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# Development of cognition decline in non-acute symptomatic patients with cerebral small vessel disease: Non-Acute Symptomatic Cerebral Ischemia Registration study (NASCIR)—rationale and protocol for a prospective multicentre observational study

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## ABSTRACT

**Introduction** Headaches, dizziness and memory loss of unspecific causes are the most common non-acute ischemia symptoms in the ageing population, which are often associated with cerebral small vessel disease (CSVD) imaging markers; however, there is insufficient evidence concerning their association with the development of cognitive decline. This study aims to investigate risk factors, clinical course, cerebral and retinal imaging changes, proteomics features of non-symptomatic ischaemia symptomatic patients with cognitive decline.

**Methods and analysis** The Non-Acute Symptomatic Cerebral Ischemia Registration study is a multicentre, registry-based, prospective observational study, is designed to investigate the cognitive decline in non-acute ischaemia symptomatic patients. We will recruit 500 non-acute ischaemia symptomatic patients from four tertiary hospitals in China. For this study, non-acute ischaemia symptoms will be defined as headaches, dizziness and memory loss. Patients with headaches, dizziness or memory loss over 50 years of age will be included. Clinical features, cognitive assessment, cerebral and retinal imaging data, and a blood sample will be collected after recruitment. Patients will be followed up by structured telephone interviews at 1, 2, 3, 4, 5 years after recruitment. This study will improve our knowledge of the development of cognitive decline in non-acute ischaemia symptomatic patients and factors affecting the cognitive outcomes, which will eventually elucidate underlying pathways and mechanisms of cognitive decline in these patients and facilitate the optimisation of individualised interventions for its prevention and treatment.

**Ethics and dissemination** Ethics approval is obtained from The Biomedical Research Ethics Committee of West China Hospital, Sichuan University (Reference No. 2016 (335)). We will present our findings at national and international conferences and peer-reviewed journals in stroke and neurology.

## Strengths and limitations of this study

- This is a large multicentre registry-based prospective cohort study to investigate the risk factors, clinical features and course of the cognitive decline in patients with non-acute ischaemia symptoms, including headaches, dizziness and memory loss.
- Retinal swept-source optical coherence tomography scan is included in this study to monitor the structural and microvascular retinal ischaemic changes in patients with non-acute ischaemia symptoms.
- Plasma proteomics and metabolomics is applied in this study to elucidate the underlying ischaemia-associated pathway and discover novel plasma biomarkers.
- We will provide intensive in-hospital assessments and long-term follow-up to understand the natural history of cognitive decline in patients with non-acute ischaemia symptoms.
- As an observational study, our study may be subject to selection bias and inaccurate data recording. To overcome such limitations, we will provide standard training and regular monitoring to site researchers to promote adherence to protocol.

**Trial registration number** ChiCTR-COC-17013056.

## INTRODUCTION

Dementia is currently a huge burden on families and society due to the increase in the ageing population.[1] Vascular dementia is one of the most common causes of dementia after Alzheimer's disease, causing around 15% of cases and there is merging evidence to support the role of vascular mechanisms

in the development of dementia.[2–4] Cerebral small vessel disease (CSVD) is commonly observed on neuroimaging among elderly individuals and is recognised as the most common vascular cause of dementia, a major contributor to mixed dementia.[5 6] Alzheimer's disease and CSVD share common risk factors[7 8] and both lead to cognitive decline and dementia.[9–11]

CSVD is present to some extent in virtually every individual aged 60 years or older.[12] Common radiological imaging markers of CSVD such as white matter lesions (WMLs), lacunes, enlarged perivascular spaces (EPVS), microbleeds and brain atrophy, encompass a group of age-related neuropathological processes affecting the small perforating arteries, arterioles and capillaries ranging from around 100 to 200 µm in diameter.[13 14] Cognitive decline or dementia is one of the major consequences of CSVD. For instance, in the RUN DMC study, a prospective cohort among 503 non-demented participants with ages between 50 and 85 years at baseline, 43 (8.6%) participants developed dementia after 5 years of follow-up.[15–18] However, recent prospective cohort studies of CSVD are usually population-based studies with a low prevalence of cognitive decline or dementia and there is a lack of evaluation of other imaging variables and novel plasma biomarkers.

