Serum YKL-40 as a biomarker related to white matter fiber damage and cognitive impairment in patients with cerebral small vessel disease

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Serum YKL-40 as a biomarker related to white matter fiber damage and cognitive impairment in patients with cerebral small vessel disease

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Abstract

Chitinase-3-like protein-1 (YKL-40) is recognized as a novel neuroinflammatory biomarker related to neurodegenerative diseases. We aimed to explore whether serum YKL-40 can serve as a potential biomarker in patients with cerebral small vessel disease (CSVD), and possible neural correlates with white matter fiber damage and cognitive impairment. Blood samples were collected from 100 CSVD patients and 80 healthy controls (HCs) to measure serum YKL-40 levels. All subjects underwent diffusion tensor imaging (DTI) scanning and cognitive function assessment, and the CSVD group was classified into CSVD-no cognitive impairment (CSVD-NCI) and CSVD-mild cognitive impairment (CSVD-MCI) two subgroups. Binary logistic regression analysis was conducted to determine the independent risk factors for CSVD and CSVD-MCI. Receiver operating characteristic analysis was performed to assess the diagnostic value of YKL-40. Partial correlation analysis was carried out to explore the associations of DTI indices with YKL-40 and cognition. The serum YKL-40 levels of CSVD were significantly higher than those of the HCs, and the CSVD-MCI was higher than that in HCs and CSVD-NCI. YKL-40 was further identified as an independent risk factor for CSVD and CSVD-MCI, in addition, serum YKL-40 provided high diagnostic accuracy for CSVD and CSVD-MCI. Comparison of DTI indices showed that patients with CSVD accompanied by a significant decrease in white matter fibers integrity. Moreover, the altered DTI indices were significantly correlated with the elevated level of YKL-40 and worse cognitive performance. Our study suggests that YKL-40 may be a biomarker of CSVD and may cause cognitive impairment by disrupting white matter fiber integrity.

Key words: cerebral small vessel disease, YKL-40, white matter fiber, cognitive impairment, diffusion tensor imaging

Introduction

Cerebral small vessel disease (CSVD) is frequently found on MRI images, and mainly manifests as white matter hyperintensitie (WMH), lacunes, cerebral microbleed (CMB), perivascular spaces, and brain atrophy(Wardlaw et al., 2013). The prevalence of CSVD increases with age, according to the statistics, the prevalence of WMH for people aged 50 years is 5%, increasing to nearly 100% by the age of 90 years(de Leeuw et al., 2001). Similarly, the prevalence of CMB increases from 6.5% for people in the age of 45-50 years to about 36% for people aged 80–89 years(Poels et al., 2010). Besides, approximately 45% of dementia cases are caused by CSVD(Pantoni, 2010). The pathogenesis of CSVD is complex, which may be related to the inflammatory mechanism, but there is no specific inflammatory marker yet.

Previous studies suggest that patients with CSVD have extensive loss of microstructural integrity of white matter fibers and are associated with cognitive impairment(Tuladhar et al., 2015). However, conventional neuroimaging is limited to observing late pathological changes. Diffusion tensor imaging (DTI) can quantify

early subtle white matter fiber microstructure changes in CSVD. Among the indices derived from DTI, fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) values are considered to best reflect the integrity of white matter fiber microstructure. Studies have confirmed that elevated peripheral inflammation can affect brain structure, especially the loss of white matter integrity(Bettcher et al., 2015; Satizabal, Zhu, Mazoyer, Dufouil, & Tzourio, 2012). Studies have observed that elevated C-reactive protein (CRP) levels in middle age may lead to the destruction of white matter integrity later in life. It is believed that systemic inflammation can promote changes in the microstructure of white matter(Walker et al., 2017). To date, few studies have investigated the integrity of white matter fibers and their relationship with inflammation in CSVD and associated cognitive impairment.

YKL-40, also known as chitinase-3 like protein-1 (CHI3L1) or human cartilage glycoprotein 39, is a 40 kDa heparin and chitin-binding glycoprotein(Renkema et al., 1998). It can be synthesized and secreted by a variety of cells such as macrophages, chondrocytes, endothelial cells, astrocytes and microglia(Connor et al., 2000; Rehli et al., 2003). Recently, a growing body of studies have shown that YKL-40 is a specific neuroinflammation marker in neurodegenerative diseases(Llorens et al., 2017), and is related to biomarkers such as nerve damage, large myelinated axons, and synaptic damage(Baldacci, Lista, Cavedo, Bonuccelli, & Hampel, 2017). YKL-40 exists in the systemic circulation and plays a role in atherosclerosis, cardiovascular and cerebrovascular diseases. Evidence has shown that the level of plasma YKL-40 is positively correlated with the severity of carotid and coronary atherosclerosis(Michelsen et al., 2010; Sciborski et al., 2018). In the general population, elevated plasma YKL-40 levels were associated with an increased risk of ischaemic cerebrovascular disease(Kjaergaard, Bojesen, Johansen, & Nordestgaard, 2010). In addition, serum YKL-40 was associated with all-cause mortality of cardiovascular disease and can be used as a biomarker of patient prognosis(Harutyunyan et al., 2012; Kastrup et al., 2009). In central nervous system diseases, YKL-40 can be used as a predictor of disease progression in multiple sclerosis and Alzheimer's disease(Alcolea et al., 2015; Varhaug et al., 2018). In addition, basic experiments have also found the possible inflammatory mechanism of YKL-40 in white matter lesions(Yuan et al., 2022), however, there are currently few studies on YKL-40 and CSVD and their associated cognitive impairment in the population. We speculate that YKL-40 may be a potential biomarker in patients with CSVD and CSVD-MCI and related to the damage of white matter fibers.

In this study, we aimed to explore whether serum YKL-40 could be a biomarker for CSVD and its early cognitive impairment, and its neural correlates with microstructural damage to white matter fibers and cognitive impairment.

