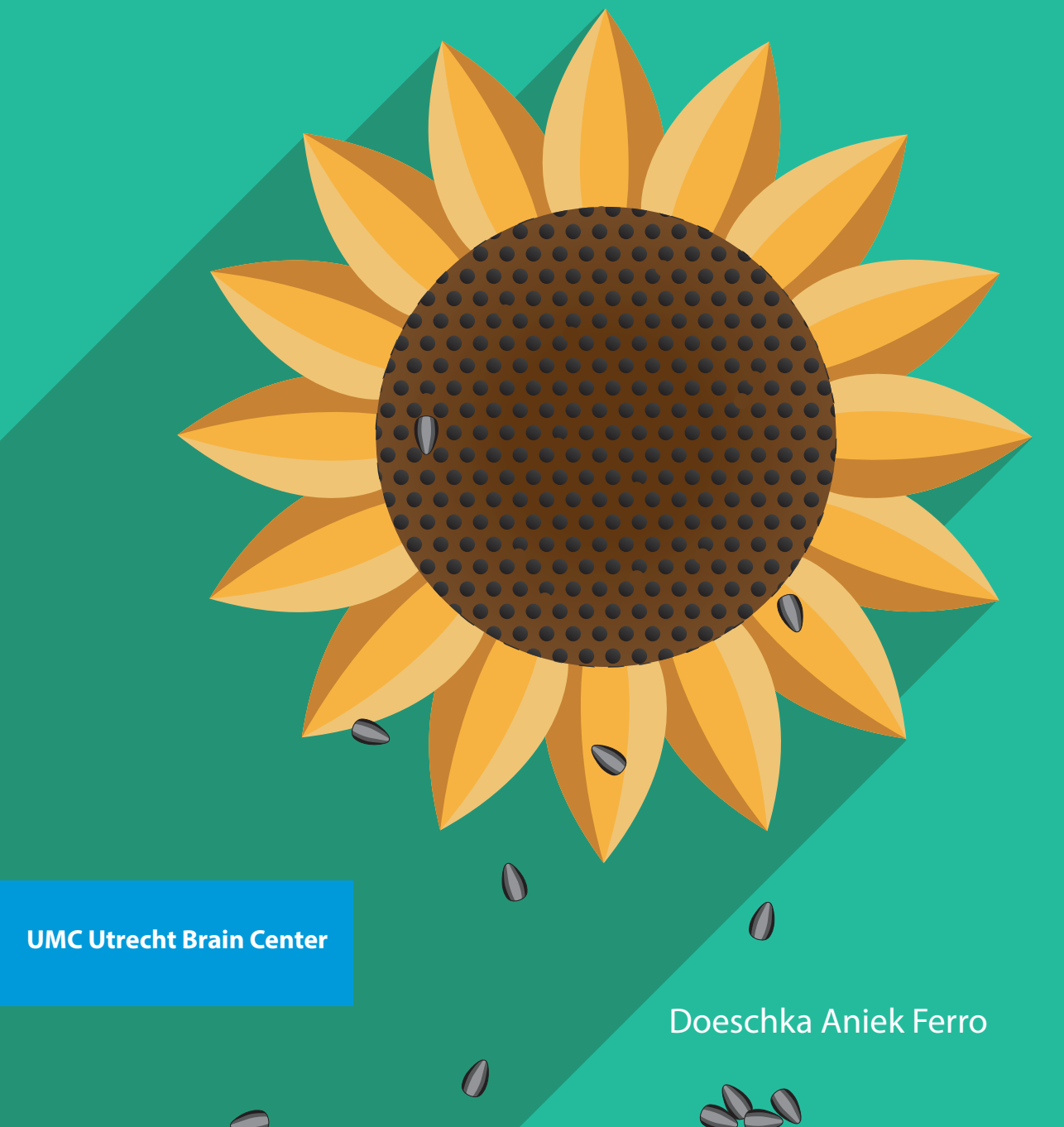
CAUSES AND CONSEQUENCES OF CEREBRAL MICROINFARCTS

Characterizing the "(in)visible" lesion



UMC Utrecht Brain Center

Doeschka Aniek Ferro

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Characterizing the "(in)visible" lesion

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Causes and consequences of cerebral microinfarcts

Characterizing the "(in)visible" lesion

Oorzaken en gevolgen van cerebrale microinfarcten
De "(on)zichtbare" afwijking in kaart gebracht

(met samenvatting in het Nederlands)

Proefschrift

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Doeschka Aniek Ferro

geboren op 31 juli 1984 te Zwolle

Promotoren:
Prof. dr. G.J. Biessels
Prof. dr. J. Hendrikse

Copromotor:
Dr. J.J.M. Zwanenburg

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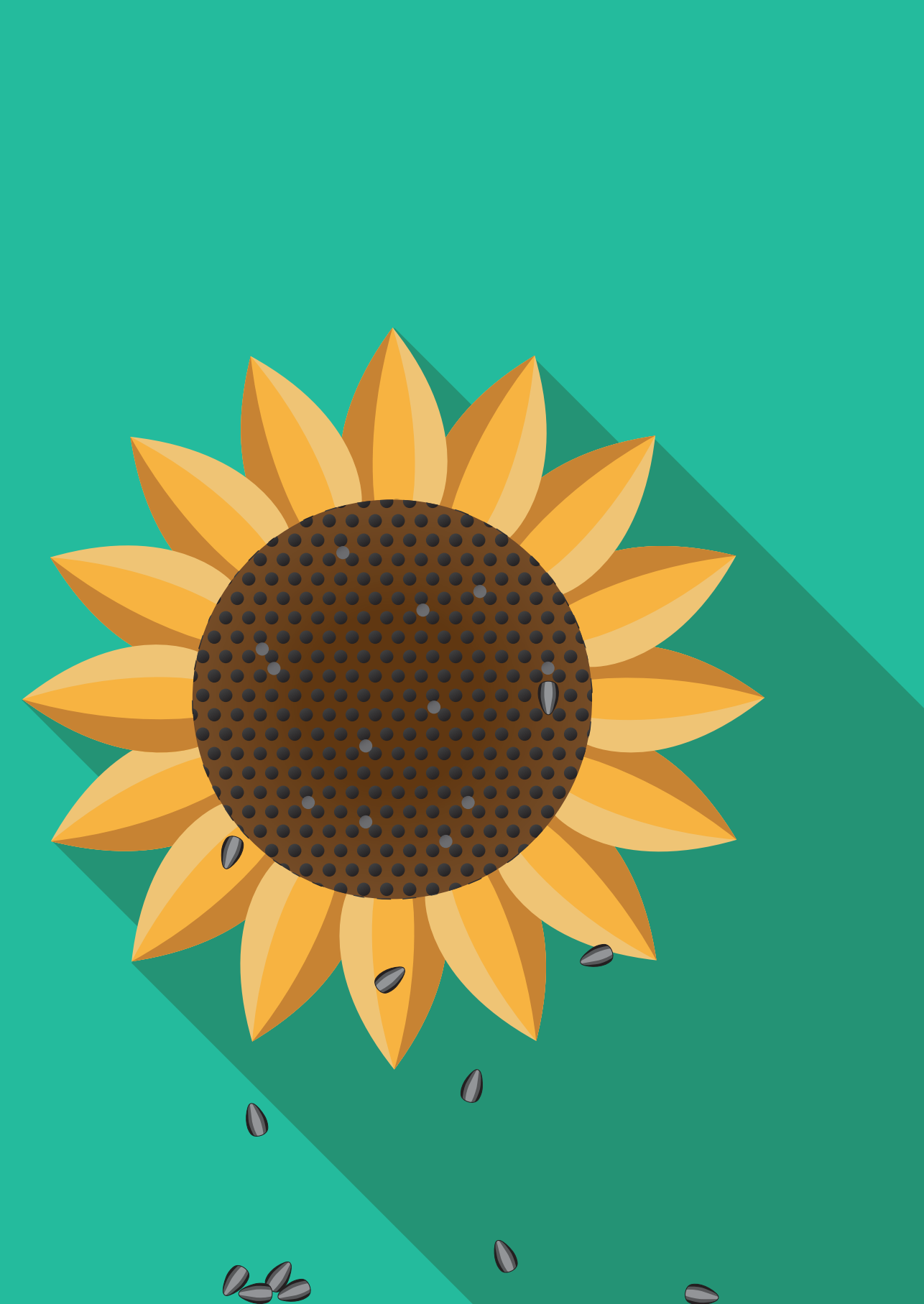
Voor oma Haag en oma Fokje,