It was established that individuals with subjective memory loss are at higher risk of cognitive decline.[19] Apart from subjective memory loss, in our preliminary study, we found that a substantial proportion of patients with headaches, dizziness had cognitive decline using global cognitive tests. Headache, dizziness and memory loss are the most common unspecific complaints in middle-aged patients of neurology clinics, which have been considered as characteristic of cerebrovascular insufficiency due to CSVD such as WMLs.[20 21] In accordance with our preliminary study, it was reported that headache disorders were associated with a 24% greater risk of all-cause dementia.[22] It was also reported that 7818 patients with dizziness and vertigo symptoms exhibited a 1.24-fold higher risk of dementia over 20 years, compared with 31 272 controls after adjusting for gender, age and index year.[23] It was also reported that the WML detected by MRI contributed to vertigo in the patients with dizziness and visual vertigo.[24] Based on the above evidence, we hypothesise that cognitive decline is prevalent in patients with headaches, dizziness and memory loss over 50 years old; moreover, in these patients, small vessel ischaemic changes, such as CSVD imaging markers and retinal small vessel changes, may be associated with the cognitive decline and could predict the development and deterioration of cognitive decline in these patients.

Previous reports using retinal imaging tools such as the fundus camera have suggested that retinal vascular signs such as retinal haemorrhage and microaneurysms are associated with cerebral WMLs, and other cerebrovascular diseases.[25–28] Optical coherence tomography angiography (OCTA) is an imaging tool that can non-invasively image the structure and microvasculature of the retinal and choroidal microcirculation in vivo; it enables the visualisation of the capillary plexuses in the retina and choroid at a high resolution. OCTA is considered as a potential screening tool for CSVD, stroke, and neurodegenerative disease such as AD[29–32]; however, few studies investigated the relationship between retinal small vessel changes and cognitive decline in patients with non-acute ischaemia symptoms. Proteomics and metabolomics analysis identified new biomarkers and networks in hereditary CSVD like cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy and cerebral amyloid angiopathy[33–35]; however, few studies investigated the proteomics and metabolomics changes in sporadic CSVD patients with cognitive decline.

In the current study, we aim to recruit patients with non-acute ischaemic symptoms such as headaches, dizziness and memory loss, to investigate the course and development of cognitive decline as well as its relationship with the neuroimaging markers of CSVD and retinal small vessel changes, to explore the underlying vascular molecular pathway and novel biomarkers.

## METHODS AND ANALYSIS

This is a multicentre, registry-based, prospective cohort study, which started in September 2016 and will end in December 2021 (figure 1). Patients will be recruited from the neurological clinics of West China Hospital, Peking Union Medical College Hospital, Beijing Tian Tan Hospital, Capital Medical University, Third Military Medical University affiliated Xinqiao Hospital. Control participants will be individuals without any neurological deficits and are willing to participate in this research.

### Ethics and dissemination

The study was approved by the Biomedical Research Ethics Committee and the Committee on Human Research of West China Hospital, Sichuan University (Reference No. 2016 (335)). Appropriate local ethics committee approvals have been obtained from all the centres which are participating in this study and proof of local approval will be sent to the leading centre before recruitment can start in each centre (The Medical Review Ethics Committee of Peking Union Medical College Hospital, reference number: JS-2822; Institutional Review Board of Beijing Tian Tan Hospital, Capital Medical University, reference number: KY-2017-039-01; Medical Ethics of Second Affiliated Hospital of Army Medical University, reference number: PLA, 2020-131-01). Potential participants who will show a willingness to take part in the research will provide all needed information. All patients (or their legal proxies) will be provided with a written patient information sheet and be fully informed of the aim and content of this study. Each patient (or their proxies if the patient is incapable of decision making) will be asked to sign an informed consent form before they are recruited.