Methods

Study design and participants

In this study, a total of 100 patients with CSVD were included from the Department of Neurology of the First Affiliated Hospital of Anhui Medical University, Hefei, China. The inclusion criteria were as follows: (1) age between 50 and 80 years; (2) at least one risk factor for arteriosclerosis, including hypertension, diabetes, hyperlipidaemia, smoking, drinking; (3) at least one common clinical symptom of CSVD, including cognitive decline, gait and balance disorders, mood or sleep disorders, and urinary and faecal disorders; or a history of symptomatic stroke; (4) magnetic resonance neuroimaging meets the imaging standards for CSVD diagnosis(Wardlaw et al., 2013), at least meet one of the following; WMH: periventricular Fazekas score=3 or subcortical Fazekas score $\geqslant$ 2; cerebral microbleeds (CMB): $\geqslant$1, CMBs were defined as small areas (2-10 mm in diameter) of signal void with associated blooming in SWAN sequence; and lacunes: $\geqslant$1, a symptomatic lacunar infarct was defined as circular or oval hyperintense lesions <20 mm on T2WI with corresponding hypointense lesions with a hyperintense rim on FLAIR.

The exclusion criteria were as follows: (1) new infarction with a larger area of diameter >2cm; (2) acute ischaemic stroke caused by atrial fibrillation or other cardiogenic embolism; (3) CT and other imaging examinations of cerebral haemorrhage; (4) patients with brain tumours or other systemic malignancies; (5) history of traumatic brain injury or craniocerebral surgery; (6) acute or chronic infectious status; (7) history of asthma and chronic obstructive pulmonary disease; (8) history of osteoarthritis and rheumatoid arthritis; (9) dysfunction of liver, kidney, heart, lung or other vital organs; (10) mental or consciousness disorders; (11) suspected cognitive impairment due to Alzheimer's disease or Parkinson's disease; (12) history of medication that clearly affects serum YKL-40 levels and cognitive function ; (13) patients with other CSVD aetiologies secondary to genetics, infections, autoimmune inflammation, tumours, trauma, poisoning, radiation, metabolic encephalopathy and sporadic cerebral amyloid angiopathy.

The study included 80 healthy controls (HCs) during the same period. They matched the demographic data of the CSVD group, including sex, age, and years of education, and did not meet the inclusion criteria of the CSVD group. This cross-sectional study was approved by the Ethics Committee of The First Affiliated Hospital of Anhui Medical University, and the study conformed to the World Medical Association Declaration of Helsinki. All participants signed an informed consent form before participating. The study flow diagram is shown Fig. S1.

Clinical assessment and classification of cognitive function

Demographic and clinical data of all subjects were collected, including sex, age, education level; history of stroke, hypertension, diabetes, hyperlipidaemia, drinking and smoking; medical history related to YKL-40 and vascular risk factors. In addition, detailed neurological physical examination and clinical interview were performed on all subjects by two neurologists. The diagnosis of MCI was based on previously published criteria and was judged by cognitive assessors based on neuropsychological

test results(Espinosa et al., 2013), and meet the diagnostic criteria for vascular-related cognitive impairment(Gorelick et al., 2011). First, participants complained of cognitive decline, and this performance lasted at least several months to several years; second, in the cognitive test, at least one or more cognitive domains were impaired but did not reach the level of dementia (compared with the healthy control, the composite z-score of at least one cognitive domain was lower than the adjusted average of 1.5SD); in addition, the participants' ability of daily living was basically unaffected, and only complex activities were impaired or defective. Therefore, those who did not meet the MCI standard were defined as no cognitive impairment (NCI).

Neuropsychological test

The neuropsychological tests of this research were uniformly evaluated by doctoral students in neurology who had undergone rigorous training. The Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess global cognitive function(Folstein, Folstein, & McHugh, 1975; Nasreddine et al., 2005). Additionally, we also assessed the following six main cognitive areas. (1) Information processing speed test was assessed using the Trail Making Test-A (TMT-A)(Reitan, 1955), the Stroop's Colour Word Test-A (SCWT-A: dot), the Stroop's Colour Word Test-B (SCWT-B: word)(Kynast et al., 2018) , and the Symbol Digit Modalities Test (SDMT)(Silva, Spedo, Barreira, & Leoni, 2018). (2) The Trail Making Test-B (TMT-B)(Selnes et al., 1991) and Stroop's Colour Word Test-C (SCWT-C: colour word)(Kynast et al., 2018) were used to measure executive function. (3) The forwards and backwards aspects of the Digital Span test (DS), were used to evaluate the attention of the subjects(Yamamoto, Kazui, & Takeda, 2011). (4) The auditory verbal learning test (AVLT) was used to evaluate memory function and consists of four parts: immediate memory, five-minute delay memory, twenty-minute delay memory, and recognition memory(Guo, Zhao, Chen, Ding, & Hong, 2009). (5) The Verbal Fluency Test (VFT) was used to evaluate language function(Thurstone, 1948). (6) The Weschler memory scale-revised (WMS-R) visual reproduction copy task was used to assess visuospatial ability(Yamamoto et al., 2011). In addition to various cognitive domain tests, Activities of Daily Living (ADL) scale was used to judge subjects' ability of daily living, and the Hamilton Anxiety (HAMA) and Hamilton Depression (HAMD) scales were assesed to rule out the influence of anxiety and depression on subjects' cognition. To facilitate the correlation analysis of DTI parameters and cognitive fields, and more intuitive reading results, we performed z-transformation on the neuropsychological test of each subject. Then, the subitems of each cognitive domain were averaged to obtain the composite score of a single domain.

Laboratory biomarker measurements

Subjects fasted for more than 12 hours. On the day of performing the brain MRI, 3 ml of peripheral venous blood was drawn on an empty stomach in the morning, and immediately sent to the laboratory of the First Affiliated Hospital of Anhui Medical University to complete liver and kidney function, triglycerides (TG), total cholesterol

(TCH), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), homocysteine (Hcy), and hs-CRP detection. Additionally, 2 ml of peripheral venous blood was taken at 3000 rpm/separation of the heart for 8 minutes, the serum was taken in the Eppendorf tube and placed in the refrigerator at -80 °C until it was used for testing. Using a two-site sandwich enzyme-linked immune sorbent assay to determine serum YKL-40 (lowest detection limit 3 ng/L), the intra-assay and interassay coefficient changes were less than 9% and 11%, respectively (Huada Biotech Corporation Ltd., Wuhan, China).