beiden overleden aan de gevolgen van dementie

CONTENTS



CHAPTER 1

INTRODUCTION

INTRODUCTION

Cerebral microinfarcts are small brain lesions presumed to be caused by ischemia. Since the beginning of the 21st century there has been an increasing interest in cerebral microinfarcts, especially in the context of aging and cognitive decline. Various post-mortem neuropathology studies found that microinfarcts occurred highly frequent in patients with dementia and cerebrovascular disease [1]. For a long time microinfarcts were considered too small to be visible on in vivo brain imaging, hence being called "the invisible lesions" [2]. This changed with the arrival of high field strength (7 tesla) MRI, allowing visualization of cerebral microinfarcts in living patients for the first time [3]. Moreover, once identified at 7 tesla, microinfarcts also appeared to be visible on widely available 3 tesla MRI [3], which opened up the opportunity to study cerebral microinfarcts on an epidemiological scale. This is an important development, since causes and consequences of microinfarcts have remained elusive. Characterizing these aspects would contribute to our understanding of the role of microinfarcts in (vascular) cognitive impairment and their value as potential clinical biomarker.

In this thesis, I will therefore explore the causes and consequences of cerebral microinfarct on 3 tesla MRI. Within this introduction I will address the following:

1) The different modalities available for microinfarct detection
2) Risk factors and mechanisms of cerebral microinfarcts
3) Perilesional injury associated with cerebral microinfarcts
4) Functional impact of cerebral microinfarcts

DETECTION METHODS OF CEREBRAL MICROINFARCTS

In recent years the detection of cerebral microinfarcts has evolved from histopathological post-mortem detection to identification of microinfarcts during life, either in the chronic phase on structural MRI or in the acute phase on diffusion-weighted MRI (DWI). As will be explained below, it is important to acknowledge that these three modalities differ not only in their size definition of microinfarcts, but also in their temporal and spatial detection resolution and whether or not they allow whole brain detection [4]. They can therefore not be directly compared to each other. Within this thesis I will use detection of chronic microinfarcts on structural MRI and acute microinfarcts on DWI on 3 tesla MRI.

Neuropathology studies have characterized microinfarcts "as sharply delineated regions of cellular death or tissue necrosis with or without cavitation". Their size is typically between 50 μ to a few mm, although no official criteria have been proposed [1]. An example of a cerebral microinfarct on neuropathological examination is shown in figure 1. They have been found particularly frequent in patients with vascular dementia (weighted-average 62%) and patients with Alzheimer's disease (43%) compared to non-demented elderly (24%) [1]. A major strength of neuropathological detection is its high spatial resolution. However, since detection can clearly only be performed post-mortem, it limits the ability to explore the (future) consequences of microinfarcts. Secondly, as neuropathological sampling is performed on selected brain regions (covering less 0.1% of the total brain tissue [5]), it provides insufficient information on spatial dispersion of microinfarcts throughout the brain.

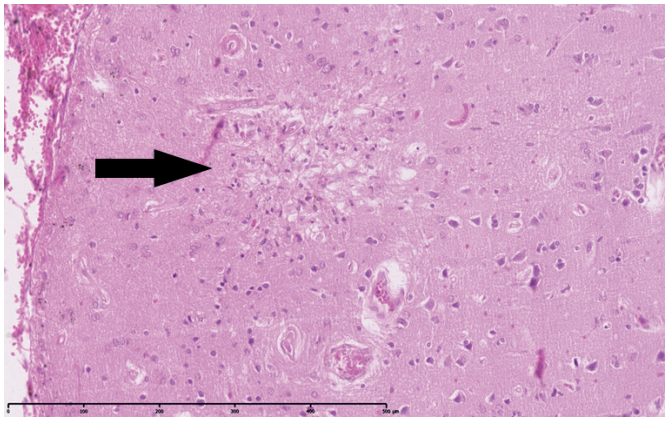


Figure 1: Cerebral microinfarct on neuropathological examination (Haematoxylin and eosin-stained). (Courtesy of Wilmar Jolink).

In 2013 our group succeeded in visualizing cerebral microinfarcts in vivo on 7 tesla MRI. Using a rigorous histopathologic validation protocol including ex vivo (e.g. post mortem brain tissue) 7 tesla [3], we established that chronic microinfarcts could be identified in the human cortex. Of note, subcortical microinfarcts were not evaluated, based on the assumption that they could not be readily resolved from other lesion types, such as enlarged perivascular spaces (PVS) or white matter hyperintensities (WMHs). Approximately one-in-four chronic cortical microinfarcts on 7 tesla was also visible on 3 tesla [3], in line with to the lower spatial resolution of 3 tesla compared to 7 tesla. An example of a chronic cortical microinfarct on 3 tesla MRI is shown in figure 2 in text box 1. Official rating criteria have been proposed for detection of chronic cortical microinfarcts on 3 or 7 tesla MRI, including size of less than 4 mm in greatest dimension (see text box 1 for criteria) [4].

Cerebral microinfarcts can also be visualized in their acute phase using diffusion-weighted MRI (DWI). An example of an acute cerebral microinfarct on DWI is shown in figure 3 in text box 1. A major advantage of this MRI technique is that it allows truly whole brain coverage, including subcortical regions. However, this technique has a limited temporal resolution, as it only provides a snapshot on ischemic events from the last two weeks. See texts box 1 for the proposed detection criteria of acute cerebral microinfarcts, which include a size of less than 5 mm in the greatest dimension.

Text box 1: Detection criteria for chronic cortical microinfarcts and acute cerebral microinfarcts.

Chronic cortical cerebral microinfarcts on structural MRI should meet all of the following criteria:

- Hyperintense on T2-weighted MRI (ie, FLAIR, T2), with or without cavitation on FLAIR.
- Hypointense on T1-weighted MRI
- Isointense on T2*-weighted MRI or blood-sensitive scans (eg, GRE or SWI)
- Operationally defined as strictly intracortical and less than 4 mm in greatest dimension
- Distinct from enlarged perivascular spaces*
- Visible in at least two planes (eg, sagittal, transversal, coronal)

*Important mimics for cortical cerebral microinfarcts are enlarged perivascular spaces in the immediate underlying juxtacortical areas that might extend into the cortical ribbon, leptomeningeal vessels (especially in the temporal lobes), anatomical variations (eg, gyral curvatures), and cortical cerebral microbleeds (signal on blood-sensitive scans should always be verified). We suggest that cerebral microinfarcts in close proximity (ie, <1 cm in the same gyrus) to larger strokes should not be considered as independent cerebral microinfarcts.

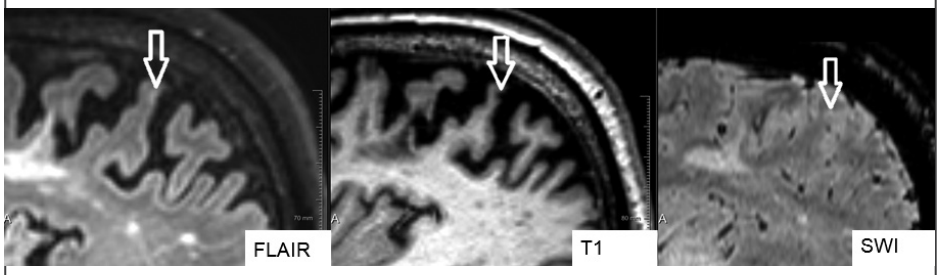


Figure 2: Example of a chronic cortical microinfarcts (white arrow) on 3 tesla MRI, being hyperintense in FLAIR, hypointense on T1 and isointense (e.g. non-distinct) on SWI.