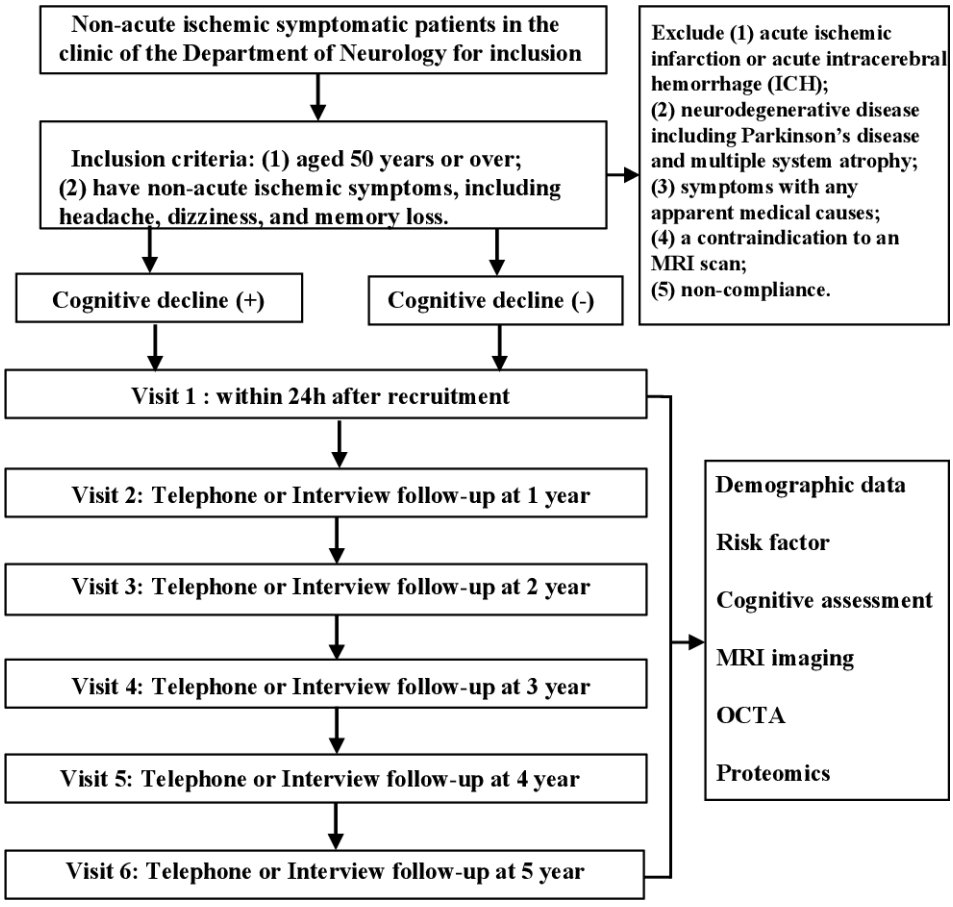


**Figure 1** Flow chart of the process for patients' inclusion, collection of clinical features and outcomes of patients with non-acute ischaemia symptoms. Cognitive decline is defined as a MoCA score smaller than 14 for illiterate individuals, 20 for individuals with 1–6 years of education and 25 for individuals with 7 or more years of education. OCTA, optical coherence tomography angiography; MoCA, Montreal Cognitive Assessment.

## Study population

### Inclusion criteria

In each participating hospital, patients visiting the clinic of the Department of Neurology will be screened for eligibility by their responsible doctors. Inclusion criteria: (1) aged 50 years or over; (2) have non-acute ischaemic symptoms, including headache, dizziness and memory loss.

### Exclusion criteria

Potential participants are excluded if they have (1) acute ischaemic infarction or acute intracerebral haemorrhage (ICH); (2) neurodegenerative disease including Parkinson's disease and multiple system atrophy; (3) symptoms with any apparent medical causes as in traumatic, vascular or haemodynamic causes such as hypotension, haematological, metabolic, visual, dental causes or certain drugs; (4) a contraindication to an MRI scan (such as a permanent pacemaker), are unable to tolerate an MRI (due to claustrophobia) or comorbidity that limits their ability to take part in the study; (5) non-compliance.

## Structured case report form

A case report form (CRF) will be used to collect the information for each participant. Data collected at the first visit and subsequent follow-up are summarised in table 1, including patient demographic characteristics, vascular risk factors, cardiovascular disease, current medications, and clinical features.