Image acquisition

MRI scans were obtained using a 3.0-Tesla MR system (Discovery MR750w, General Electric, Milwaukee, WI, USA) with a 24-channel head coil. The detailed image acquisition parameters and processing steps of the structural and diffusional MRI data were described in Supplementary File.

Structural MRI data processing

The three-dimensional (3D) structural MRI images were processed using the Computational Anatomy Toolbox 12 (CAT12) software (http://www.neuro.uni-jena.de/cat) based on SPM8. Firstly, all the structural images were corrected for bias-field inhomogeneities. Secondly, the structural images were segmented into three density components including gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Thirdly, these concentration images of GM, WM and CSF were spatially co-registered into the MNI standard space using DARTEL method(Ashburner, 2007). Fourthly, the total volumes of three components were obtained via multiplying the transformed concentration images by the parameters generated from the abovementioned normalization step(Ashburner & Friston, 2000). Total brain volume (TBV) was calculated as the sum of total GM and WM. Intracranial volume (ICV) was the summation of all tissue classes (total GM, total WM and CSF volume). The whole white matter hyperintensities volum (WMHV) were detected using these processed 3D T1-weighted images, and volumes of total WMH were estimated as direct volumes in mm^3.

DTI data processing

The DTI datasets were pre-processed with the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). First, eddy current distortion and head motion were corrected by registering the diffusion-weighted images to the first b0 image through the affine transformations(Smith et al., 2004). Second, the data were skull-stripped by using the FMRIB Brain Extraction Tool(Smith, 2002). Then, diffusion parameters maps of FA were calculated by using the DTIFIT toolbox. Third, we applied non-linear registration of all FA images to the Montreal Neurological Institute (MNI) 152 standard space, provided by MNI(Jenkinson, Bannister, Brady, & Smith, 2002). Thereafter, the mean FA images were drawn and thinned to create a mean FA skeleton, which represented the centers of all tracts common to the group (threshold FA value of 0.2). The aligned FA, AD, RD, and MD images for each subject were

then projected onto this common skeleton(Smith et al., 2006). Following analysis with randomize and the creation of the white matter skeleton, region of interest (ROI) areas were created using a semi-automated procedure that incorporated 50 structures identified probabilistically by the Johns Hopkins University white matter tractography Atlas(Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). We divided these white matter fibers of interest into four categories according to their functional type based on the white matter atlas ICBM-152(Mori et al., 2008). Including, Brainstem fibers: middle cerebellar peduncle, pontine crossing tract, bilateral corticospinal tract, bilateral medial lemniscus, bilateral inferior cerebellar peduncle, bilateral superior cerebellar peduncle; Commissural fibers: corpus callosum (divided into genu, body and splenium), bilateral tapetum; Projection fibers: bilateral cerebral peduncle, bilateral internal capsule (divided into anterior limb, posterior limb and retrolenticular), bilateral corona radiata (divided into anterior, posterior and superior), bilateral posterior thalamic radiation; Association fibers: fornix, bilateral sagittal stratum, bilateral external capsule, bilateral cingulum (cingulate gyrus), bilateral cingulum (hippocampus), bilateral fornix (cres)/stria terminalis, bilateral superior longitudinal fasciculus, bilateral superior fronto-occipital fasciculus, bilateral inferior fronto-occipital fasciculus, bilateral uncinate fasciculus. Intersections between anatomical masks and the skeleton were used in order to extract mean FA, AD, RD and MD values in these regions. Mean FA, MD, RD and AD from ROIs of the same fiber types were averaged to obtain the overall FA, MD, RD and AD estimates of each fiber type.

Statistical analysis

Statistical analysis of all data was performed using SPSS version 25. The comparison of continuous variables between the HC and CSVD groups used independent samples t-tests and Mann-Whitney U tests, and categorical variables were analyse using the chi-square test. The comparison of continuous variables between the HC, CSVD-NCI and CSVD-MCI groups used one-way analysis of variance (ANOVA) followed by a post hoc Bonferroni test and Kruskal-Wallis tests, categorical variables were tested by chi-square tests, and the significant difference between the three groups was corrected by Bonferroni method to control false-positive, P <0.0167 was considered statistically. Binary logistic regression analysis was performed to determine the independent risk factors for CSVD. To evaluate the diagnostic value of serum level of YKL-40 in distinguishing HC from CSVD, and CSVD-NCI from CSVD-MCI, receiver operating characteristic (ROC) analysis was performed, the maximum Youden index was calculated to obtain the corresponding sensitivity, specificity and optimal cut-off values, and the area under the curve (AUC) was calculated to provide better tools for early diagnosis. Partial correlation analysis was used to analyze the correlation between DTI indices and YKL-40 and Z-scores for each cognitive domain. The statistical significance threshold was set at P <0.05.

Results

Participant characteristics, clinical data, and neuroimaging manifestations

A total of 100 CSVD patients [divided into CSVD-NCI (n=45) and CSVD-MCI (n=55) subgroups based on the results of neuropsychological tests] and 80 HCs matched for age, sex, and education level were included in this study. The demographic and clinical data are shown in Table 1. CSVD had a higher proportion of hypertension, diabetes, lacunes, and microbleeds, as well as higher levels of Hcy and and larger volum of WMHV. Most noteworthy was that the level of YKL-40 in the CSVD group was significantly higher than that in the HC group (125.47±6.20 ng/L vs. 120.60±7.70 ng/L). Compared with the HCs, patients with CSVD-MCI had higher rates of hypertension, diabetes, lacunes and microbleeds, and higher levels of Hcy and YKL-40, and larger volum of WMHV. Compared with CSVD-NCI, the CSVD-MCI WMHV and serum YKL-40 levels (128.41±7.65 ng/L vs. 123.91±6.76 ng/L) were significantly higher.