Text box 1 (Continued): Detection criteria for chronic cortical microinfarcts and acute cerebral microinfarcts.

Acute cerebral microinfarcts on DWI should meet all of the following criteria:

- Hyperintense on DWI
- ADC should be isointense or hypointense at the same location (to rule out so-called T2-shine-through of high-T2 signal)
- Isointense or hyperintense on T2*-weighted MRI or blood-sensitive scans (eg, GRE or SWI)
- Any brain parenchymal location
- Operationally defined as less than 5 mm in greatest dimension.

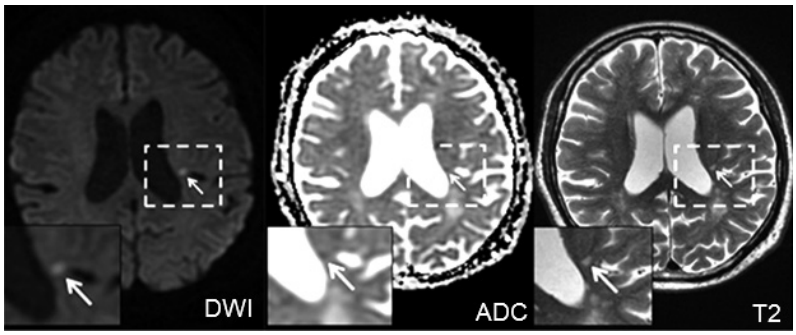


Figure 3: Example of an acute cerebral microinfarct (white arrow) on 3 tesla MRI, being hyperintense in DWI, hypointense on ADC and hyperintense on T2*.
Abbreviations: FLAIR= fluid-attenuated inversion recovery; GRE=gradient echo; SWI=susceptibility-weighted imaging; DWI=diffusion-weighted imaging; ACD=Apparent diffusion coefficient.
Source: Van veluw et al (2017) *Lancet neurology*.

RISK FACTORS AND MECHANISMS OF CEREBRAL MICROINFARCTS

It is currently unclear what risk factors relate to cerebral microinfarcts and which underlying mechanisms are involved. Since cerebral microinfarcts are presumed to be of ischemic origin, a logical starting point would be to explore if "classical" vascular risk factors, such as hypertension, hypercholesterolemia and diabetes are associated with microinfarct presence. To obtain a complete picture of the epidemiology of microinfarcts, the association with basic demographics, such as age and sex, in different populations, are also of major relevance.

It has been suggested that cerebral microinfarcts do not a have a single, but multiple underlying causes, including cerebral small vessel disease (SVD) and micro-emboli due to large vessel disease and cardiac disease [4]. The term SVD refers to a group of pathological processes with various etiologies that affect the small arteries, arterioles, venules and capillaries of the brain [6].Hypertension-related SVD and (sporadic) cerebral amyloid angiopathy (CAA) are the most common forms of SVD. MRI-manifestations of SVD include recent small subcortical infarcts, lacunes, WMHs, PVS and microbleeds. Their MRI-characteristics have been described in the STRIVE-criteria [7]. The role of SVD-pathology in the development of microinfarcts can be further explored by assessing the link with these MRI-manifestations in high risk populations. Microinfarcts have not been exclusively linked to SVD, but were also found to co-occur with macroscopic infarcts, suggestive of a trombo-embolic origin [1]. This finding warrants further exploration of the relation with cerebral large vessel disease markers and cardiac disease. Finally, an interesting and recurring observation is that cortical microinfarcts on neuropathological evaluation appear more frequently in watershed areas [8,9]. While watershed stroke is considered a relatively rare cause of macroscopic infarctions, these studies thus suggest that hypoperfusion may be an important underlying cause of cerebral microinfarcts. Moreover, it can be of clinical interest to observe the effects of hypoperfusion on microinfarcts in populations were cerebral hypoperfusion originates at different levels of the vascular tree.

A key aim of this thesis is to explore the risk factors and causes of cerebral microinfarcts:

- The relation between cerebral microinfarcts and "classical" vascular risk factors
- Observing co-occurring MRI-manifestations of SVD and trombo-embolic stroke
- Explore the role of cerebral hypoperfusion

PERILESIONAL INJURY ASSOCIATED WITH MICROINFARCTS

Although cerebral microinfarcts can occur in abundance in individual patients, with 100s or even 1000s of these lesions in a single individual, the total sum of injured tissue still remains a tiny percentage of total brain volume due to their small size. It has, however, been suggested that microinfarcts may cause more extensive brain impairment

due to additional remote effects on cerebral tissue, a *perilesional effect*. Perilesional effects have been described in various other (vascular) lesions types, for example for SVD MRI-manifestations [10,11].

A proposed site of microinfarct-related perilesional injury is the (adjacent) cortex. This is supported by reports of disproportional smaller cortical volumes in patients with microinfarcts [12,13], that simply cannot be explained by the cumulative lesion volume of all microinfarcts. Recently, evidence has emerged of perilesional injury in mice in cortical area far greater than the microinfarct-core [14]. A next step would be to translate these results to humans by demonstrating a local cortical perilesional injury in in vivo.

A second site that may be affected by perilesional damage are white matter connective tracts. MRI-manifestations of SVD, including WMHs and lacunes, have shown to disrupt white matter connective tracts leading to brain network impairment. These network alteration provide in turn a (partial) explanation of the cognitive symptoms in these patients [11]. A small study of acute subcortical microinfarcts has demonstrated changes in the local white matter integrity [15]. It is unclear whether cortical microinfarcts also affect white matter integrity and whether these alterations lead to measurable impaired brain network structure and function. Answering these questions with regards to perilesional injury would be an important step towards the understanding of the functional impacts of microinfarcts.

- **A key aim of this thesis is to identify perilesional injury associated with microinfarcts on MRI:**
  - Identify cortical and subcortical perilesional injury around cerebral microinfarcts

ASSESSING THE FUNCTIONAL IMPACT OF CORTICAL MICROINFARCTS

Previous neuropathology studies have established a relation between ante-mortem cognitive decline and post-mortem microinfarct presence [1]. However, post-mortem evaluation is hampered in the ability to assess future outcome. This thesis will therefore assess the consequences of microinfarcts detected during life.

A key functional outcome of microinfarcts is thus cognitive impairment, making it a potential relevant MRI-marker of vascular cognitive impairment (VCI). VCI is a term which describes all cognitive dysfunction associated with and presumed to be caused by vascular brain damage [16]. The cognitive profile of VCI is typically characterized by impairment of attention and executive function with relative sparing of memory function [16]. It is unclear whether microinfarct presence is associated with a similar cognitive profile. Another matter of clinical significance is whether microinfarcts are in fact predictors of cognitive decline, a question which should ideally be researched in a longitudinal design. The prognostic value of microinfarcts should ideally also be examined in a broader context with clinically relevant endpoints such as institutionalization, occurrence of stroke or cardiovascular events and death.

- **A key aim of this thesis is to assess the functional impact of microinfarcts:**
  - Establish the cognitive profile of patients with microinfarcts
  - Identify the predictive value of microinfarcts on clinical outcome

OUTLINE OF THE THIS THESIS

In this thesis I will explore the causes and consequences of cerebral microinfarct on 3 tesla MRI. The three key research aims will be addressed in the three corresponding parts of this thesis:

Part I: Causes and mechanisms of cerebral microinfarcts

The vascular risk factors and MRI-markers of vascular brain injury associated with cortical microinfarcts in patients with VCI -with a high burden of SVD- will be reported in chapter 2. These risk factors and MRI-markers will also be addressed in two populations with a high risk of embolic stroke, namely in patients with carotid occlusive disease in chapter 4 and patients with heart failure in chapter 5.