### Demographics information

Patients' demographics will be obtained by in-person interviews at the first visit, including age, sex, ethnicity, marital status, main lifetime occupation type, education levels and current basic status. The educational status will be measured using years. The current basic health status will include weight, height, current blood pressure (BP) and heartbeat rate. Systolic BP (SBP), diastolic BP (DBP) and heartbeat rate is recorded with an electronic arm type BP device, in the resting status 15 min after the patient arrives at the clinics about 15 min. If the abnormal BP is detected, the whole measurement procedure will be repeated after 10 min and the highest values will be recorded.

### Vascular risk factor, cardiovascular disease, current medication and basis status

At the first visit, each participant will be asked for any history of hypertension, diabetes mellitus, hyperlipidaemia, atrial fibrillation, transient ischaemic attack, stroke and coronary heart disease including angina or myocardial infarction and rheumatic heart disease. Hypertension will be defined

**Table 1** Study timeline and investigations

| All patients | Baseline | Follow-up 1 1 year | Follow-up 2 2 years | Follow-up 3 3 years | Follow-up 4 4 years | Follow-up 5 5 years |
|---|---|---|---|---|---|---|
| Demographics | ○ | ○ | ○ | ○ | ○ | ○ |
| Medical history | ○ | ○ | ○ | ○ | ○ | ○ |
| Routine laboratory tests | ○ | ○ | ○ | ○ | ○ | ○ |
| NIHSS | ○ | ○ | ○ | ○ | ○ | ○ |
| mRS | ○ | ○ | ○ | ○ | ○ | ○ |
| Non-acute CSVD symptoms | | | | | | |
| Headache | ○ | ○ | ○ | ○ | ○ | ○ |
| Dizziness | ○ | ○ | ○ | ○ | ○ | ○ |
| Memory loss | ○ | ○ | ○ | ○ | ○ | ○ |
| Cognitive assessment | | | | | | |
| MoCA | ○ | ○ | ○ | ○ | ○ | ○ |
| MMSE | ○ | ○ | ○ | ○ | ○ | ○ |
| Neuropsychological test battery | ○ | ○ | ○ | ○ | ○ | ○ |
| ADL | ○ | ○ | ○ | ○ | ○ | ○ |
| HAMD | ○ | ○ | ○ | ○ | ○ | ○ |
| TICSm | ○ | ○ | ○ | ○ | ○ | ○ |
| MRI | | | | | | |
| WML | ○ | ○ | ○ | ○ | ○ | ○ |
| Lacunes | ○ | ○ | ○ | ○ | ○ | ○ |
| EPVS | ○ | ○ | ○ | ○ | ○ | ○ |
| Microbleeds | ○ | ○ | ○ | ○ | ○ | ○ |
| Brain atrophy | ○ | ○ | ○ | ○ | ○ | ○ |
| Plasma multi-omics assay | | | | | | |
| Proteomics | ○ | ○ | ○ | ○ | ○ | ○ |
| Metabolomics | ○ | ○ | ○ | ○ | ○ | ○ |
| OCTA | | | | | | |
| SVP | ○ | ○ | ○ | ○ | ○ | ○ |
| ICP | ○ | ○ | ○ | ○ | ○ | ○ |
| DCP | ○ | ○ | ○ | ○ | ○ | ○ |
| CC | ○ | ○ | ○ | ○ | ○ | ○ |

The blue little circle indicated subgroup analysis.
ADL, activity of daily life; CC, choriocapillaris; CSVD, cerebral small vessel disease; DCP, deep capillary plexus; EPVS, enlarged perivascular spaces; HAMD, Hamilton Depression Scale; ICP, intermediate capillary plexus; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; mRS, modified Rankin Scale score; NIHSS, National Institutes of Health Stroke Scale; OCTA, Optical coherence tomography angiography; SVP, superficial vascular plexus; TICSm, modified telephone interview for cognitive status; WML, white matter lesions.