Binary logistic regression analysis of the risk factors for patients with CSVD and CSVD-MCI

To further examine the risk factors for CSVD and CSVD-MCI, we performed binary logistic regression model (Table 2) for the factors that may affect CSVD and CSVD-MCI. The result showed that hypertension (odds ratio [OR] =3.203; 95% Confidence interval (CI=1.193-8.590); p =0.021) and diabetes (OR=7.074; 95%CI =1.025-48.844 p =0.047) could significantly affect the appearance of CSVD. In addition, the level of YKL-40 was significantly associated with the risk of CSVD with the adjusted OR of 1.067 (95% CI =1.012-1.126; p =0.017). Furthermor, we found that only YKL-40 levels (OR=1.146; 95%CI =1.048-1.255; p =0.047) and WMHV (OR=1.250; 95%CI =1.073-1.353; p =0.002) were independent risk factors for CSVD-MCI after adjusting for confounders such as vascular risk factors and imaging markers of small vessel disease.

ROC analysis of the serum YKL-40 levels in the diagnosis of the HCs, CSVD-NCI, and CSVD-MCI

Considering the above findings, we speculated that serum YKL-40 may be a potential marker of CSVD and cognitive impairment, we thus further determined the diagnostic value of serum YKL-40 levels by constructing the ROC curve. We found that when the maximum Youden index was 0.3158, the optimal cut-off value for YKL-40 to discriminate between HC and CSVD was 123.40 ng/L, at which the sensitivity and specificity were 71.58% and 66.80%, respectively, and the AUC was 0.663 (p <0.01, 95%CI: 0.569-0.756) (Fig. 1a). Similarly, when the maximum Youden index was 0.4182, the optimal cut-off value for YKL-40 to discriminate between CSVD-NCI and CSVD-MCI was 127.70 ng/L, at which the sensitivity and specificity were 71.82% and 80.10%, respectively, and the AUC was 0.725 (p <0.001, 95%CI: 0.624-0.830) (Fig. 1b).

Mean differences in white matter fiber DTI indices between the HCs, CSVD-NCI, and CSVD-MCI

We compared the DTI indices between the three groups and found that there was no significant difference in FA, MD, RD and AD of brainstem fibers. Howere, we found that subjects in CSVD-MCI exhibited significant disruption of microstructural integrity in commissural, projection, and association fibers than those of the HCs and CSVD-NCI. In addition, association and projection fibers showed significant microstructural impairment in CSVD-NCI patients. Details of the intergroup comparison of the FA, MD, RD, and AD values were presented in Fig. 2a-d.

Association of serum YKL-40 level with white matter fiber DTI indices in CSVD-NCI and CSVD-MCI

To further determine whether the increase of serum YKL-40 level was related to the destruction of white matter fiber integrity, we performed partial correlation analysis (after adjustment for age, sex, education, vascular risk factors and CSVD markers) on serum YKL-40 levels with significant changes in DTI index in CSVD-NCI and CSVD-MCI groups. The correlations between white matter fiber DTI indices and serum YKL-40 levels in CSVD-MCI patients were illustrated in Fig. 3. Interestingly, elevated serum YKL-40 levels were significantly correlated with lower FA and higher MD, RD, and AD in the three fiber types (commissural, projection, association) (Fig. 3a-d). The correlations between white matter fiber DTI indices and serum YKL-40 levels in CSVD-NCI patients were illustrated in Supplementary file Fig. S2. The results showed that elevated serum YKL-40 levels were significantly correlated with lower FA and higher MD, RD in the projection and association fibers (Fig. S2 a-c). It indicated that there was a certain relationship between elevated serum YKL-40 levels and decreased white matter fiber integrity.

Association between altered white matter fibers DTI indices and cognitive function in CSVD-NCI and CSVD-MCI

To further determine whether disruption of white matter fiber integrity was associated with cognitive impairment, we performed partial correlation analysis (after adjustment for age, sex, education, vascular risk factors and CSVD markers) on z-score of major cognitive domains with DTI index in CSVD-NCI and CSVD-MCI groups. The correlations between white matter microstructure integrity and cognitive z-score in CSVD-MCI patients are illustrated in Table 3. We found that among the three diffusion indices of FA, MD, and RD, the decreased integrity of commissural fibers was significantly related to worse information processing speed; the decreased integrity of association fibers was significantly related to worse information processing speed, excutive and language function, respectively; and the decreased integrity of projection fibers was significantly related to worse information processing speed. However, no white matter fibers were found to be associated with cognitive function in the AD index of DTI.

The correlations between white matter microstructure integrity and cognitive z-score in CSVD-NCI patients are showed in Table S1. Similarly, we found that among the three diffusion indices of FA, MD, and RD, the decreased integrity of associattion fibers was significantly related to worse information processing speed, excutive and

language function, respectively; and the decreased integrity of projection fibers was significantly related to worse information processing speed.

Discussion

CSVD is one of the most important causes of stroke and cognitive impairment(Gorelick et al., 2011; Nam et al., 2017), and white matter damage is very common in CSVD. As a novel inflammatory marker, YKL-40 considered being closely related to cardiovascular, cerebrovascular, and neurodegenerative diseases(Dichev, Kazakova, & Sarafian, 2020; Zhao, Su, Li, Zhang, & You, 2020), bue few studies have explored the relationship between YKL-40 and CSVD and associated cognitive impairment. In this study, we identified for the first time the diagnostic value of YKL-40 in patients with CSVD, and its association with white matter fiber damage and cognitive impairment, with the following important results. First, the serum YKL-40 levels of CSVD were significantly higher than those of the HCs, and the CSVD-MCI was higher than that in HCs and CSVD-NCI; Second, YKL-40 was an independent risk factor for CSVD and CSVD-MCI; Third, serum YKL-40 can be used for the early diagnosis of CSVD and cognitive dysfunction; Finally, the elevated level of YKL-40 was correlated with damage of white matter fibers, and disruption of specific white matter fiber integrity was associated with corresponding cognitive impairment.