The relationship of cortical microinfarcts with cerebral hypoperfusion will be assessed using arterial spin labelling (ASL), a non-invasive MRI-method to measure cerebral blood flow, in memory clinic patients in chapter 3. As a clinical model of hypoperfusion, cortical microinfarct presence will be researched in patients with compromised cerebral blood flow due to carotid occlusive disease in chapter 4 and with cardiac-pump dysfunction in patients with heart failure in chapter 5.

Part II: Identifying perilesional injury associated with cerebral microinfarcts

Perilesional injury will be explored in cortex surrounding the microinfarcts using a MRI-method based assessment of cortical thickness in memory clinic patients in chapter 6. Perilesional injury leading to disruption of white matter tracts will be investigated using diffusion imaging-based tractography (DTI) memory clinic patients in chapter 7.

Part III: Functional impact of cerebral microinfarcts

The cognitive profile associated with cortical microinfarct presence in patients with VCI will be explored in chapter 1. The predictive value of acute microinfarcts on clinical outcome, including cognitive deterioration, institutionalization, stroke and death, will explored in patients with VCI in chapter 7.

Chapter 8 provides a reflection on findings in this thesis on these three research aims and a discussion of the future direction of microinfarct research.

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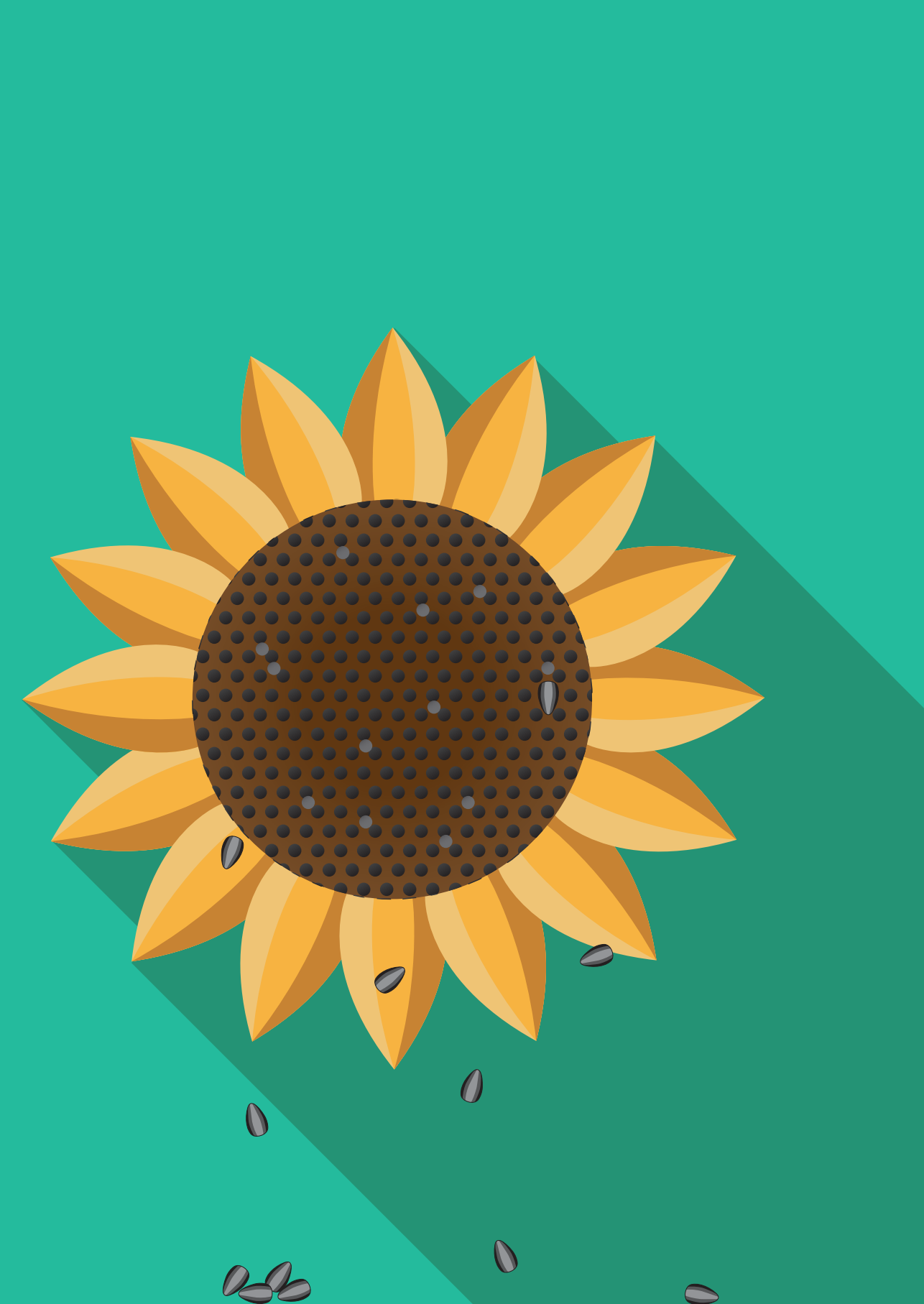
PART I

CAUSES AND MECHANISMS OF CEREBRAL MICROINFARCTS



CHAPTER 2

CORTICAL CEREBRAL MICROINFARCTS ON 3 TESLA MRI IN PATIENTS WITH VASCULAR COGNITIVE IMPAIRMENT

Doeschka A. Ferro
Susanne J. van Veluw
Huiberdina L. Koek
Lieza G. Exalto
Geert Jan Biessels
On behalf of the Utrecht Vascular Cognitive Impairment (VCI) study group.

Journal of Alzheimer's Disease. 2017; 60: 1443–1450

ABSTRACT

Background: Cerebral microinfarcts (CMIs) are small ischemic lesions that are a common neuropathological finding in patients with stroke or dementia. CMIs in the cortex can now be detected in vivo on 3 Tesla MRI.

Objective: To determine the occurrence of CMIs and associated clinical features in patients with possible vascular cognitive impairment (VCI).

Method: 182 memory-clinic patients (mean age 71.4±10.6, 55% male) with vascular injury on brain MRI (i.e. possible VCI) underwent a standardized work-up including 3 Tesla MRI and cognitive assessment. A control group consisted of 70 cognitively normal subjects (mean age 70.6±4.7, 60% male). Cortical CMIs and other neuroimaging markers of vascular brain injury were rated according to established criteria. **Result:** Occurrence of CMIs was higher (20%) in patients compared to controls (10%). Among patients the presence of CMIs was associated with male sex, history of stroke, infarcts and white matter hyperintensities. CMI presence was also associated with a diagnosis of vascular dementia and reduced performance in multiple cognitive domains.

Conclusion: CMIs on 3 Tesla MRI are common in patients with possible VCI and co-occur with imaging markers of small and large vessel disease, likely reflecting a heterogeneous etiology. CMIs are associated with worse cognitive performance, independent of other markers of vascular brain injury.

INTRODUCTION

Cerebral microinfarcts (CMIs) are small ischemic lesions and a common neuropathological finding in patients with a history of stroke or dementia [1]. With the advent of high field strength MRI cortical CMIs can now be visualized in living patients [2]. Recently it has been established that CMIs are also detectable on 3 Tesla MRI [3]. Despite the lower detection sensitivity, the widespread availability of 3 Tesla MRI scanners offers the opportunity to assess CMI burden in larger study cohorts. In two recent studies, assessing an Asian population-based and a memory-clinic cohort, CMIs were associated with worse cognitive functioning [3,4]. These findings highlight CMIs as a clinically relevant imaging marker in Vascular Cognitive Impairment (VCI).

This study aims to determine the occurrence of CMI in patients with VCI at a memory-clinic compared to controls. The second aim is to identify the associated features of CMI presence among the patients, including vascular risk factors, other neuroimaging markers of vascular brain injury, cognitive performance, and clinical diagnosis.