as any recorded hypertension diagnosis or pre-existing BP ≥140/90 mm Hg on at least three measurements at rest on at least two separate healthcare visits for more than 1 month before the first visit either on or off antihypertensive therapy. Diabetes mellitus will be defined as a fasting plasma glucose level >126 mg/dL (7.0 mmol/L) or a casual plasma glucose >200 mg/dL (11.1 mmol/L) or a previous diagnosis of diabetes mellitus. Hyperlipidaemia will be considered present if a subject was treated for hyperlipidaemia at the time of examination or if the fasting triglyceride level >1.7 mmol/L or cholesterol level >5.7 mmol/L. Drinking will be confirmed if the patients drank alcohol at least once per week during the past 12 months. Alcohol consumption is defined as units per day and the age at which alcohol consumption had started (and if stopped) was noted. Current medication includes oral antiplatelet drugs, oral anticoagulants, antihypertensive drugs, oral antidiabetic drugs and insulin injection, oral lipid-lowering agents, cognition-related drugs. The routine blood tests at the first visit will be recorded, including complete blood count, liver and kidney function, coagulation routine.

### Clinical symptoms assessment: definition of non-acute ischaemic symptoms

We searched PubMed with search terms (dizziness OR vertigo) OR (chronic OR non-acute OR cerebral small

vessel disease and symptom) for clinical trials, observational studies and reviews. 'non-acute ischaemic symptoms' are defined as headaches, dizziness and memory loss, which occurs more than 1 day per week for a period of 1 month or more. Headaches, dizziness and memory loss in the current study are further detailed as following: (1) mild to moderate headache without any definite migraine or post-traumatic headache; (2) dizziness, which will be defined as any equivalent complaints like vertigo, light-headedness, loss of balance/equilibrium, spinning/swimming of the head without any definite vestibular origin or peripheral disorders; (3) memory loss will include subjective memory loss, which will be defined as self-reported memory decline with or without a decline in the ability to work or daily life. A headache will be considered as the headache that happened repeatedly, measured by the duration of each time and the frequency per month. A headache will be qualified by its frequency and duration each time and scaled according to the criteria: light-frequency between 3and 7 days per month, moderate between 8 and 15 days per month, severe: more than 15 days per month.

## MRI

MRI scanning will be performed on a 3-T MRI unit (Singa 750W GE Healthcare, Milwaukee, Wisconsin, USA). The scanning protocol includes (1) whole brain 3 D-T1 BRAVO sequence (TR/TE 8.5/3.2 ms; Prep time: 450 ms, flip angle 12°; voxel size 1.0×1.0×1.0 mm); (2) T2-FLAIR (TR/TE/TI 9000/95/2474 ms; voxel size 0.93×0.93×5.0 mm; gap 1 mm); (3) T2 propeller (TR/TE 5039/110 ms; voxel size 0.58×0.58×5.0 mm; gap 1 mm); (4) 3D-ASL (TR/TE 4809/10.7 ms; slice thickness 4 mm; post label delay 2024 ms; arms 8; number of excitation 3). During resting state, subjects will be told not to concentrate on any particular subject, but just to relax with their eyes closed. The complete scanning protocol takes 20 mininutes.

### White matter lesion

WML will be defined as hyperintense lesions on FLAIR MRI without corresponding cerebrospinal fluid (CSF)-like hypointense lesions on the T1-weighted image. Gliosis surrounding lacunar and territorial infarcts will not be considered as WML.[36] Deep WMLs will be specified as high signal intensity areas on T2-weighted images but isodense with normal brain parenchyma on T1-weighted images, and graded according to Fazekas scale into grade 0, absent; grade 1, punctate; grade 2, beginning confluent; and grade 3, large confluent.