In our cross-sectional study, we found that the proportion of hypertension, diabetes, and the levels of Hcy of the CSVD were higher than in the HCs. Studies had shown that YKL-40 levels were increased in patients with essential hypertension and independently associated with arterial stiffness(Ma et al., 2012). Similarly, plasma YKL-40 was elevated in type 2 diabetes independent of hs-CRP(Rathcke, Johansen, & Vestergaard, 2006). On the one hand, it was well known that hypertension, diabetes, and high homocysteine levels were strong vascular risk factors for atherosclerosis, and the study found that both macrophages and human smooth muscle cells in atherosclerotic plaques had high expression of YKL-40(Boot et al., 1999; Michelsen et al., 2010). On the other hand, Hypertension and other factors were important factors leading to vascular endothelial injury, and previous related experiments had shown that YKL-40 was of great significance in cell proliferation(Recklies, White, & Ling, 2002), cell survival(Harutyunyan et al., 2012), extracellular matrix and tissue remodeling(Michel, Tonon, Scornet, Cock, & Kloareg, 2010), and angiogenesis(Junker, Johansen, Hansen, Lund, & Kristjansen, 2005), so it had a protective effect from a certain degree. In addition, YKL-40 may function in vascular endothelial cell generation by stimulating smooth muscle cell migration and adhesion(Malinda, Ponce, Kleinman, Shackelton, & Millis, 1999). But at the same time, YKL-40 in the pathological state or beyond a certain critical value may accelerate the process of atherosclerosis， and cause inflammatory cascade reaction, thereby leading to the occurrence of the disease. These might be the reason that the serum YKL-40 levels in CSVD patients were higher than that in HCs. Our logistic regression analysis further showed that YKL-40 was still independently associated

with CSVD after including vascular and other related risk factors, indicating that YKL-40 played an independent role in the occurrence and development of CSVD. Therefore, we tried to further determine whether YKL-40 could be used to distinguish CSVD from HC. The ROC results showed an AUC of 0.663 for YKL-40, and the optimal cut-off value to discriminate between HC and CSVD was 123.40 ng/L, at which the sensitivity and specificity were 71.58% and 66.80%, respectively. ROC analysis showed that YKL-40 could serve as an effective diagnostic marker for CSVD.

When we divided the CSVD group into CSVD-NC and CSVD-MCI groups based on neuropsychological tests, we found similar results, with higher levels of YKL-40 in the CSVD-NCI than in the HCs, and higher levels in the CSVD-MCI than that in patients in CSVD-NCI. Studies have shown that compared to healthy subjects, pre-AD patients had higher levels of YKL-40(Antonell et al., 2014); compared with subjective cognitive decline patients, subjects with MCI had higher levels of YKL-40(Nordengen et al., 2019), which was similar to our findings. Further results showed that YKL-40 was an independent risk factor for mild cognitive impairment in CSVD and was independent of imaging markers of small vessel disease. ROC results showed an AUC of 0.725 for YKL-40, and the optimal cut-off value to discriminate between CSVD-NCI and CSVD-MCI was 127.70 ng/L, at which the sensitivity and specificity were 71.82% and 80.10%, respectively. The ROC results showed that when YKL-40 reached a certain concentration, it could effectively differentiate patients in CSVD with MCI from those with NCI, and therefore it could be used as a diagnostic tool for early cognitive impairment in CSVD, but the specific neural mechanism was still unclear.

A large prospective cohort study found that cognitive impairment in patients with CSVD was primarily associated with loss of normally occurring white matter microstructure, and location-specific disruption was associated with specific cognitive impairment(Tuladhar et al., 2015). Several previous studies had shown that the destruction of white matter integrity was related to inflammatory mechanisms(Bettcher et al., 2015; Chiang et al., 2017). A recent study found that inhibition of adenosine A2a receptor could significantly increase the expression of STAT3 and YKL-40 in astrocytes, thereby aggravating white matter damage(Yuan et al., 2022). For further explore the neural correlates of YKL-40 and cognitive decline in CSVD patients, we performed analysis of white matter fiber integrity in all subjects using DTI imaging. Results showed that compared with the HCs, the integrity of association and projection fibers in CSVD-NCI patients were significantly decreased, while the CSVD-MCI had significantly disruption in commissural, association, and projection fibers integrity compared with the HCs and CSVD-NCI. We also noted that there was no significant difference in the DTI index of brainstem fibers among the three groups, indicating that the white matter integrity disruption in the early stages of CSVD and cognitive impairment was mainly concentrated in regions closely related to cognitive function. Multiple studies had also shown that the loss of white matter fiber integrity in CSVD was more manifested in cognitively relevant regions such as the corpus callosum and hippocampus(Tuladhar et al., 2015; van Norden et al., 2012).

White matter microstructure was susceptible to vascular risk factors, especially hypertension. The RUN DMC study found that increased blood pressure and hypertension were associated with loss of white matter integrity in both normal appearing white matte and WMH(Gons et al., 2010). In addition, a cross-sectional study of white matter microstructure in diabetic patients had shown decreased white matter microstructural integrity in patients with type 2 diabetes compared with healthy subjects(Del Bene et al., 2015). In our study, the CSVD and subgroups had higher rates of hypertension and diabetes, which may partly explain the disruption of white matter integrity, but some of the reasons remain unclear. Therefore, after controlling for related vascular risk factors, we found that elevated YKL-40 levels were still associated with reduced FA, elevated MD, RD, and AD. It suggested that the elevated levels of YKL-40 may be related to the destruction of white matter fibers. Some researchers found that in Alzheimer's disease patients, higher cerebrospinal fluid (CSF) YKL-40 levels were associated with elevated CSF light chain protein (NFL), suggesting that YKL-40 plays an important role in neurological damage(Melah et al., 2016). Two large longitudinal follow-up cohort studies found that YKL-40 was associated with an increase in mean diffusivity (MD) over time. These findings suggest that YKL-40 is an important indicator of decreased white matter integrity(Racine et al., 2019). On the other hand, some studies have pointed out that YKL-40 may lead to decreased blood brain barrier stability and increased permeability(Muszyński et al., 2017). While circulating YKL-40 entering the brain parenchyma, it can activate microglia and astrocytes to secrete YKL-40, interleukin (IL)-6, tumor necrosis factor-α (TNF-α), activity Oxygen, and other inflammatory mediators, and then cause damage to nerve cells and white matter fibers(Chitnis & Weiner, 2017; Lucas, Rothwell, & Gibson, 2006). Therefore, we suppose that the disruption of white matter integrity in CSVD may be partly caused by YKL-40, and the specific mechanism remains to be further explored.

