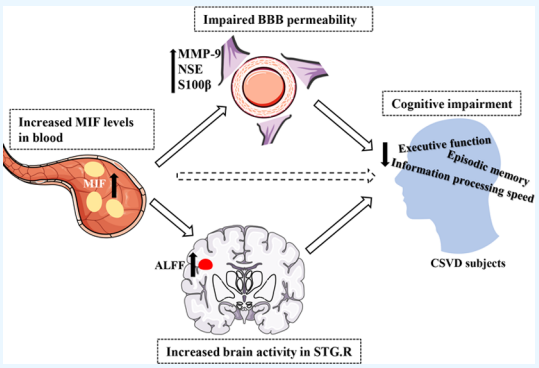
ABSTRACT: As the pathogenesis of cerebral small vessel disease with cognitive impairment (CSVD-CI) remains unclear, identifying effective biomarkers can contribute to the clinical management of CSVD-CI. This study recruited 54 healthy controls (HCs), 60 CSVD-CI patients, and 57 CSVD cognitively normal (CSVD-CN) patients. All participants underwent neuropsychological assessments and multimodal magnetic resonance imaging. Macrophage migration inhibitory factors (MIFs) were assessed in plasma. The least absolute shrinkage and selection operator model was used to determine a composite marker. Compared with HCs or CSVD-CN patients, CSVD-CI patients had significantly increased plasma MIF levels. In CSVD-CI patients, plasma MIF levels were significantly correlated with multiple cognitive assessment scores, plasma levels of blood−brain barrier (BBB)-related indices, white matter hyperintensity Fazekas scores, and the mean amplitude of low-frequency fluctuation in the right superior temporal gyrus. Higher plasma MIF levels were significantly associated with worse global cognition and information processing speed in CSVD-CI patients. The composite marker (including plasma MIF) distinguished CSVD-CI patients from CSVD-CN and HCs with >80% accuracy. Meta-analysis indicated that blood MIF levels were significantly increased in CSVD-CI patients. In conclusion, plasma MIF is a potential biomarker for early identification of CSVD-CI. Plasma MIF may play a role in cognitive decline in CSVD through BBB dysfunction and changes in white matter hyperintensity and brain activity.



INTRODUCTION

Cerebral small vessel disease (CSVD) is a common cause of vascular cognitive impairment in the elderly and is associated with progressive cognitive decline.[1] Early clinical manifestations of CSVD with cognitive impairment (CSVD-CI) are often atypical,[2] with executive dysfunction and impaired information processing speed as common core symptoms.[3] Prior magnetic resonance imaging (MRI) studies have provided several valuable imaging indicators for assessing the risk of CSVD-CI. Among these, white matter hyperintensity (WMH) is considered a risk marker of incident CSVD-CI,[4,5] and the Fazekas score, a qualitative tool commonly used to estimate the severity of WMH, is significantly associated with impaired cognitive function in CSVD patients.[4] The underlying pathogenic basis may also include hypoperfusion, oxidative stress, and inflammation, which can cause endothelial damage, blood−brain barrier (BBB) breakdown, impaired neurovascular coupling, and neuronal injury in CSVD.[6] These pathological changes in the brain can further mediate the occurrence of CSVD-CI. Although markers of these various pathological mechanisms, e.g., tumor necrosis factor-alpha (TNF-α) reflecting inflammation response, and matrix metalloproteinase-9 (MMP-9) reflecting BBB integrity, show significant changes in CSVD,[7−9] it is difficult to precisely identify CSVD-CI. Hence, there is a need to identify sensitive and accurate biomarkers for clinical diagnosis of CSVD-CI.

Macrophage migration inhibitory factor (MIF) is an evolutionarily highly conserved 12.5 kDa secretory protein involved in inflammation and immune response.[10] As a proinflammatory cytokine, MIF promotes endothelial dysfunction and leads to enhanced BBB permeability.[11] Prior research has shown that an MIF antagonist plays a neuroprotective role in the pathology of stroke[12] and that increased MIF production can expand the neuroinflammatory process and amyloid β-mediated neurotoxicity in Alzheimer's disease, which is associated with cognitive decline.[13,14] MIF is also associated with chronic vascular dysfunction and neurodegeneration pathology,[15] indicating a possible relationship of MIF with CSVD-CI. Recently, Zhao et al. reported that serum MIF levels

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Table 1. Comparison of Clinical Features, Cognitive Scores, and Plasma Index Levels Among the HC, CSVD-CI, and CSVD-CN Groups[a]