### Lacunes

Lacunar infarcts will be defined as hypointense areas >2 mm and ≤15 mm on FLAIR and T1, ruling out EPVSs (≤2 mm, except around the anterior commissure, where perivascular spaces can be large) and infra-putaminal pseudo-lacunes. Territorial infarcts will be defined as hyperintense lesions on FLAIR and hypointense lesions on T1 images >15 mm.[36]

### Enlarged perivascular space

EPVS will be rated on axial T2-weighted MRI using a validated visual rating scale.[37 38] EPVS will be defined as ≤2 mm round or linear CSF isointense lesions (T2-hyperintense and T1/FLAIR hypointense to the brain) along the course of penetrating arteries. They will be distinguished from lacunes by the latter's large size (>2 and ≤15 mm) and a surrounding rim of FLAIR hyperintensity.[13 39 40] A sum score of EPVS will be calculated as the sum of EPVS in basal ganglion (BG) and centrum semiovale (CSO) regions. For this analysis, EPVS will be categorised into 0–2 versus 3–4 grades. We will define high BG-EPVS or CSO-EPVS (grades 3–4) as >20, in line with the most severe category of EPVS used in previous studies.[41 42]

### Microbleeds

Microbleeds will be defined as small, homogeneous, round foci of low signal intensity on sensitivity weighted imaging of less than 10 mm in diameter. Microbleeds will be counted per hemisphere separately. Lesions will not be considered as microbleeds when they are symmetric hypointensities in the globus pallidus, most likely calcifications or iron deposits, flow voids artefacts of the pial blood vessels or hyposignals in T2* inside a lesion compatible with an infarct, likely to be haemorrhagic transformation.[43]

### Brain volume

All the images will be processed using AccuBrain IV1.2.0 (Brainnow Medical Technology, Shenzhen, China). AccuBrain IV1.2.0 performed brain structure and tissue segmentation and quantification in a fully automatic mode using T1-weighted MRI data. Several brain structures (eg, hippocampus, lateral ventricle, amygdala, etc) and three major brain tissues (ie, white matter, grey matter (GM) and CSF) will be segmented automatically based on prior anatomical knowledge specified by experienced radiologists. The cortical regions will be measured by GM volumes and regional atrophy indices calculated as the ratio of the volume of CSF to the cortical volume of specific lobar regions.

## Plasma proteomics and metabolomics

Plasma proteomics will be carried out on a high-resolution mass spectrometer (Thermo Fisher, Q Exactive Plus). Two plasma samples with high abundant proteins removed or unremoved will be used for proteomics. The proteins will be precipitated and digested and further labelled with Tandem mass tag (TMT) reagents. A TMT-labelled internal standard will be used to evaluate and eliminate the batch variations. The combined samples will be desalted and analysed by mass spectrometry. The proteomics data will be extracted and used for further bioinformatics analysis.

Untargeted metabolomics and lipidomics will be performed. The hydrophilic metabolites will be extracted

with methanol, and lipids are extracted with dichloromethane/methanol (2:1). Isotopic chemicals will be added to each sample to monitor the extraction efficiency. The extracted metabolites and lipids will then be analysed by a mass spectrometer (Thermo Fisher, Q Exactive HF) under positive and negative ion modes. Quality controls samples will be used to monitor the stability of the machine. The metabolomics and lipidomics data will be extracted and used for further bioinformatics analysis.

### Imaging of the retinal vascular structure using the swept-source optical coherence tomography

Swept-source optical coherence tomography (SS-OCT) (VG 200; SVision Imaging Limited, Luoyang China) will be used to scan and image the macula of all participants. Using a three-dimensional protocol, a high-resolution image (512×512 A-scans) of the macula and choroid (6×6 mm) will be imaged; the three-dimensional Projection Artifact Removal, will be incorporated in the imaging tool to reduce projection artefacts while preserving the true layout. An examiner will observe the segmentation of each image. The quality of the macula images will be assessed objectively and subjectively, rejecting images with a signal quality less than 6 on a scale of 10.[44] Images with artefacts were also excluded. Early Treatment Diabetic Retinopathy Study will be applied to the analysed area the mean microvascular blood flow of the macula and choriocapillaris and mean values were recorded, respectively.