METHODS

Population

The patients were selected from the Vascular Cognitive Impairment cohort of the University Medical Center (UMC) Utrecht ([5]). The study cohort consists of 196 consecutive patients who received a standardized memory-clinic evaluation of the neurology or geriatric department of the UMC Utrecht between 2009 and 2013. For this study, analyses were carried out on 182 patients as 14 participants were excluded from analysis due to insufficient scan quality or lacking MRI sequences. All patients had evidence of vascular brain injury on MRI. Vascular brain injury was operationalized as white matter hyperintensities (WMH) on the Fazekas scale grade ≥ 2 [6] or; ≥ 1 lacunar infarct(s); or ≥ 1 non-lacunar infarct(s); or ≥ 1 cerebral microbleed(s); or ≥ 1 intracerebral hemorrhage(s); or the presence of Fazekas scale grade 1 and an increased vascular risk defined as the presence of ≥ 2 vascular risk factors (hypertension, hypercholesterolemia, diabetes mellitus, obesity, current smoking or self-reported history of a vascular event other than stroke (e.g. ischemic heart disease, peripheral arterial disease or carotid artery stenting). The cohort includes patients with evidence of co-existing neurodegenerative disorders (such as Alzheimer dementia), in line with proposed VCI criteria [5]. Patients visiting the memory-clinic and proved to have vascular brain injury, but did not have objective cognitive impairment were also included. We excluded patients with primary non-vascular/non-degenerative causes of cognitive dysfunction (e.g. brain tumors, traumatic brain injury) or psychiatric disease (other than depression). Patients with monogenetic (non-) vascular (e.g. NOTCH3) causes cognition dysfunction were also excluded.

The control group consisted of individuals that were recruited through general practitioners between April 2010 and June 2011 as part of the Second Utrecht Diabetes Encephalopathy Study [7]. We included all 60 individuals without diabetes from this case control study. We also randomly selected 10 subjects with type 2 diabetes mellitus from that study, to obtain a reference group with a diabetes prevalence representative of the Dutch population in this age group [8] . Exclusion criteria were a history of stroke and neurological or psychiatric disease that were likely to affect cognition.

Ethical approval was provided by the institutional review board of the UMC Utrecht. Informed consent was obtained from all participants prior to research related procedures.

Vascular risk factors

For all patients the followings risk factors were identified. *Hypertension* was defined as either present in medical history, use of antihypertensive medication or current blood pressure above 140/90 mmHg. *Hypercholesterolemia* was identified based on medical history or use of cholesterol lowering medication. *Diabetes mellitus* was identified based on medical history or use of appropriate medication. *Obesity* was defined as a baseline body mass index (BMI) ≥ 30, calculated as weight in kilograms divided by height in meters squared. *History of stroke* was based on a history of clinical hemorrhagic or ischemic stroke. *History of atrial fibrillation* was based on a history (obtained from interview and screening of medical records) of paroxysmal and permanent atrial fibrillation and *history of cardiac disease* included myocardial infarction, congestive heart failure, atrial fibrillation or cardiac intervention (e.g. percutaneous coronary intervention).

MRI protocol and assessment of conventional neuroimaging markers

MRI scans of the brain were performed on a Philips 3 Tesla MRI scanner (Intera; Philips, Best, the Netherlands) with a scan protocol that included 3D T1-weighted images (TR/TE: 7.2/2.9ms; reconstructed voxel size 1.0x1.0x1.0mm^3), 2D T2-weighted turbo spin echo (TSE) images (TR/TE: 3194/14.0ms; reconstructed voxel size 0.96x0.95x3.0mm^3), T2*-weighted (TR/TE: 1653/20ms; reconstructed voxel size 0.96x0.95x3.0mm^3) and fluid-attenuated inversion recovery (FLAIR) images (TR/TE/TI: 11000/125/2800ms; reconstructed voxel size 0.96x0.95x3.0mm3). Scans were evaluated for *medial temporal lobe atrophy* (MTA) by visual assessment according to the Scheltens Scale for both hemispheres separately and averaged [9]. *WMH* according to the Fazekas scale [6]. Both the presence and number of *(non-) lacunar infarcts* and *microbleeds* were rated based on the STRIVE criteria [10].

Rating of CMIs

The cortical CMIs were identified by visual inspection according to criteria that have been recently proposed by a group of international collaborators and previously validated [11,12]. CMIs were rated on high resolution 3 Tesla MRI and had to be hypointense on 3D T1, hyperintense or isointense on 2D FLAIR/T2-weighted images and isointens on T2*-weighted MRI. Restricted to the cortex and ≤ 4mm in the greatest dimension on T1. If on FLAIR/T2-weighted image the lesion was substantially larger than 4mm, the lesion was disregarded as the region was considered part of a larger infarction. CMIs had to be identified in at least two views of the brain (e.g. sagittal, transversal, coronal

plane) and distinct from other structures and lesions such as arteries, veins, enlarged perivascular spaces or microbleeds. Lesions neighboring a larger stroke (i.e. , <1cm in the same gyrus) were not classified as CMIs. A video instruction and methodological details on the detection and visual rating criteria of cortical cerebral microinfarcts on high resolution 3 and 7 Tesla MRI is available [11]. Figure 1 shows an example of a typical CMI in the cohort. Ratings took on average between 15-30 minutes per subject and were performed in MeVisLab (MeVis medical solutions, Bremen, Germany) by one trained rater (DF) blinded to the clinical condition of the subjects. With the applied criteria DF had a good intra-rater agreement (test set 40 scans; intra-class correlation coefficient (ICC)=.95; Dice's similarity index (DSC)=.63) and inter-rater agreement compared to another trained rater (SvV) (ICC=.99 DSC=.68).

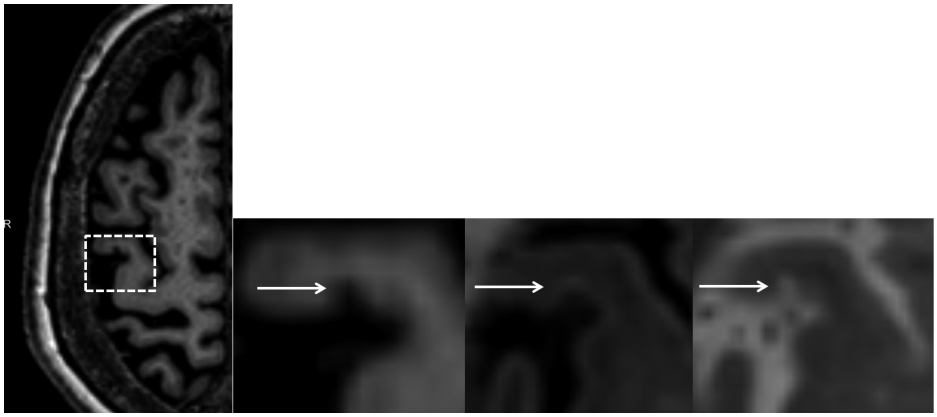


Figure 1: Example of a CMI in the cohort. On the far left a T1 image of right hemisphere. The dotted area is enlarged showing the CMI in detail below (arrow) on T1, Flair and T2 (left to right).

Neuropsychological testing battery and level of education

A Dutch version of the *Mini-Mental State Examination* (MMSE) was used as a measure of global cognitive functioning [13]. The neuropsychological testing battery consisted of several tests covering 4 cognitive domains: 1) *Memory* using the Visual Association Test [14] and a Dutch version of the Rey Auditory Verbal Learning Test [15]. 2) *Attention and executive functioning* using the Trail Making Test part B and A (TMT-A, TMT-B) [16], the Stroop Color-Word Test part III [17] and verbal fluency [18]. 3) *Processing speed* using scores on the TMT-A, the Stroop Color Word Test I and II and the subtest Digit Symbol of the WAIS-III. 4) *Perception and construction* using the Incomplete Letters and Dot Counting, which are two separate tests of the Visual Object and Space Perception

Battery. Raw test scores of individual patients were standardized into z-scores (reversed z-scores for the TMT and Stroop Colour Word Test) using mean and standard deviation within the study cohort. To create a domain score per patient the individual z-scores for the subtests of that domain were averaged and again standardized into z-scores for this cohort. Missing variables were not included in the formation of domain scores. Level of education was ranked according to the Verhage criteria, according to a 7-point rating scale [19].