	HC (N = 54)	CSVD-CI (N = 60)	CSVD-CN (N = 57)	P value
age (years)	67.44 ± 7.46	70.22 ± 4.84	68.72 ± 5.81	0.054[b]
sex (male/female)	21/23	35/25	25/22	0.562[d]
education (years)	9.07 ± 2.51	8.82 ± 2.63	9.75 ± 2.45	0.068[c]
total WMH Fazekas scores	0.00 ± 0.00	2.92 ± 1.53	2.14 ± 1.17	<0.001[c]
MMSE score	28.74 ± 1.03	26.65 ± 2.30	28.56 ± 1.17	<0.001[c]
MoCA score	28.04 ± 1.35	22.13 ± 3.87	27.68 ± 1.26	<0.001[c]
AVLT-IR (raw score)	6.15 ± 1.20	4.50 ± 1.54	6.18 ± 1.41	<0.001[b]
AVLT-IR (Z score)	0.36 ± 0.75	−0.68 ± 0.96	0.37 ± 0.88	<0.001[b]
AVLT-20 min DR (raw score)	5.68 ± 1.96	3.18 ± 2.33	5.33 ± 2.28	<0.001[c]
AVLT-20 min DR (Z score)	0.40 ± 0.80	−0.61 ± 0.95	0.26 ± 0.95	<0.001[c]
TMT-A (raw score)	57.09 ± 18.58	102.52 ± 47.61	59.67 ± 17.66	<0.001[c]
TMT-A (Z score)	−0.44 ± 0.49	0.75 ± 1.25	−0.37 ± 0.46	<0.001[c]
Stroop-A (raw score)	28.13 ± 5.42	37.80 ± 11.75	29.60 ± 6.14	<0.001[c]
Stroop-A (Z score)	−0.41 ± 0.58	0.62 ± 1.25	−0.26 ± 0.65	<0.001[c]
Stroop-B (raw score)	45.44 ± 11.24	73.68 ± 39.36	49.88 ± 12.90	<0.001[c]
Stroop-B (Z score)	−0.41 ± 0.40	0.60 ± 1.40	−0.25 ± 0.46	<0.001[c]
information processing speed	−0.42 ± 0.35	0.66 ± 1.05	−0.29 ± 0.39	<0.001[c]
TMT-B (raw score)	138.52 ± 31.62	306.55 ± 143.92	149.02 ± 31.45	<0.001[c]
TMT-B (Z score)	−0.53 ± 0.27	0.90 ± 1.22	−0.44 ± 0.27	<0.001[c]
Stroop-C (raw score)	86.04 ± 20.85	152.70 ± 65.37	90.58 ± 26.50	<0.001[c]
Stroop-C (Z score)	−0.47 ± 0.39	0.79 ± 1.24	−0.38 ± 0.50	<0.001[c]
DST-backward (raw score)	4.44 ± 0.74	3.68 ± 0.95	4.32 ± 0.74	<0.001[c]
DST-backward (Z score)	0.35 ± 0.84	−0.51 ± 1.08	0.21 ± 0.84	<0.001[c]
executive function	−0.22 ± 0.32	0.39 ± 0.63	−0.21 ± 0.29	<0.001[c]
CDT (raw score)	9.02 ± 0.84	8.18 ± 1.33	8.68 ± 1.30	0.001[c]
CDT (Z score)	0.33 ± 0.68	−0.35 ± 1.09	0.06 ± 1.06	0.001[c]
plasma MIF(ng/mL)	4.28 ± 1.31	6.44 ± 1.97	5.60 ± 1.36	**<0.001**[b]
plasma MMP-9 (ng/mL)	22.27 ± 11.31	33.92 ± 24.70	24.59 ± 10.40	0.025[c]
plasma NSE (ng/mL)	14.85 ± 8.10	56.15 ± 21.63	52.73 ± 17.49	<0.001[b]
plasma S100β (pg/mL)	78.00 ± 30.06	91.03 ± 26.00	97.84 ± 22.25	<0.001[b]
plasma TNF-α (pg/mL)	6.62 ± 1.81	9.70 ± 5.04	8.11 ± 3.49	0.001[c]

[a]NOTE: Data are presented as the mean ± stand deviation. The information processing speed total scores were calculated by TMT-A, Stroop-A, and Stroop-B scales (Z scores), and the executive function total scores were calculated by TMT-B, Stroop-C, and DST-backward scales (Z scores). Abbreviations: HC, healthy control; CSVD-CI, cerebral small vessel disease with cognitive impairment; CSVD-CN, cerebral small vessel disease with cognitively normal; P-WMH, periventricular white matter hyperintensities; D-WMH, deep white matter hyperintensities; SVD, small vessel disease; NIHSS, National Institutes of Health Stroke Scale; MMSE, Mini-mental State Examination; MoCA, Montreal Cognitive Assessment; AVLT-IR, Auditory Verbal Learning Test-immediate recall; AVLT-20 min DR, Auditory Verbal Learning Test-20 min delayed recall; TMT-A, Trail Making Test A; Stroop-A, Stroop Color and Word Test A; Stroop-B, Stroop Color and Word Test B; TMT-B, Trail Making Test B; Stroop-C, Stroop Color and Word Test C; DST, Digit Span Test; MIF, macrophage migration inhibitory factor; MMP-9, matrix metalloproteinase-9; NSE, neuron-specific enolase; S100β, S100beta protein; and TNF-α, tumor necrosis factor-α. [b]P values were obtained by the one-way ANOVA test. [c]P values were obtained by the Kruskal−Wallis H test. [d]P values were obtained by the Chi-square test.

are increased in CSVD-CI patients as compared to CSVD-CN ones and are closely correlated with impaired executive function.[16] However, at present, the evidence of MIF as a peripheral biomarker of CSVD-CI is preliminary. In particular, the unsatisfactory diagnostic power of MIF for CSVD-CI should be addressed.

In the present study, we aimed to assess differences in plasma MIF among healthy controls (HC), CSVD-CI patients, and CSVD cognitively normal (CSVD-CN) patients. Potential associations between plasma MIF levels and clinical characteristics of CSVD-CI (e.g., cognitive phenotype, pathological molecules, and MRI features) were also explored. Specifically, the diagnostic value of plasma MIF for CSVD-CI was thoroughly assessed.

RESULTS

Clinical Characteristics of Participants. As shown in Table 1, there were no significant differences in age, sex, or education years among the HC, CSVD-CI, and CSVD-CN groups (all $p > 0.05$). However, the total WMH Fazekas scores and multiple neuropsychological assessments showed significant group differences (all $p < 0.05$; Table 1).

Significant group differences were also observed in plasma levels of MIF, MMP-9, neuron-specific enolase (NSE), S100beta protein (S100β), and TNF-α (all $p < 0.05$; Table 1). As shown in Figure 1A, CSVD-CI patients had significantly higher plasma MIF levels than the other groups, while CSVD-CN had significantly increased plasma MIF levels compared with HCs. The plasma levels of MMP-9, NSE, S100β, and TNF-α were also significantly different between the HC and CSVD-CI groups. However, there were no differences in these four plasma indices between the CSVD-CI and CSVD-CN groups (Supporting Figure 1A−D).