It was also found in our findings that disruption of white matter fiber integrity was associated with cognitive decline and that different types of white matter fibers are associated with specific cognitive domains. Our datas showed that the decrease in fiber integrity was related to the decrease in executive function. Previous studies have reported that the destruction of the white matter microstructure of the corpus callosum was related to the decline of executive function, which may be due to the connection of the corpus callosum to the prefrontal brain area related to executive function(Johnson et al., 2017), it was consistent with our results. Meanwhile, the decline in the integrity of the association fibers was related to the decline of information processing speed, executive and language function, which is mainly attributed to the function of the association fibers to transfer information with the frontal, temporal and occipital lobes and the limbic system(Wakana, Jiang, Nagae-Poetscher, van Zijl, & Mori, 2004). In addition, the decline in the integrity of the projection fibers was also related to the decline of information processing speed. Previous studies have shown that damage to the sub-frontal cortical circuits and thalamic projection fibers is an important predictor of deficits in information processing speed(Bai et al., 2020). It is worth noting that in our findings, white matter

fiber damage in CSVD-NCI and CSVD-MCI patients was mainly related to information processing speed and executive function, which was also similar to previous studies, and the early cognitive decline in CSVD was mainly manifested in these two fields(Prins et al., 2005). Based on the above results, we have reason to speculate that there might be some neural correlation between YKL-40 and cognitive impairment in CSVD, which was through disruption of the integrity of white matter fibers.

It is worth mentioning that few previous studies have focused on the relationship between YKL-40 and CSVD, especially YKL-40 is an independent predictor of CSVD and CSVD with mild cognitive impairment. In addition, a correlation between YKL-40 and decreased white matter fiber integrity was also found in our study, suggesting that YKL-40 and CSVD cognitive impairment may be partly mediated through neuroinflammation. If more relevant experiments are confirmed, the results of this part of the study can provide a new method for the early diagnosis of CSVD and early identification of cognitive decline in clinical practice. Second, it can be used as a therapeutic target to reduce the incidence of CSVD and preventing and delaying the development of cognitive impairment in CSVD.

However, there were several limitations in the present study. First, our study did not include the enlarged perivascular space, which may have an impact on the results. Second, the samples we tested were serum, which may be affected by many factors, although we had excluded as much as possible related factors affecting YKL-40 levels, there were still some unexplained. Future studies are suggested to collect cerebrospinal fluid to detect the level of YKL-40. Third, we only focused on the damage of white matter fibers and ignored neuroimaging factors such as cerebral perfusion that may affect cognitive function. Future studies need to further explore the effect of YKL-40 on cognitive impairment in CSVD by multimodal magnetic resonance imaging. Finally, this study was cross-sectional with a small number of subjects, we could not determine the causal relationship between serum YKL-40, white matter fibre integrity and cognitive function, and a larger prospective follow-up study is needed. Future randomized drug trials are expected to further confirm this theory by reducing serum YKL-40 levels can improve white matter fiber integrity and delay cognitive decline.

Conclusion

In conclusion, our research showed that serum YKL-40 was an important biomarker of CSVD, which provided a reliable means for the early recognition of CSVD and CSVD with mild cognitive impairment. The disruption in the microstructure of white matter fibers might be the neural basis for the relationship between serum YKL-40 and decline in cognitive function, which provides new insight into the treatment and prevention of CSVD and cognitive impairment by exposing serum YKL-40 as a promising biomarker.

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Declarations

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Conflict of interest: The authors declare that they have no conflict of interest related to this work.

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The studies involving human participants were reviewed and approved by the Ethics Committees of the First Affiliated Hospital of Anhui Medical University (reference number: PJ 2022-01-43).

Availability of data and material: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions: Wei Zhang and Mingxu Li acquired and analyzed data; conceptualized study; drafted the manuscript for intellectual content.
Chaojuan Huang, Xin Yuan, Wenwen Yin, Ke Wan, Mengzhe You, and Xianfeng Yu contributed to the acquisition and analysis of data.
Wenming Zhao and Cun Zhang, MRI scan and image data analysis.
Xia Zhou, Xiaoqun Zhu, Zhongwu Sun designed and conceptualized the study; obtained funding and revised the manuscript for intellectual content.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Figure legends

Fig. 1 ROC analysis of the serum YKL-40 levels in the diagnosis of the HCs, CSVD-NCI and CSVD-MCI. **a** ROC analyses of serum YKL-40 level for CSVD patients versus HC. **b** ROC analyses of serum YKL-40 level for CSVD-NCI versus CSVD-MCI. ROC receiver operating characteristic, HC healthy control, CSVD cerebral small vessel disease, NCI no cognitive impairmen, MCI mild cognitive impairment, vs versus.

Fig. 2 Mean differences in white matter fiber DTI indices between the HCs, CSVD-NCI and CSVD-MCI**. a** Comparison of the differences of white matter fiber FA values among the three groups. **b** Comparison of the differences of white matter fiber MD values among the three groups. **c** Comparison of the differences of white matter fiber RD values among the three groups. **d** Comparison of the differences of white matter fiber AD values among the three groups. HC healthy control, CSVD cerebral small vessel disease, NCI no cognitive impairment, MCI mild cognitive impairment, FA fractional anisotropy, MD mean diffusivity, RD radial diffusivity, AD axial diffusivity, *P <0.05, **P <0.01, ***P <0.001, respectively.

Fig. 3 Scatter plots of correlation between serum YKL-40 level and DTI indices in patients with CSVD-MCI. **a** Negative correlations between serum YKL-40 level and FA in the commissural, association and projection fibers. **b** Positive correlations between serum YKL-40 level and MD in the commissural, association and projection fibers. **c** Positive correlations between serum YKL-40 level and RD in the commissural, association and projection fibers. **d** Positive correlations between serum YKL-40 level and AD in the commissural, association and projection fibers. pr partial correlation coefficient, FA fractional anisotropy, MD mean diffusivity, RD radial diffusivity, AD axial diffusivity, *P <0.05, **P <0.01, respectively.