Clinical diagnosis

As part of the clinical work-up, patients received a clinical diagnosis in a multidisciplinary consensus meeting. *Mild cognitive impairment* (MCI) was diagnosed when there was a decline in cognitive function from a prior baseline and impairment in at least 1 cognitive domain. Instrumental activities of daily living were normal or only mildly impaired [20]. *Dementia* was diagnosed is patients suffered a decline in cognitive function defined as a deficit in ≥ 2 cognitive domains at neuropsychological testing and interference in daily living [20]. Patients with dementia were further classified according to the etiological diagnosis based on internationally established diagnostic criteria. *Alzheimer's disease* (AD) [21], *Vascular Dementia* (VaD) [22] or *other* (including frontotemporal dementia, Lewy body dementia, Parkinson plus syndromes or unknown etiology). Patients with cognitive complaints but no objective cognitive impairment (i.e. a domain score below the 5th percentile on normative values) on the neuropsychological testing battery were classified as *No objective cognitive impairment* (NOCI).

Data analysis

Statistical comparisons between patients and controls, diagnostic subgroups, patients with and without CMIs were respectively analyzed with one-way ANOVA (for continuous normally distributed data), Mann-Whitney U tests (for non-parametric data) and χ-square tests (for proportions) including odds ratios.

A linear regression model was applied to investigate the relationship between CMI occurrence and cognitive functioning (Model I). Additional adjustments were made for age, sex, education (Model II) and age, sex, education and other neuroimaging markers (WMH-score, MTA-score, (lacunar) infarcts, microbleeds) (Model III). All data analyses were carried out in IBM SPSS statistics (version 22) and a p-value < .05 was considered significant.

RESULTS

Demographics and CMI occurrence in patients and controls

Patients and controls were similar with respect to age, sex, and education (Table 1). In the patient group 180 CMIs were found in a total of 37 subjects (20%). In the control group, 12 CMIs were found in a total of 7 subjects (10%). Patients had higher numbers of CMIs than the controls (p<.05). The spatial distribution of CMIs is plotted for both patients and controls in Figure 2, showing a clustering in the pre- and postcentral gyri and middle frontal gyri in both hemispheres, possibly with a preference in watershed areas.

Table 1: Characteristics of patient group and controls.

	Controls (N=70)	Possible VCI group (N=182)
Age, (years)	70.4±4.6	71.4±10.6
Male sex, (%)	43 (61.4)	100 (54.9)
Level of education	4.5 [1-7]	5 [1-7]
Subjects with CMIs	7 (10.0)	**37 (20.3)***
Subjects with multiple (≥2) CMIs	2 (2.9)	**21 (10.7)**$^{\pm}$

Data are presented as means ± SD, n (%), or median [range]. * p=.05 $^{\pm}$ p<.01

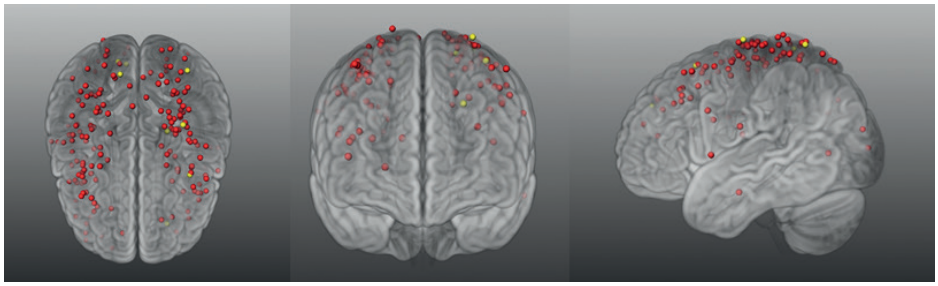


Figure 2: 3D representation of the spatial distribution of CMIs across the brain in patients (red dots) and controls (yellow dots) in transversal, coronal and sagittal views (left to right). Map was configured by registering the CMIs to MNI152 standard brain.

Vascular risk factors and neuroimaging markers in patients with possible VCI

Table 2 shows the demographics, vascular risk factors and neuroimaging markers in patients with and without CMIs. Patients with and without CMIs were similar with respect to age and education. Patients with CMIs were more likely to be male (OR 2.7, CI 1.2-5.9, p<.02) and to have a history of stroke (OR 4.1, CI 1.9-8.8, p<.001) than patients without CMIs. CMIs presence was also associated with more frequent occurrence of

severe WMHs (OR 2.6, CI .99-6.7, $p<.05$) and infarcts (OR 7.01, CI 3.2-15.5 $p<.001$), but not with other neuroimaging markers.

Table 2: Demographics, vascular risk factors and neuroimaging markers for patients with and without CMIs.

	Patients without CMIs (N=145)	Patients with CMIs (N=37)
Demographics		
Age (years)	71.6±10.5	70.4±11.0
Sex (% male)	73 (50.3)	**27 (73.0)***
Level of education	5 [1-7]	5 [3-7]
Vascular Risk profile		
Hypertension	137 (94.5)	34 (91.9)
Hypercholesterolemia	101 (69.7)	29 (78.4)
Diabetes mellitus	51 (35.2)	14 (37.8)
Obesity	26 (18.2)	4 (10.8)
History of stroke	41 (28.5)	**23 (62.2)**$^{\pm}$
Atrial fibrillation	9 (6.2)	1 (2.7)
History of cardiac disease	53 (36.8)	13 (35.1)
Neuroimaging markers		
WMHs (Fazekas 3)	14 (9.7)	**8 (21.6)***
Large infarcts	30 (20.7)	**24 (64.9)**$^{\pm}$
Lacunar infarcts	50 (34.5)	17 (45.9)
Microbleeds	49 (34.0)	13 (36.1)
Medial temporal lobe atrophy	1 [0-4]	2 [0-3]

Data are presented as means ± SD, n (%), or median [range]. $^{\pm}$ $p<.001$; * $p<.05$

Cognitive performance in the patients with possible VCI

MMSE scores were similar for patients with (25.7±2.9) and without CMIs (26.0±3.3). Table 3 presents the regression models of CMI presence on 4 cognitive domains in patients with possible VCI. CMI presence was associated with lower performance on cognitive domains attention & executive functioning and perception & construction, with a trend towards a slower processing speed (Model I). The association between presence of CMIs and cognitive domains was independent of age, sex and level of education (Model II). After an additional adjustment for conventional neuroimaging markers, a significant association remained between CMIs and the domain perception & construction and a trend for attention & executive functioning (mainly due to widening of the confidence interval, rather than attenuation of effect size after adjustment).

Table 3: Regression analysis of CMIs on cognitive performance.

	Model I		Model II		Model III	
	B [CI]	*p*	B [CI]	*p*	B [CI]	*p*
Memory (n=180)	-.14 [-51; .22]	.443	-.17 [-.50; .15]	.292	-.08 [-.44; .29]	.673
A&EF (n=179)	-.37 [-.73; -.01]	**.044**	-.42 [-.78; -.06]	**.022**	-.38 [-.80; .04]	*.073*
PS (n=178)	-.33 [-.70; .04]	*.076*	-.15 [-.72; -.04]	**.027**	-.14 [-.53; .26]	.494
P&C (n=166)	-.49 [-.92; .60]	**.026**	-.46 [-.91; -.01]	**.043**	-.51 [-.10;-.01]	**.046**

Abbreviations: A&EF: Attention and executive functioning; PS: processing speed; P&C: perception and reconstruction.
Model I: CMI presence on cognitive domain (z-scores). Model II: adjusting for age, gender and education. Model III: Additionally adjusting for other MRI markers (WMH, MTA, (non-) lacunar infarcts, microbleeds).