Correlation of Plasma MIF with Cognitive Assessments and Plasma Indices in CSVD Patients. In the CSVD-CI group, plasma MIF levels and cognitive assessments

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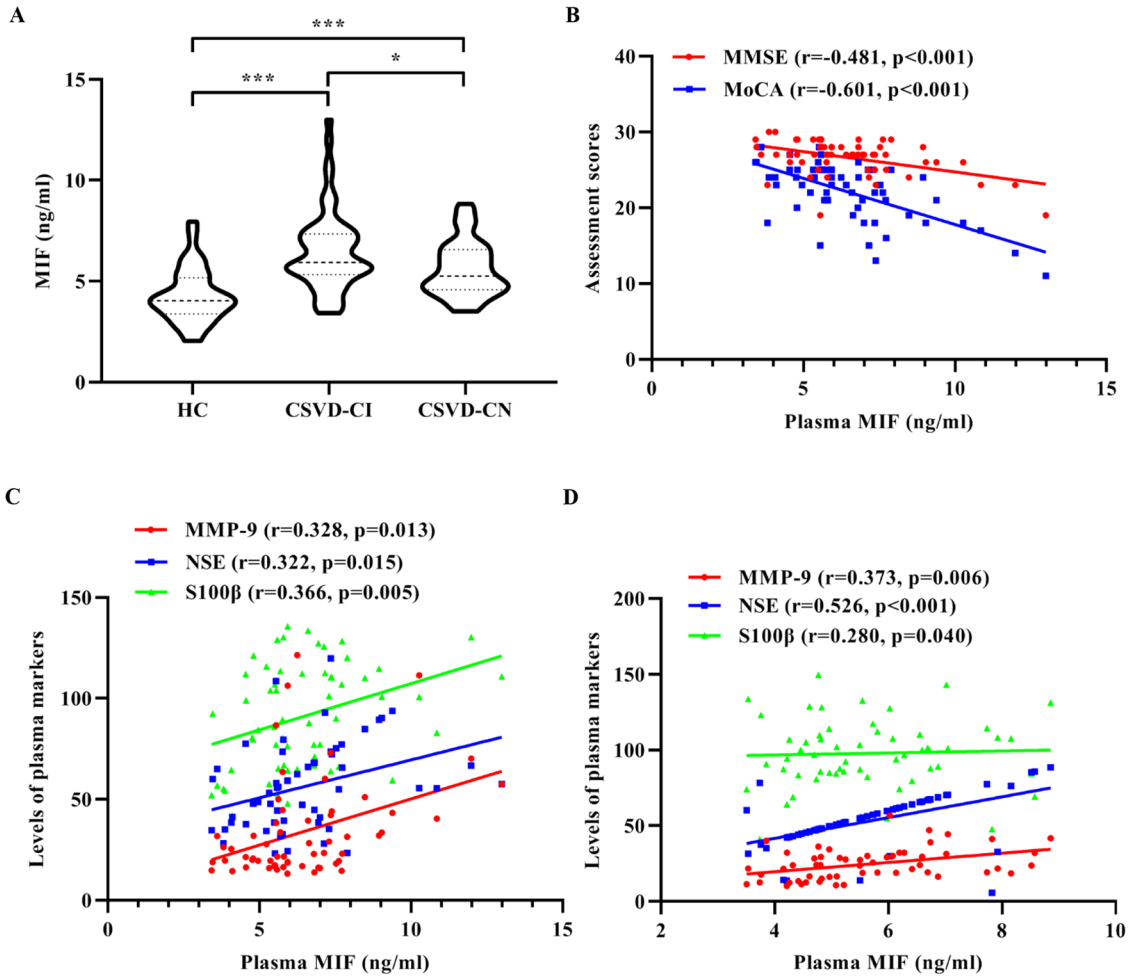

Figure 1. Clinical expression of plasma MIF. (A) Differences in plasma levels of MIF among the HC, CSVD-CI, and CSVD-CN groups. Bonferroni correction for the post hoc test. ***P value < 0.001; **0.001 ≤ p value < 0.01; *0.01 ≤ p value < 0.05. (B) Correlations of plasma MIF with global cognitive associations (MMSE and MoCA scales) in CSVD-CI patients. (C) Correlations of plasma MIF with three plasma makers (MMP-9, NSE, and S100β) in CSVD-CI patients. (D) Correlations of plasma MIF with three plasma makers (MMP-9, NSE, and S100β) in CSVD-CN patients. Age, sex, and education years as covariates were controlled in each correlation analysis. Abbreviations: MIF, macrophage migration inhibitory factor; HC, healthy control; CSVD-CI, cerebral small vessel disease with cognitive impairment; CSVD-CN, cerebral small vessel disease with cognitively normal; MMSE, Mini-mental State Examination; MoCA, Montreal Cognitive Assessment; MMP-9, matrix metalloproteinase-9; NSE, neuron-specific enolase; and S100β, S100beta protein.

were significantly correlated; however, plasma MIF levels in CSVD-CN patients were not correlated with any cognitive assessments (Figure 1B and S2). Furthermore, plasma levels of MMP-9, NSE, and S100β were significantly correlated with plasma MIF levels in both the CSVD-CI and CSVD-CN groups (Figure 1C,D and S2).

Association of Plasma MIF with MRI Features in CSVD Patients. In CSVD patients, plasma MIF levels showed a significant positive correlation with total WMH Fazekas scores (Figure 2A). According to the distribution of total WMH Fazekas scores, CSVD patients were divided into six subgroups. Significant differences were found in plasma MIF levels between these six groups (F = 5.387, p < 0.001). Specifically, CSVD patients with a score of 4 or 5 had significantly higher plasma MIF levels than those with scores of 1, 2, or 3 (Figure 2B and Table S1). A significant correlation between plasma MIF levels and total WMH Fazekas scores was observed in the CSVD-CI group but not in the CSVD-CN group (Figure 2C). Subgroup analysis of total WMH Fazekas scores in the CSVD-CI group was not performed due to the small sizes of the subgroups.

The mean amplitude of low-frequency fluctuation (mALFF) values showed significant group differences in three brain regions, i.e., left postcentral gyrus (PoCG.L), right median cingulate and paracingulate gyri (DCG.R), and right superior temporal gyrus (STG.R), among the HC, CSVD-CI, and CSVD-CN groups (Figure 2D, Alphasim corrected p < 0.001). As shown in Figure S3, CSVD-CI patients had significantly reduced mALFF values in the PoCG.L and significantly increased mALFF values in DCG.R and STG.R compared with HCs or CSVD-CN patients. In the CSVD-CI group, plasma MIF levels showed a significant positive correlation with mALFF values in STG.R (Figure 2E).