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Figures

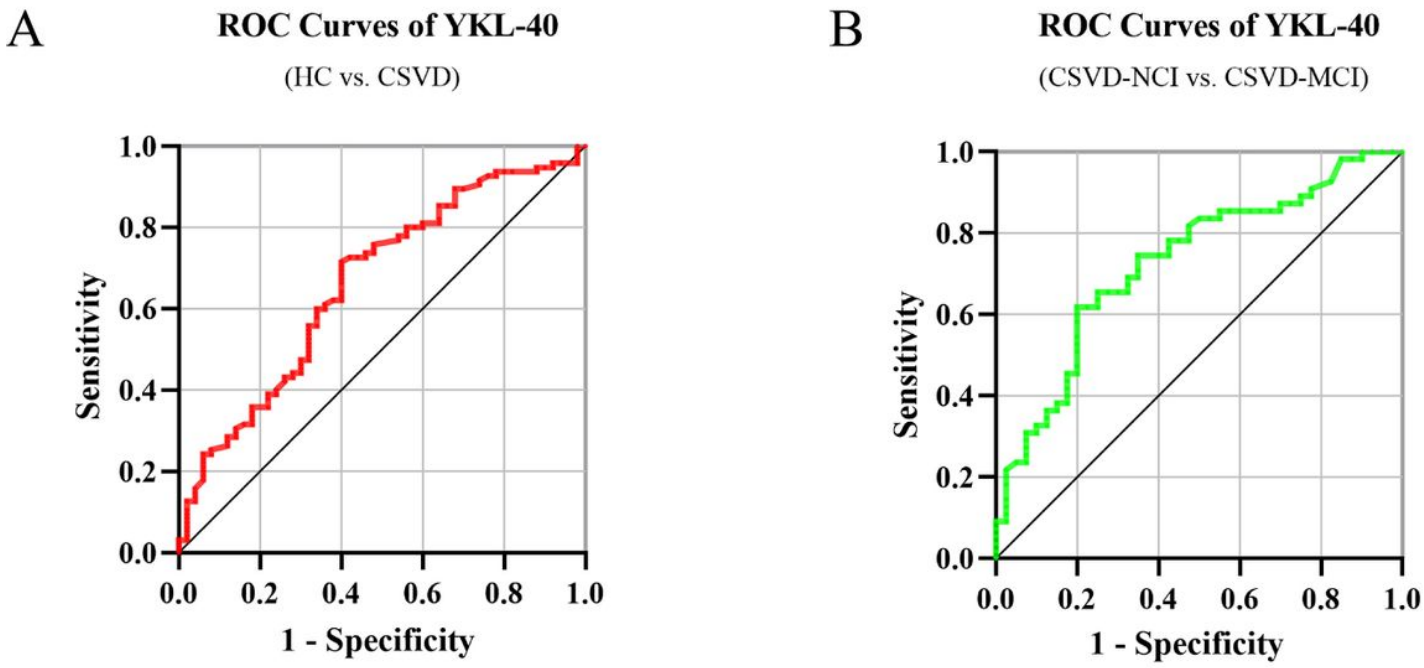

Figure 1

ROC analysis of the serum YKL-40 levels in the diagnosis of the HCs, CSVD-NCI and CSVD-MCI. a ROC analyses of serum YKL-40 level for CSVD patients versus HC. b ROC analyses of serum YKL-40 level for CSVD-NCI versus CSVD-MCI. ROC receiver operating characteristic, HC healthy control, CSVD cerebral small vessel disease, NCI no cognitive impairmen, MCI mild cognitive impairment, vs versus.