Clinical subgroups in patients with possible VCI

The patient group was further categorized into clinical subgroups NOCI, MCI, AD, VaD and other dementia. The clinical subgroups did not differ from each other with respect to sex, but patients were older in the AD (76.2±7.8), MCI (72.6±10.9) and other dementia group (71.4±9.1) than in the NOCI (62.3±8.9) and VaD group (61.6±9.4), $p<.0001$). The percentage of subjects with CMIs per clinical diagnosis is displayed in Figure 3. There was a significant association between CMI occurrence and clinical diagnosis ($p<.05$). In particular, CMIs were more common in patients with a diagnosis of VaD compared to all other diagnoses combined (OR 5.4, CI 1.6-18.9, $p<.005$).

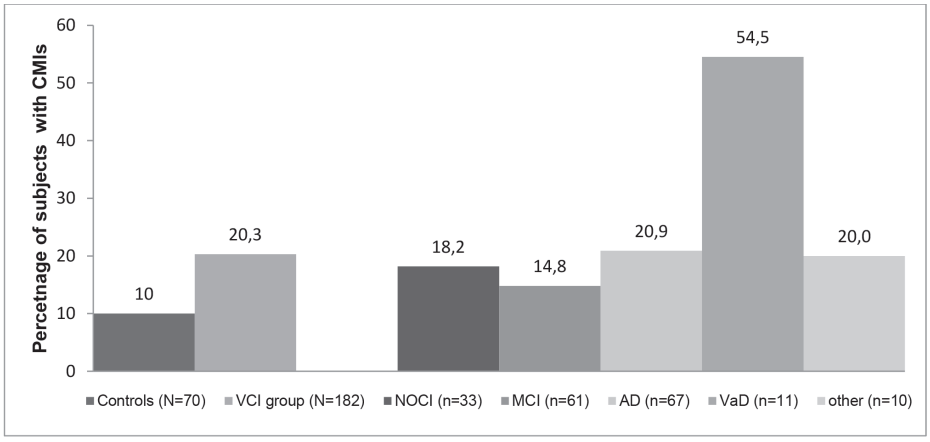


Figure 3: Percentage of subjects with CMIs in the controls and patients, as well as per patient clinical subgroups. Abbreviations: NOCI: no objective cognitive impairment; MCI: mild cognitive impairment; AD: Alzheimer's disease; VaD: vascular dementia.

DISCUSSION

CMI burden was higher in memory-clinic patients with vascular brain injury (20.3%) compared to controls (10.0%). Among patients, cortical CMIs were associated with male sex, history of stroke and the presence of both small vessel disease (SVD) and large vessel disease MRI markers. Moreover, we found that patients with CMIs had reduced performance in multiple cognitive domains and were more likely to have the clinical diagnosis VaD. These findings add to a growing body of research highlighting CMIs as relevant vascular imaging markers in cognitive impairment and dementia.

Over the last few years there is an emerging literature on CMIs detected with MRI. CMIs were found in 16-32% in a memory-clinic cohorts, 15% in an acute stroke cohort and 6% in a population based cohort, which is in line with the 20% reported in our VCI cohort [3,4,23–26]. Our findings are also in agreement with a systematic review of pathology studies, concluding that the CMI burden is increased in cerebrovascular disease and dementia, especially in VaD [27].

In this study we only addressed cortical CMIs, because subcortical CMIs on MRI cannot yet be readily resolved from other lesions such as WMHs or enlarged perivascular spaces [11]. The preferential location of cortical CMIs is not yet firmly established. This study found a strong clustering of CMIs around the pre- and postcentral gyri and medial frontal areas in both hemispheres. Two previous MRI studies reported a predominance in parietal areas [3,4], while in pathology studies a predilection for cortical watershed areas was shown [28]. Although there is interpersonal variation in the location of watershed regions, a majority of the CMIs displayed in Figure 2 are indeed located in the watershed area between the middle cerebral artery and the anterior and posterior arteries.

CMIs were related both to markers of SVD and large vessel disease in this study, but not with classical vascular risk factors such as hypertension or hypercholesterolemia. Other studies have also found that CMIs were associated with intracranial atherosclerosis [4,24,29], microbleeds [23,24,30] and atrial fibrillation [25]. A recent pathology study suggested that etiology of CMIs might differ per brain location [31] Taken together, these studies indicate that CMIs have a heterogeneous etiology in memory-clinic patients.

We found that CMIs were associated with worse cognitive functioning in multiple domains; perception & construction, attention & executive functioning and possibly

processing speed. The association with perception & construction was independent of other markers of vascular injury, which was also previously established in both MRI and pathology studies [3,4,25,32]. Perception & construction ability is linked to functioning of the parietal cortex, a location with a high density of CMIs in this study. Deficits in attention & executive functioning and processing speed are the core cognitive features of advanced SVD.. At this stage it is too early to link this cognitive profile to the lesion distribution as show in figure 2, also given the limited sample size. Moreover, it has been proposed that the functional impact of CMIs might also be determined by more widespread disruption of white matter networks. [33]. Yet, lesion burden and location, and cognition relations in CMIs is an emerging area of research that requires further study.

The size of a single cortical CMI is most likely not sufficient to cause substantial cognitive deficits. It is not yet clear to which extent MRI visible cortical CMIs are accompanied smaller cortical and subcortical lesions. Hence, one cortical CMI on MRI could be indicative of a more widespread burden of hundreds or even thousands of smaller CMIs that escape detection on in vivo MRI. Evidence for this notion comes from two studies mathematically estimating total CMI burden, using the prevalence of acute CMIs detected with diffusion-weighted imaging [34] and pathology data [35]. These findings suggest that MRI visible CMIs may reflect a more wide spread form of vascular brain injury. .

Strength of this study is the availability of high quality imaging and cognitive data from a memory-clinic cohort with the full spectrum of vascular brain injury and cognitive impairment. This study also has some limitations. We used an unselected group of memory clinic patients with MRI evidence of vascular brain injury in our study, without prior selection of certain diagnostic subgroups or severity of cognitive impairment. Although this may benefit the generalizability of the results, the study population is heterogeneous due to this design. As a consequence, the CMIs in the cohort probably have different etiologies. This may be of prognostic relevance, however due to limited sample size this could not be further explored. Moreover, the cross-sectional design of this study limits the causal inferences can made regarding cognitive decline.

In conclusion, CMIs are common findings on 3 Tesla MRI in memory-clinic patients with vascular brain injury and a relevant vascular imaging marker in cognitive impairment and dementia.