Multivariate Analysis of Plasma MIF with Cognitive Impairment. Because the plasma levels of MMP-9, NSE, S100β, and TNF-α were significantly correlated with global cognitive function in CSVD-CI patients (Figure S1E), they were included in the multivariate analysis. As the total WMH Fazekas score was a high-risk indicator of cognitive decline in CSVD patients,[17] and the mALFF values of the PoCG.L, DCG.R, and STG.R showed significant changes in CSVD-CI

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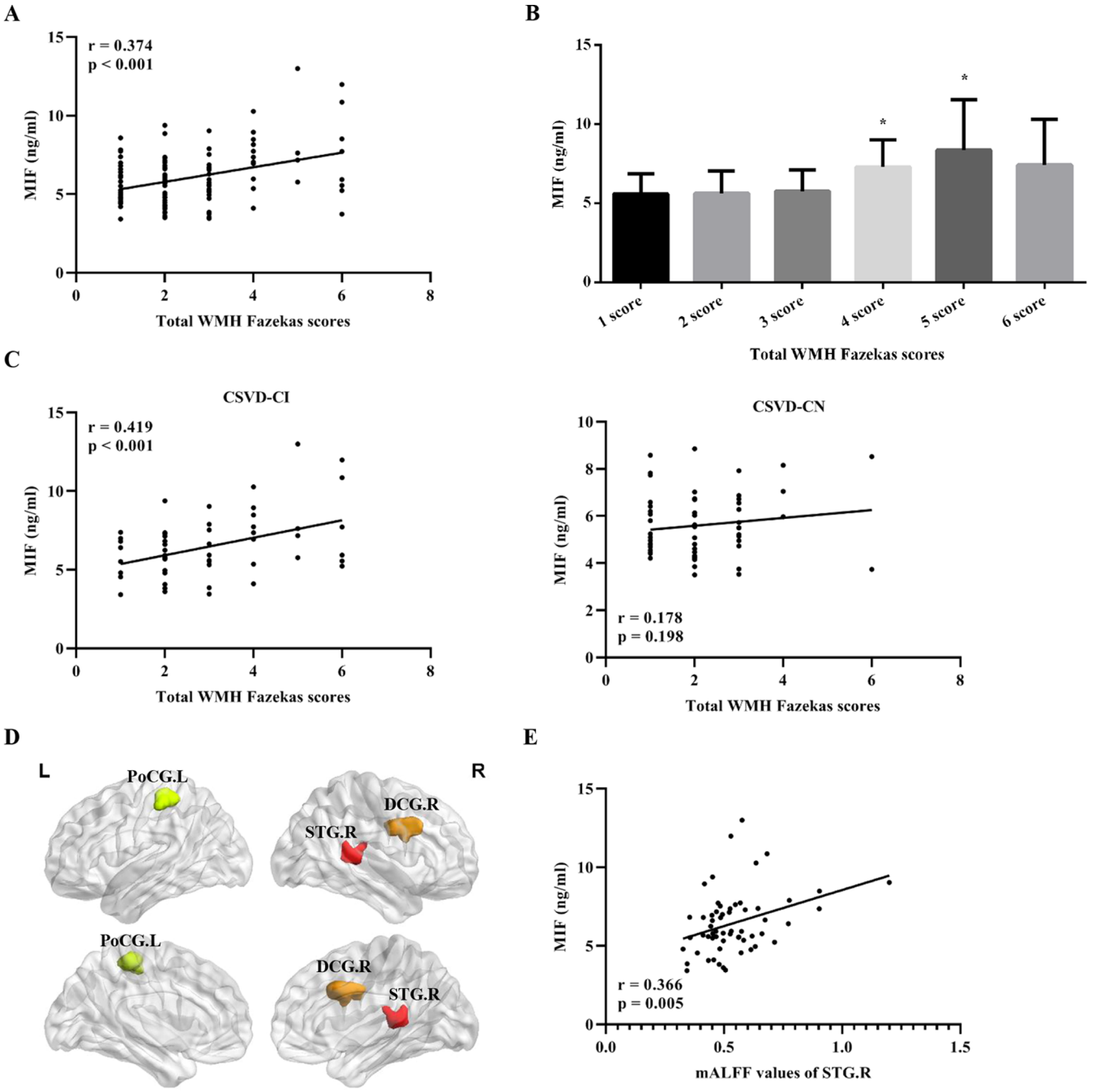

Figure 2. Associations between plasma MIF and MRI features. (A) Correlation of plasma MIF with total WMH Fazekas scores in 117 CSVD patients. (B) Difference of plasma MIF levels among six CSVD subgroups with total WMH Fazekas scores 1−6. One-way ANOVA analysis was used for the present analysis. Bonferroni correction for the post hoc test. * $0.01 \leq p$ value < 0.05. (C) Correlation of plasma MIF with total WMH Fazekas scores in the CSVD-CI group (right) and the CSVD-CN group (left). (D) Three brain regions indicated significantly different mALFF values among the HC, CSVD-CI, and CSVD-CN groups ($p < 0.001$, Alphasim multiple comparison correction, voxel number: 100). (E) Correlation of plasma MIF with mALFF values of the STG.R region in CSVD-CI patients. The above analyses were conducted with adjusting age, sex, and years of education. Abbreviations: MIF, macrophage migration inhibitory factor; CSVD-CI, cerebral small vessel disease with cognitive impairment; CSVD-CN, cerebral small vessel disease with cognitively normal; WMH, white matter hyperintensities; HC, healthy control; mALFF, mean amplitude of low-frequency fluctuation; PoCG.L, left postcentral gyrus; DCG.R, right median cingulate and paracingulate gyri; and STG.R, right superior temporal gyrus.

patients (Figure S3), these four MRI-related indices were also included.