### Outcomes events and assessments

#### Cognitive decline

Cognitive decline will be the primary outcome of the observational study. Global cognitive assessments at the first visit will include the validated Chinese Beijing version of the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Examination. Cognitive decline is defined as a MoCA score smaller than 14 for illiterate individuals, 20 for individuals with 1–6 years of education, and 25 for individuals with 7 or more years of education.[45] A neuropsychological test battery will be done to assess the specific cognitive domains, including Stroop test, digit span test, trail marking test, verbal fluency test. Neuropsychiatric Inventory and Hamilton Depression Scale will be applied to exclude the existence of psychiatry disease and severe depression. During the follow-up, the cognitive function will be assessed by the modified telephone interview for cognitive status. For the subgroup of patients who could visit the clinics, face-to-face cognitive assessment will be performed additionally.

#### Acute cerebral vascular event

The incidence of the acute cerebral vascular events, including acute ischaemic stroke and acute ICH, will be the secondary outcomes.

### Follow-up

The following-up period is 5 years. Each year after the initial recruitment, a trained researcher blind to all relevant medical information will contact all eligible patients to deliver a structured telephone interview and collect the data on survival status (survival or death and cause for death), non-acute ischaemic symptoms (headaches, dizziness and memory loss), cognitive status, functional outcomes (assessed by scores on modified Rankin Scale) (table 1). All patients will be invited by telephone to visit our research centre. During their visit to the research centre, a cognitive, structured interview, neurological examination, and an extensive MRI protocol and OCTA protocol will be performed. All tests will be performed by the same two trained neurology residents and all MRI scans and OCTA scans will take place on the same scanners or with the same protocols.

### Provisional analysis plan

We have planned the following analyses:

- Clinical features, cerebral MRI imaging and retinal OCTA imaging markers characterisation of non-acute ischaemia symptomatic patients with or without cognitive decline.

The clinical features, cerebral MRI imaging and retinal OCTA imaging markers characterisation will be compared between non-acute ischaemia symptomatic patients with cognitive decline and those without cognitive decline. The clinical features include demographics, hypertension, diabetes, hyperlipidaemia, medication history, complete blood count, liver and kidney function, coagulation routine, and non-acute ischaemia symptoms (headaches, dizziness, memory loss). MRI and OCTA imaging markers characterisation focus on the prevalence and grade of these markers.

- Bioinformatics differences between non-acute ischaemia symptomatic patients with or without cognitive decline.

Plasma proteomics and metabolomics will be performed in all patients and compared between patients with or without cognitive decline. We will investigate the difference between the two groups and particularly explore whether these significant pathways are vascular factor-associated pathways.

- Course and development of the cognition decline, cerebral MRI and retinal OCTA imaging markers in non-acute ischaemia symptomatic patients with or without cognitive decline.

All patients will be included in the analysis of the telephone-based cognition scale of each 5 years, which will be compared between patients with or without cognitive decline. For a subgroup of patients who receive a face-to-face cognitive evaluation in the neurological clinics, the global cognitive scale and extensive neuropsychological battery of each 5 years will be described and compared between patients with or without cognitive decline on registration. In addition, for patients who have a subsequent follow-up of MRI and OCTA scans, the course and development of all MRI and OCTA imaging markers will be described and compared between patients with or without cognitive decline on registration.

## Statistical analysis

### Sample size estimation

This is an observational study with a primary aim to describe the clinical features and outcomes of patients with non-acute ischaemic symptoms, which would often not require sample size estimation. Therefore, we will calculate the sample size for the logistic analysis for predicting mild cognitive impairment, for which we anticipate testing 5–10 variables as potential predictors. Based on the criteria of at least 10 events per variable,[46 47] we will need 50–100 patients with cognitive decline. Based on the literature and preliminary data, we expect about 25% of non-acute ischaemia symptomatic patients would develop cognitive decline, a sample of 200–400 patients are required. In this observational study, we aim to recruit 500 patients to allow a possible withdrawal rate of 10% and provide sufficient information on different subgroups of patients for further analyses.

### Statistical analyses

Categorical variables will be presented as counts (%), and the continuous or discrete variables will be presented as mean (SD) or median (IQR). Student's t-test, the $\chi^2$ test, Analysis of Variance (ANOVA), Mann-Whitney U test, Fisher's exact test, and Kruskal-Wallis test were used for univariate analysis among groups with relevant variables as appropriate. Associations of clinical characteristics with death will be analysed using logistic regression models, whereas associations of clinical characteristics with ordinary outcomes will be analysed using ordinal logistic regression. Data will be reported as ORs and 95% CIs. Where appropriate, adjusted ORs will be reported. Two-sided p values will be reported, with $p<0.05$ considered statistically significant in all tests unless another threshold will be given. All statistical analyses will be performed in R Core Team (2017) (R: A language and environment for statistical computing. R Foundation for Statistical Computing).