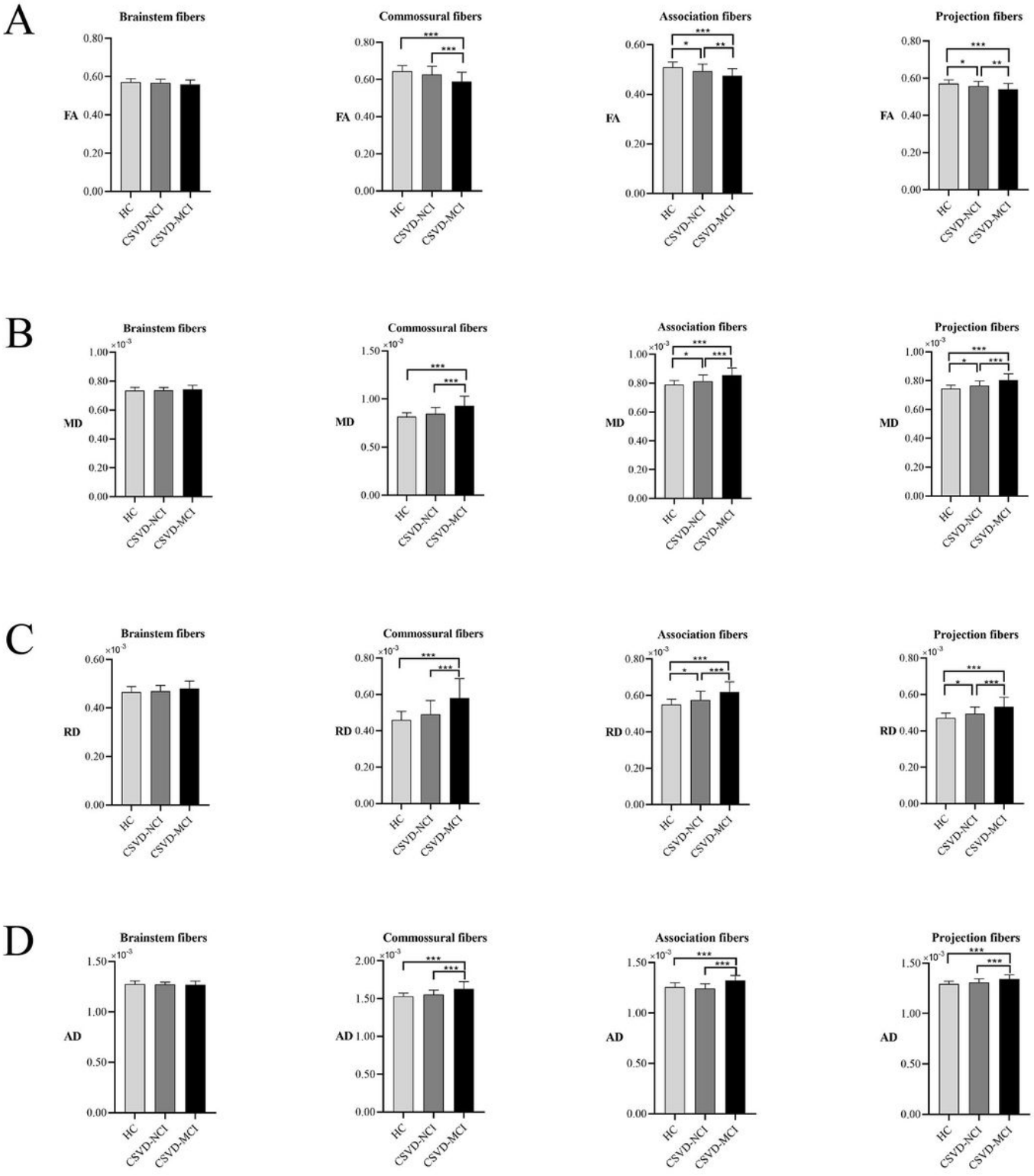

Figure 2

Mean differences in white matter fiber DTI indices between the HCs, CSVD-NCI and CSVD-MCI. a Comparison of the differences of white matter fiber FA values among the three groups. b Comparison of the differences of white matter fiber MD values among the three groups. c Comparison of the differences of white matter fiber RD values among the three groups. d Comparison of the differences of white matter fiber AD values among the three groups. HC healthy control, CSVD cerebral small vessel disease, NCI no

cognitive impairment, MCI mild cognitive impairment, FA fractional anisotropy, MD mean diffusivity, RD radial diffusivity, AD axial diffusivity, *P <0.05, **P <0.01, ***P <0.001, respectively.

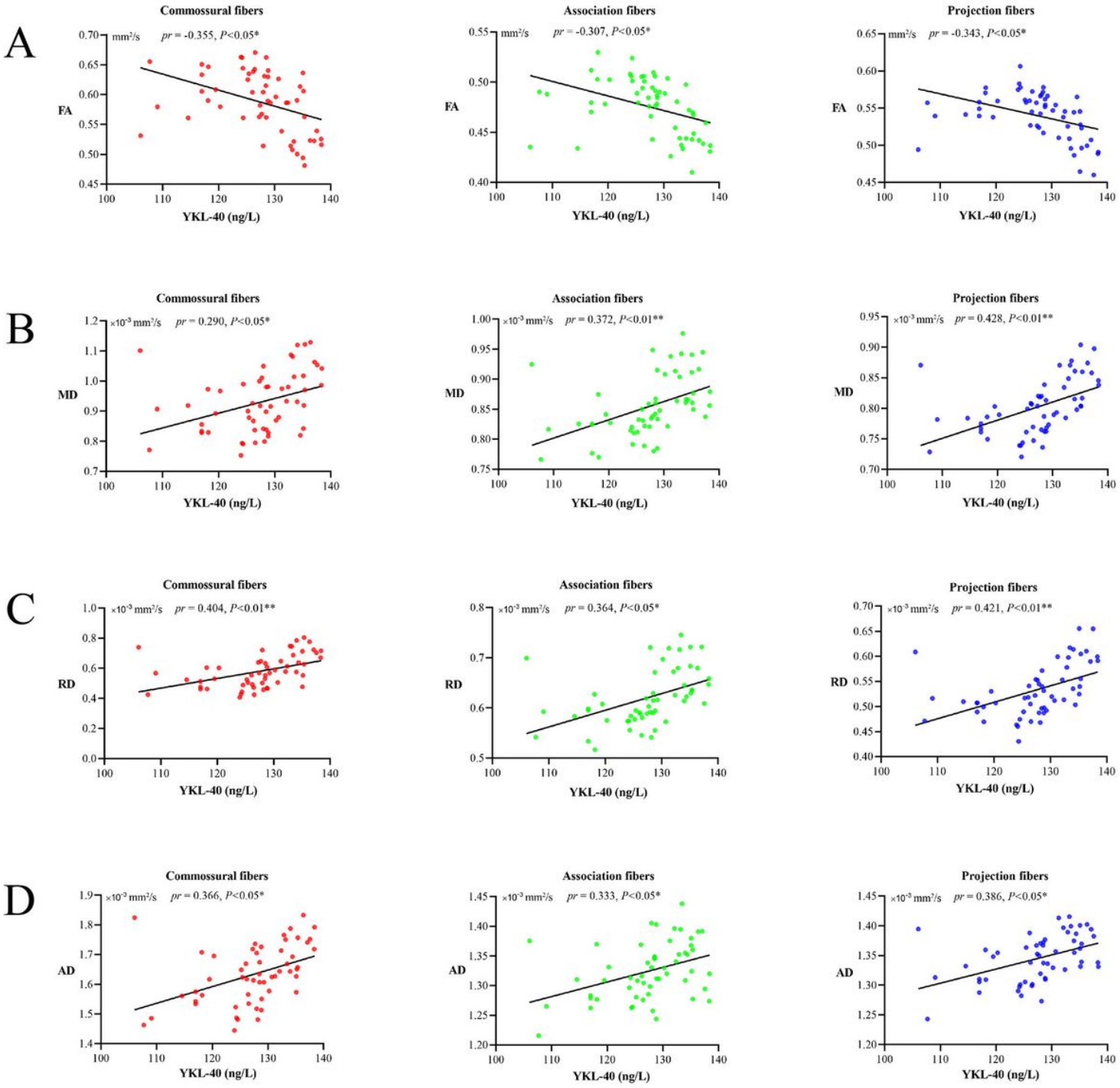

Figure 3

Scatter plots of correlation between serum YKL-40 level and DTI indices in patients with CSVD-MCI. a Negative correlations between serum YKL-40 level and FA in the commissural, association and projection fibers. b Positive correlations between serum YKL-40 level and MD in the commissural, association and projection fibers. c Positive correlations between serum YKL-40 level and RD in the commissural,

association and projection fibers. d Positive correlations between serum YKL-40 level and AD in the commissural, association and projection fibers. pr partial correlation coefficient, FA fractional anisotropy, MD mean diffusivity, RD radial diffusivity, AD axial diffusivity, *P <0.05, **P <0.01, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Table3.pdf
- AuthorChecklist.pdf
- Supplementaryfile.pdf