Multivariate analysis was performed in CSVD-CI patients for the cognitive impairment-related indices with age, sex and education years as covariates (Table S2). The results indicated that higher plasma MIF levels and higher DCG.R mALFF values were significantly associated with worse Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) scores. A worse information processing speed was associated with higher plasma MIF and MMP-9 levels. However, higher plasma MIF levels were not associated with poor executive function.

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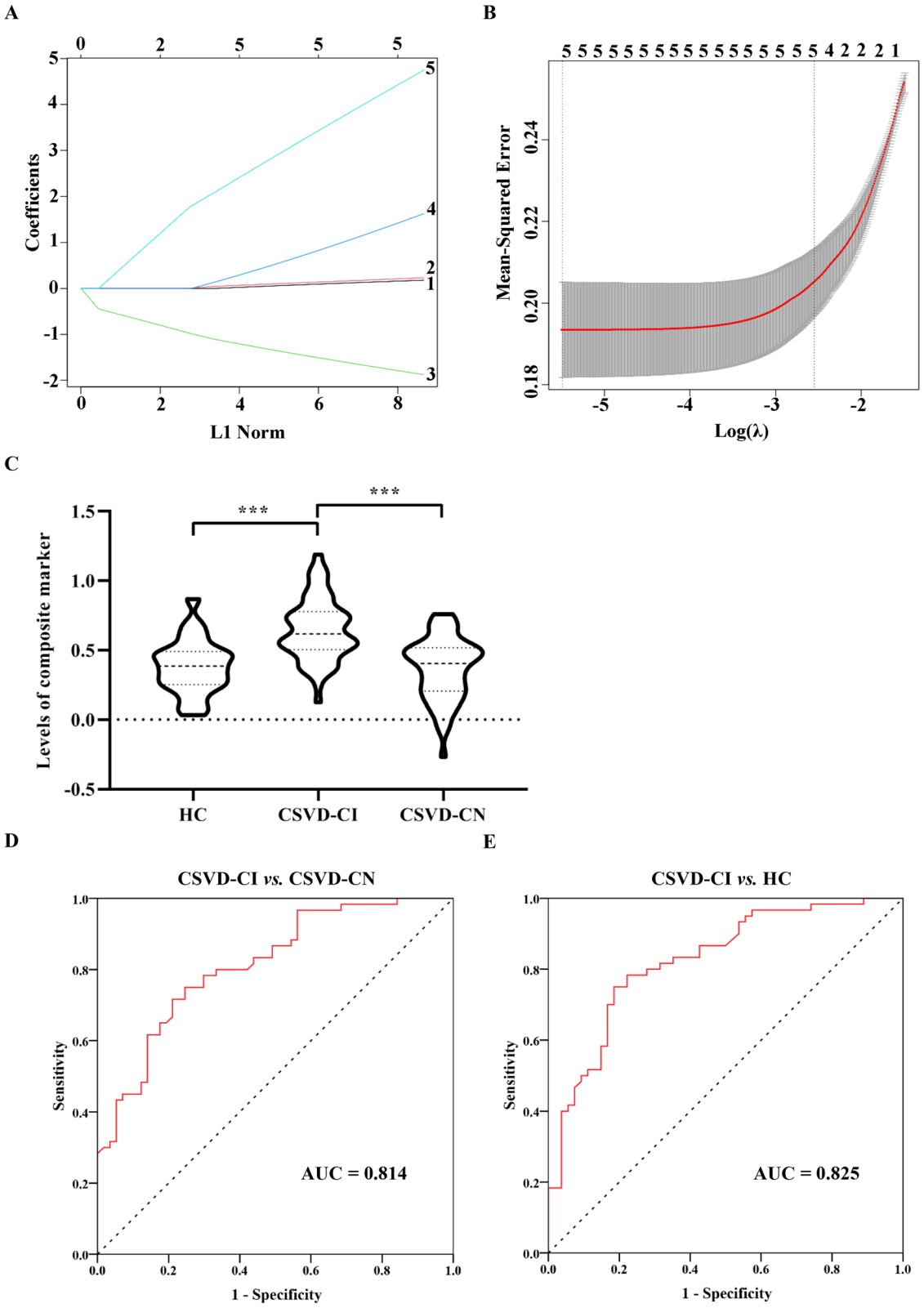

Figure 3. Diagnostic value of plasma MIF for CSVD-CI patients. (A) Coefficients of five indices in the LASSO model (1 = plasma MIF, 2 = total WMH Fazekas scores, 3 = mALFF values of PoCG.L, 4 = mALFF values of DCG.R, and 5 = mALFF values of STG.R). (B) Mean-squared error of the LASSO model. (C) Significant difference in the levels of composite markers among the HC, CSVD-CI, and CSVD-CN groups. Bonferroni correction for the post hoc test. ***P value < 0.001. (D) ROC curve of the composite marker for identifying CSVD-CI patients from CSVD-CN patients. (E) ROC curve of the composite marker for identifying CSVD-CI patients from HCs. Abbreviations: MIF, macrophage migration inhibitory factor; CSVD-CI, cerebral small vessel disease with cognitive impairment; CSVD-CN, cerebral small vessel disease with cognitively normal; WMH, white matter hyperintensities; HC, healthy control; mALFF, mean amplitude of low-frequency fluctuation; PoCG.L, left postcentral gyrus; DCG.R, right median cingulate and paracingulate gyri; STG.R, right superior temporal gyrus; ROC, receiver operating characteristic; and AUC, area under the curve.