## Data and safety monitoring

The steering committee consists of the principal investigator (ML, committee chair) based on West China Hospital, Sichuan University (the leading research centre) and chief investigators of four participating hospitals (SZ, ZZ, JN, QY; details in the Acknowledgements section). The committee will be responsible for overseeing the conduct of the study to ensure compliance with the study protocol. Interim analyses will be performed annually to assess the adherence of study administration to protocol and the progress of study conduction. The committee will advise the central management team on necessary modifications or discontinuation of the study.

The central management team (ML, SZ, YC) based on the leading research centre will be responsible for all aspects of management of the study, including coordinating the recruitment of participating hospitals, providing training and study materials, organising training and study meetings, conducting central data collection and checking, performing data analysis and coordinating the production of study reports and publications. With advice on study design modification from the steering committee, the central management team will report to the ethics committee and obtain approval before implementing the changes. The ethics committee, which is independent of investigators and the sponsor of this study, will perform annual audits for the study conduct.

## DISCUSSION

This is a multicentre, registry-based, prospective observational study, which was designed to investigate the cognitive decline in non-acute ischaemia symptomatic patients. To our knowledge, this will be the first study investigating the cognitive decline in patients with headaches, dizziness and memory loss over 50 years old. Headaches, dizziness, vertigo, light-headedness, loss of balance/equilibrium, spinning/swimming of the head, tinnitus, tinnitus cerebri, syncope, fatigue, insomnia, memory loss, depression, paraesthesia and gait instability are common non-acute, unspecific complaints in neurological clinics. Among these non-acute ischaemia symptoms, headache, dizziness and memory loss are the most common symptoms accompanied with or without other symptoms in the ageing population. Also, headaches, dizziness and memory loss are all reported to be associated with cerebral ischaemia and cognitive decline.[19–22 24 48]

By comparing the clinical features, cerebral CSVD imaging and retinal imaging markers, and plasma proteomics in non-acute ischaemia symptomatic patients with or without cognitive decline, this study will provide basic information on the association between the cognitive decline with vascular factors, clinical symptoms, cerebral CSVD imaging and retinal imaging markers and plasma proteomics biomarkers. By exploring the association between cognitive decline and CSVD imaging and retinal imaging markers, this study will inform which imaging marker is mostly correlated with cognitive decline. Using the bioinformatics method, this finding will help to elucidate the underlying pathways and mechanism of cognitive decline in these patients and provide potential targets for preventive interventions. Furthermore, by longitudinally observing the development of cognitive decline and the changes of cerebral and retinal imaging markers, we will explore imaging markers that are associated with the development of cognitive decline. Also, we will investigate the clinical factors and plasma biomarkers associated with the development of cognitive decline.

In summary, the current study will improve our knowledge of the development of cognitive decline in non-acute ischaemia symptomatic patients and factors affecting the cognitive outcomes, which will eventually elucidate underlying pathways and mechanisms of cognitive decline in these patients and facilitate prediction and individualised interventions for its prevention and treatment.

## Limitations

As a prospective cohort study, the current study cannot avoid common limitations of observational studies such as selection bias and inadequacy of data recording. To overcome these possible limitations, we select tertiary hospitals and require researchers to have clinical and research experience in stroke and dementia. In addition, only a subgroup of patients can visit the medical centre to have a face-to-face cognitive evaluation and cerebral MRI or retinal OCTA scan in the following-up.

## ETHICS AND DISSEMINATION

Appropriate local ethics committee approvals are sought for each participating hospital and proof of local approval must be sent to the leading centre before recruitment can be started in each centre. All patients will be fully informed of the study with written information and will be asked to sign the consent form before they are recruited. We will present our findings at national and international conferences and peer-reviewed journals in stroke and neurology.

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