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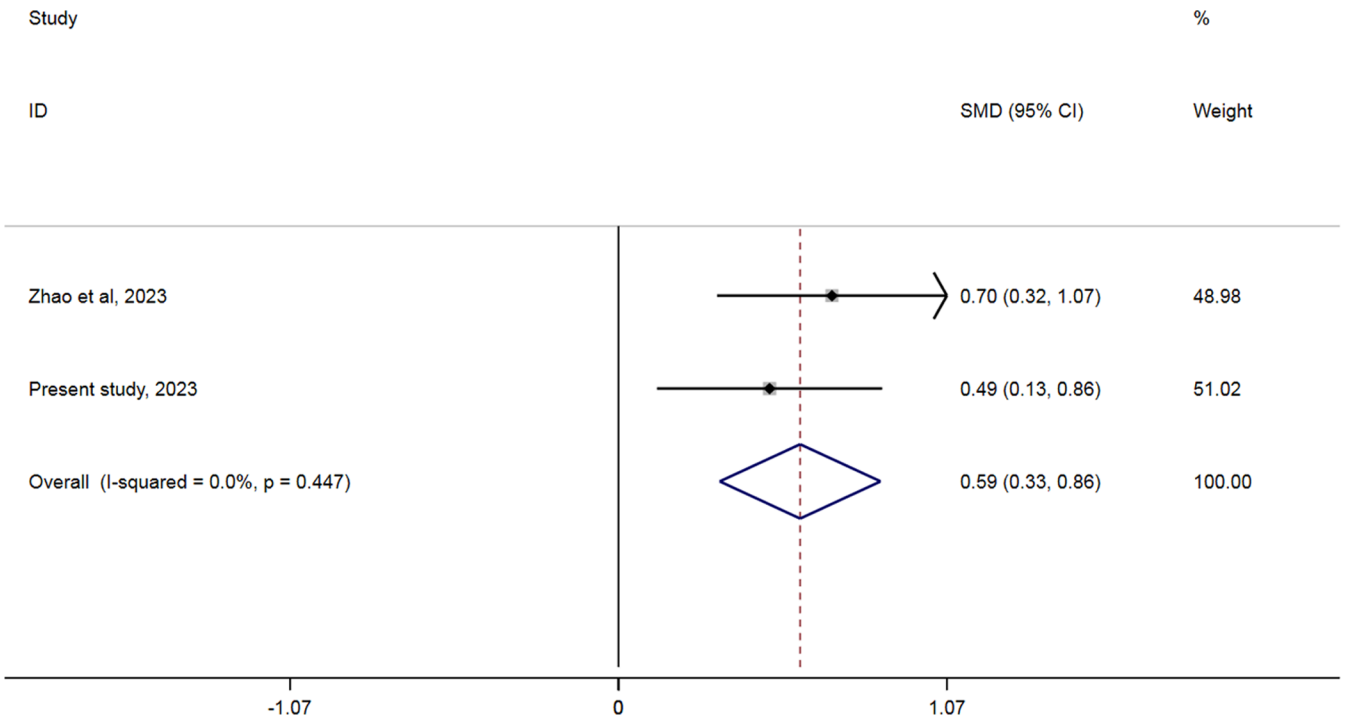

Figure 4. Forest plot for blood MIF levels in CSVD-CI patients and CSVD-CN patients using the random effects model. Abbreviations: MIF, macrophage migration inhibitory factor; CSVD-CI, cerebral small vessel disease with cognitive impairment; and CSVD-CN, cerebral small vessel disease with cognitively normal.

Diagnostic Performance of Plasma MIF. Receiver operating characteristic (ROC) curve analysis indicated that total WMH Fazekas scores exhibited the largest area under the curve (AUC) for differentiating CSVD-CI patients from HCs, followed by plasma NSE and plasma MIF (all AUC values > 0.80; Table S3). Likewise, for identifying CSVD-CN patients from HCs, total WMH Fazekas scores, plasma NSE and plasma MIF levels provided an acceptable AUC value > 0.75 (Table S3). However, no indicator could differentiate CSVD-CI from CSVD-CN patients with acceptable accuracy (Table S3).

Using the least absolute shrinkage and selection operator (LASSO) model, a composite marker was built based on indices with an AUC value > 0.60 for differentiating CSVD-CI from CSVD-CN patients (Table S3). The composite marker of the LASSO model was calculated as follows: composite marker = plasma levels of MIF × 0.03300492 + total WMH Fazekas score × 0.03891914 − mALFF values of PoCG.L × 0.34538174 + mALFF values of DCG.R × 0.16521346 + mALFF values of STG.R × 0.80967068 + 0.18797709 (Figure 3A,B).

As shown in Figure 3C, there was a significant difference in the composite marker among the HC, CSVD-CI, and CSVD-CN groups. The ROC curve indicated that the composite marker had an AUC value of 0.814 for distinguishing CSVD-CI patients from CSVD-CN patients (sensitivity = 71.67%, specificity = 78.95%; Figure 3D). The composite marker also resulted in high accuracy for differential diagnosis of CSVD-CI patients from HCs (AUC = 0.825, sensitivity = 81.48%, specificity = 75.00%; Figure 3E).

Mini Meta-Analysis. Including the present study, a total of two studies[16] reported on the blood MIF levels of CSVD patients (Table S4). These studies included a total of 234 volunteers, including 125 CSVD-CI patients and 109 CSVD-CN patients. Table S3 presents the characteristics of studies included in the present mini meta-analyses. Our findings showed that compared with CSVD-CN patients, the blood MIF levels were significantly increased in CSVD-CI patients [standard mean difference (SMD): 0.594; 95% CI: (0.331, 0.857); $P < 0.001$; $I^2 = 0.00\%$. The forest plots are presented in Figure 4].

Besides the present study, no studies were available in the two main databases that reported on blood MIF levels between CSVD and HC cohorts (data not shown). Therefore, the meta-analysis between CSVD and HC cohorts could not be performed.

DISCUSSION

The main findings of this study are as follows: (1) CSVD-CI patients showed significantly higher plasma MIF levels than HCs and CSVD-CN patients. (2) In CSVD-CI patients, higher plasma MIF levels were significantly associated with impaired cognitive function and increased plasma levels of MMP-9, NSE, and S100β. (3) Plasma levels of MIF were significantly correlated with changes in structural MRI (total WMH Fazekas scores) and functional MRI (mALFF values of STG.R) in the CSVD-CI group. (4) Plasma MIF was an essential part of the composite marker for differentiating CSVD-CI patients from CSVD-CN patients or HCs. (5) The pooled results of the present mini meta-analysis indicated a significant association between the blood MIF levels and CSVD-CI, and higher blood MIF levels may represent a potential risk signal for the development of CSVD-CI. Overall, clinical symptomatology, brain MRI, and blood markers support that MIF is a potential diagnostic biomarker of cognitive decline in CSVD.

The greatest strength of the present study is the comprehensive investigation of circulating MIF in apparent

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(symptomatology), mesoscopic (imageology), and microscopic (molecules) for the clinical value in CSVD-CI. In addition, compared with the previous study,[16] our study added a healthy cohort to determine the levels of plasma MIF in the normal state, which was helpful to reveal the pathophysiological role of MIF in the development of CSVD-CI. Furthermore, we provided an effective model to achieve the early diagnosis of CSVD-CI based on our findings of plasma MIF. Hence, the strict study design and abundant data analysis enhanced the value of our study for the clinical practice of CSVD-CI.

Consistent with previous research,[16] plasma MIF levels were significantly higher in CSVD-CI patients compared to CSVD-CN patients and HCs, suggesting a specific increase in plasma MIF in CSVD-CI patients. Furthermore, significant correlations between plasma levels of MIF and multiple cognitive assessment scores were observed in CSVD-CI patients, suggesting that plasma MIF can reflect the state of cognitive decline in CSVD. With consideration of various factors, line regression analysis still indicated that increased plasma levels of MIF were strongly associated with decreases in global cognitive function and information processing speed. Strong evidence of meta-analysis supported higher blood MIF levels for the risk of CSVD-CI. Therefore, plasma MIF levels may play an important role in cognitive impairment of CSVD.

In the present study, blood indices representing abnormal BBB function and inflammation were used to investigate the underlying mechanism of plasma MIF in CSVD-CI. A previous study reported that diminished endothelial function and arterial stiffness are associated with high MIF plasma levels,[18] suggesting that increased plasma MIF levels might be an indicator of BBB dysfunction. However, there is no evidence supporting the involvement of MIF in pathological changes in CSVD. In disease states, BBB opening could lead to elevated peripheral levels of MMP-9, NSE, and S100β,[19−21] providing an opportunity to assess the association of plasma MIF and CSVD-related pathology. In the present study, plasma MIF showed significant positive correlations with plasma MMP-9, NSE, and S100β levels in CSVD-CI patients, suggesting that plasma MIF may participate in BBB disruption in the pathological changes of CSVD-CI. Additionally, the interaction of plasma MIF and MMP-9 had a significant effect on the information processing speed, further supporting the role of plasma MIF in CSVD-CI via BBB dysfunction.

As an important risk marker of CSVD, WMH pathogenesis may involve multiple contributing factors including BBB leakage.[22,23] In our study, CSVD-CI patients showed significantly higher total WMH Fazekas scores than CSVD-CN patients, which is consistent with previous findings.[16] The significant correlation of plasma MIF levels with total WMH Fazekas scores indicated that MIF could be associated with WMH development and positively mirror the severity of WMH, which was further supported by the finding of differences in MIF plasma levels between the Fazekas score subgroups. Furthermore, previous studies have reported that decreased activity in the PoCG.L[24] and increased activity in the DCG.R[25] and STG.R[26] are closely related to cognitive impairment, which is in line with our results. The present study found that plasma MIF levels were positively correlated with mALFF values of the STG.R in the CSVD-CI group. Moreover, plasma MIF levels and mALFF values of the DCG.R showed a significant interaction that influenced global cognitive function in CSVD patients. Together, these findings indicate that plasma MIF levels may influence CSVD-CI through changes in brain activity.

Plasma MIF exhibited a good performance (accuracy > 75%) to identify CSVD patients—either CSVD-CI or CSVD-CN—from HCs, although total WMH Fazekas scores and plasma NSE had a better diagnostic performance. Of note, no single marker could distinguish CSVD-CI patients from CSVD-CN patients with satisfactory power. Thus, the LASSO model, which is a machine learning model, was used to build a composite marker based on five markers to achieve differential diagnosis. Not only did this new composite marker show a specific increase in CSVD-CI patients compared with the other two groups but it also had a better performance for identifying CSVD-CI patients from CSVD-CN patients or HCs. While plasma MIF as a biomarker could contribute to improved diagnostic accuracy of CSVD, the use of a composite marker comprising plasma MIF and MRI markers may be more valuable for clinical diagnosis of CSVD-CI.

This study is subject to several limitations. First, the dynamic features of plasma MIF in CSVD progression remain unclear—particularly in patients who have transitioned from CSVD-CN to CSVD-CI. Second, although some molecular and neuroimaging evidence are provided in this study, the explicit pathophysiological mechanism of MIF causing CSVD-CI requires further exploration. Longitudinal follow-up of CSVD patients or CSVD animal model research could help address these limitations.

CONCLUSIONS

The present study demonstrated that plasma MIF levels are significantly elevated in CSVD-CI patients and are associated with worse cognitive performance. Plasma MIF may mediate cognitive decline in CSVD via BBB dysfunction and changes in WMH and brain activity. Plasma MIF as a potential peripheral biomarker could provide satisfactory differential power for identifying CSVD patients. In particular, plasma MIF may be most valuable as part of a composite marker for achieving a sensitive and accurate diagnosis of CSVD-CI.

MATERIALS AND METHODS

Participants. A total of 171 participants of Chinese Han ethnicity were recruited from the Affiliated Wuxi People's Hospital of Nanjing Medical University and resident communities (Wuxi, China), including 117 CSVD patients and 54 HCs. Each participant completed a standardized clinical interview, including a demographic inventory and examination of physical and mental health. All participants also underwent structural and functional brain MRI, neuropsychological assessments, and blood collection.

All participants or their legal guardians provided written informed consent, and the Ethics Committee of the Affiliated Wuxi People's Hospital of Nanjing Medical University approved the present study (approval number: KY2112).

Neuropsychological Assessments. Two experienced investigators completed the neuropsychological assessments for all participants. The neuropsychological battery included: (1) global cognition: MMSE and MoCA; (2) episodic memory: Auditory Verbal Learning Test-immediate recall and Auditory Verbal Learning Test-20 min delayed recall; (3) information processing speed: Trail Making Tests A (TMT-A) and Stroop Color and Word Test A and B (Stroop-A and Stroop-B); (4) executive function: Trail Making Tests B

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(TMT-B), Stroop Color and Word Test C (Stroop-C), and Digit Span Test (DST); and (5) visuospatial function: Clock Drawing Test.

As previously described,[27−30] the raw test scores of each scale were standardized through *Z*-transformation using the mean and standard deviation for subsequent analysis. Information processing speed scores were calculated based on the composite TMT-A, Stroop-A, and Stroop-B scale scores using the *Z*-transformed averages. Executive function scores were obtained using the *Z*-transformed averages of the TMT-B, Stroop-C, and DST-backward scales.

Inclusion and Exclusion Criteria. The inclusion criteria were as follows: (1) age 50−80 years old; (2) years of education ≥ 6; and (3) good health with adequate visual and auditory acuity. The exclusion criteria were: (1) any mental disease (e.g., major depressive disorder, bipolar disorder, and schizophrenia) or family history of psychosis; (2) MRI evidence supporting cerebrovascular disorders with large intracranial vascular lesions; (3) other neurologic diseases (e.g., Parkinson's disease) or brain trauma; (4) any obvious medical problems (e.g., significantly impaired liver, kidney or thyroid function, arrhythmia, tumor, and infectious disease); and (5) abuse or dependence of alcohol or drugs; and (6) any contraindications for MRI.

According to established diagnostic criteria,[27,31] CSVD patients were diagnosed based on MRI evidence of vascular changes (details were displayed in the Supporting Information). Periventricular and deep WMH were quantified using the Fazekas scale. CSVD patients showed total WMH Fazekas scores ranging from 1 to 6.[17] CSVD patients with MCI that exhibited some degree of cognitive decrease, as determined by family members and neuropsychological assessments (i.e., solely MoCA scores < 26 or MoCA scores = 26 and TMT-B (s) > 188),[32−34] were termed the CSVD-CI group (N = 60) while those with normal cognitive function were termed the CSVD-CN group (N = 57). The matched HCs had normal cognition and showed no imaging changes reflecting cerebrovascular disease.

Assessment of Plasma Indices. Fasting peripheral venous blood was collected in EDTA-coated tubes between 8:00 am and 9:00 am. Within 30 min of collection, the samples were centrifuged at 1000*g* at 4 °C for 10 min. Subsequently, the plasma was aliquoted and stored at −80 °C.

Five plasma indices, namely MIF, S100β, MMP-9, NSE, and TNF-α, were assessed using commercial enzyme-linked immunosorbent assays kit (FineTest, Wuhan, China; Catalog Number: EH0016 for MIF, EH0543 for S100β, EH0238 for MMP-9, EH0370-HS for NSE, and EH0302 for TNF-α). The levels were measured in triplicate, and the inter- and intra-assay coefficients of variation were <5%.

MRI Data Preprocessing. Data preprocessing was performed using the Data Processing Assistant for Resting-State fMRI (DPARSFA 2.3) toolbox.[35] Based on blood oxygenation level-dependent signals, the ALFF was obtained to estimate local spontaneous neuronal activity.[36] REST software was used to calculate the mALFF values and help perform statistical analysis (ANCOVA test) among the three groups (Alphasim multiple comparison correction p < 0.001 and cluster size > 100 voxels), with age, sex, and education years as covariates. Brain regions with significant differences in mALFF values were visualized using BrainNet Viewer software. Further details are provided in the Supporting Information and our previous studies.[27,37,38]

Statistical Analyses. Statistical analyses were carried out using SPSS version 22.0 (SPSS Inc. Chicago, Illinois) and R software (version 4.2.1). Continuous variables are presented as the mean ± standard deviation. The Kolmogorov−Smirnov test was used to evaluate the normal distribution of the data. The Kruskal−Wallis H test was used if variables were non-normally distributed with the post hoc Nemenyi test; otherwise, a one-way analysis of variance was used with the post hoc Bonferroni test. Categorical variables were analyzed using the chi-square test. Partial correlation analysis was used to determine the relationships between two variables, controlling for age, sex, and education years. Multivariate analysis was performed using line regression analysis with age, sex, and education years as covariates. To identify diagnostic markers of CSVD-CI, the LASSO model was used to construct a composite biomarker.[39,40] ROC curves were performed to obtain the AUC to estimate the diagnostic accuracy of the markers. The Youden index[41] was utilized to determine the optimal value of sensitivity and specificity. Results with p < 0.05 were considered to indicate statistically significant differences.

Meta-Analysis. Meta-analysis was conducted using STATA software version 12.0 (StataCorp, College Station, Texas). Relevant references were screened from PubMed and Web of Science online databases up to July 2023 to collect eligible data for the meta-analysis study. The focus included cross-sectional case−control studies, which contained both CSVD-CI and CSVD-CN/HC cohorts. Studies that met the inclusion criteria reported on the mean and SD of blood MIF levels and included a sample size of ≥10 per group. Data from these studies were extracted, combined with new data that were obtained in our study, and analyzed. The SMD was used to estimate the pooled effect size, and the random-effect model was used to calculate variance. I^2 statistic was utilized to evaluate the heterogeneity, and the 95% confidence interval was used as a measure for continuous variables.

■ ASSOCIATED CONTENT

Data Availability Statement

The raw data analyzed in this article are not publicly available. Requests to access the data should be directed to the corresponded authors.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10126.

Method of MRI analysis and data of association analyses and ROC analyses (PDF)

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Author Contributions

#Y.S., J.D., H.M., and Y.H. contributed equally to this work as shared first authors. Y.S., X.F., and F.W. conceived and designed the study. J.S. supervised to carry out the protocols. Y.L., W.J., G.X., and Y.Y. enrolled subjects and characterized subjects. Y.S. and J.D. collected and analyzed data, prepared the tables and figures, and wrote the manuscript. H.M., Q.G., S.Z., and L.M. were responsible for multimode MRI scans. Y.H. measured plasma levels of indices. K.C. assisted with the structure design of the manuscript. All authors contributed to the article and approved the submitted version.

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Notes

The authors declare no competing financial interest.

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