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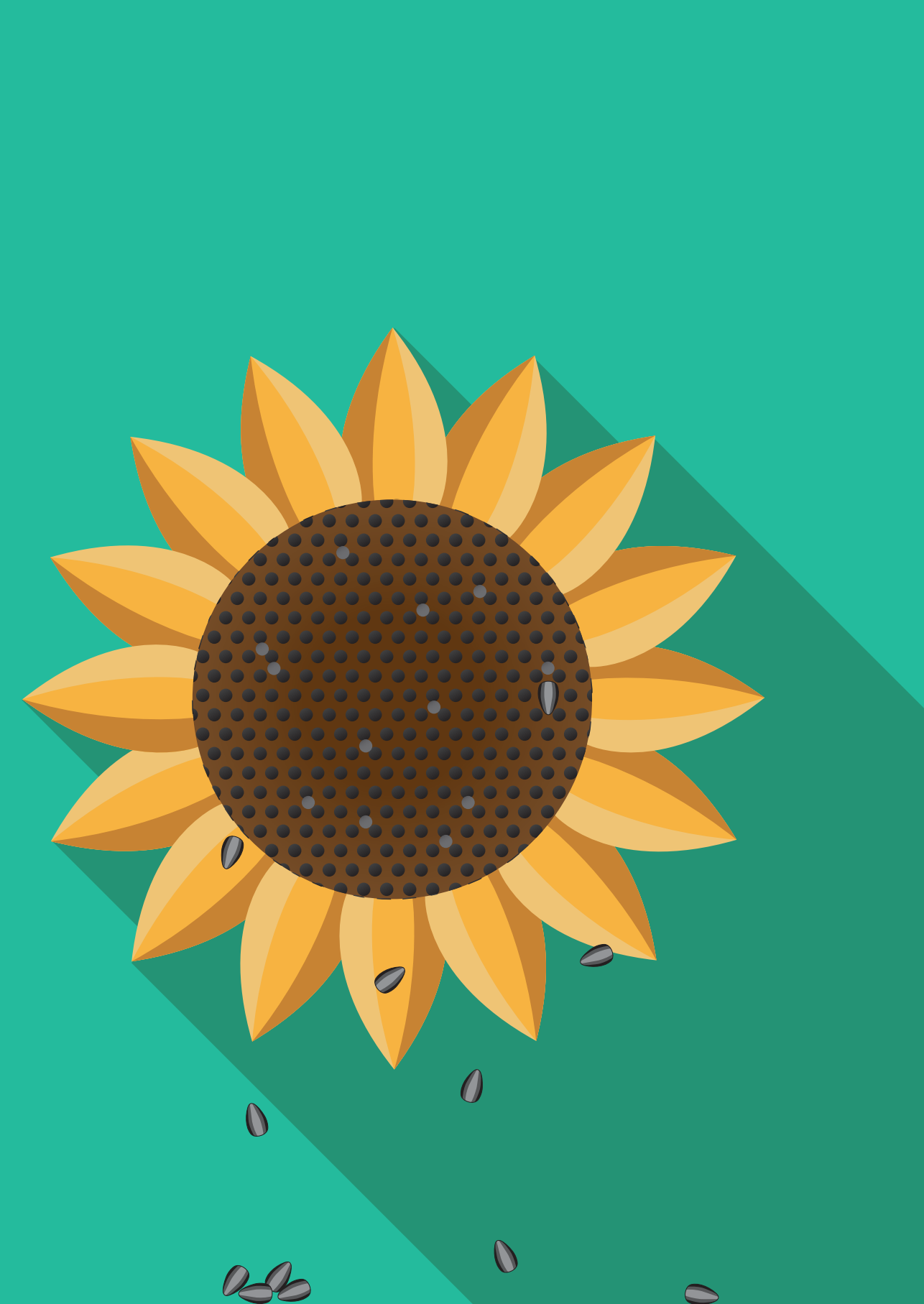

CHAPTER 3

CORTICAL MICROINFARCTS IN MEMORY CLINIC PATIENTS ARE ASSOCIATED WITH REDUCED CEREBRAL PERFUSION

Doeschka A. Ferro
Henri J.J.M. Mutsaerts
Saima Hilal
Hugo J. Kuijf
Esben T. Petersen
Jan Petr
Susanne J. van Veluw
Narayanaswamy Venketasubramanian
Tan Boon Yeow
Geert Jan Biessels
Christopher Chen

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ABSTRACT

Cerebral cortical microinfarcts (CMIs) are small ischemic lesions associated with cognitive impairment and dementia. CMIs are frequently observed in cortical watershed areas suggesting that hypoperfusion contributes to their development. We investigated if presence of CMIs was related to a decrease in cerebral perfusion, globally or specifically in cortex surrounding CMIs. In 181 memory clinic patients (mean age 72 ± 9 years, 51% male) CMI presence was rated on 3 T-MRI. Cerebral perfusion was assessed from cortical gray matter of the anterior circulation using pseudo-continuous arterial spin labeling parameters *cerebral blood flow* (CBF) (perfusion in mL blood/100g tissue/minute) and *spatial coefficient of variation* (CoV) (reflecting arterial transit time (ATT)). Patients with CMIs had a 12% lower CBF (beta=-.20) and 22% higher spatial CoV (beta= .20) (both $p<.05$) without a specific regional pattern on voxel based CBF analysis. CBF in a 2 cm region-of-interest around the CMIs did not differ from CBF in a reference zone in the contralateral hemisphere. These findings show that CMIs in memory clinic patients are primarily related to global reductions in cerebral perfusion, thus shedding new light on the etiology of vascular brain injury in dementia.

INTRODUCTION

Cerebral cortical microinfarcts (CMIs) are small ischemic lesions visible on neuropathological examination and also detectable in vivo with high field strength MRI [1,2]. CMIs commonly occur in patients with stroke and dementia and their contribution to vascular cognitive impairment is increasingly recognized [3].

Although the pathophysiological mechanisms are not fully clear, CMIs probably have multiple causes. CMIs may result from small vessel disease (SVD), including cerebral amyloid angiopathy (CAA) and hypertensive SVD [3]. Moreover, CMIs can also result from thromboemboli due to large vessel or cardiac disease [3,4]. An interesting observation is that CMIs appear to be frequently located in the cortical watershed regions both in neuropathology [5–7] and MRI studies [8,9]. Watershed regions are located at the junction of two vascular territories and are therefore sensitive to hypoperfusion [10]. These findings suggest that hypoperfusion might play a role in the pathophysiology of CMIs.

Arterial spin labeling (ASL) is a non-invasive MRI perfusion technique, whereby blood is magnetically labeled in the cervical arteries. After a short post-labeling delay, a cerebral blood flow (CBF) map is obtained from the ASL signal in brain tissue [11]. Due to this fixed delay, the CBF maps may become inaccurate when blood flowing towards the brain is slowed – prolonged arterial transit time (ATT) – for example in elderly and patients with cerebrovascular disease [12]. Recently, the ASL derived parameter *Spatial coefficient of Variation* (CoV) has been introduced that approximates ATT and is suitable for individuals with compromised cerebral blood flow [13].

In this study we aim to determine if presence of CMIs is related to decreased cerebral perfusion, assessed with pseudo-continuous ASL (pCASL) CBF and spatial CoV in memory clinic patients; and if perfusion is specifically reduced locally around CMIs.

METHODS

Population

This study involved patients who attended the memory clinics of the National University Hospital or St. Luke's Hospital in Singapore and had received one of the following diagnoses [14]: (1) *No cognitive impairment* (NCI): patients without objective cognitive impairment on formal neuropsychological tests or functional loss (2) *Cognitive impairment - no dementia* (CIND) *with* (2a) or *without* (2b*) a history of stroke*: included patients who were impaired in at least one cognitive domain on neuropsychological testing, but did not meet the DSM (4th edition) criteria for dementia. Ischemic stroke was assessed based on medical history and confirmed by neuroimaging; patients with a hemorrhagic stroke were excluded from participation. Patients with dementia received an etiological diagnosis on the basis of internationally established criteria for (3) *Alzheimer dementia* (AD) [15] and (4) *Vascular dementia* [16]. Patients with other diagnoses (e.g. FTD), significant neurological comorbidities or loss of functional independence were excluded from participation. All patients underwent a standardized clinical examination, 3-T MRI including ASL and neuropsychological testing [4,14]. For the present study we included all patients recruited between December 2010 and September 2013 and had structural MRI and ASL sequences of sufficient quality.

Ethical approval for this study was obtained from the National Healthcare Group Domain-Specific Review Board (DSRB). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained, in the preferred language of the patients, by bilingual study coordinators before recruitment into the study. Consent for patients lacking capacity was provided by their legal representative, as allowed by the DSRB.

Level of education was classified into 4 categories: no education, primary education, secondary education or tertiary education. The following vascular risk factors were recorded: hypertension was defined as a previous diagnosis of hypertension, or use of antihypertensive medication; diabetes mellitus was defined as previous diagnosis of diabetes mellitus, or use of anti-diabetic medication; hyperlipidemia was defined as previous diagnosis of hyperlipidemia, or use of cholesterol-lowering medication. Obesity was defined as a body mass index >25. A history of cardiac disease was defined as a previous diagnosis of myocardial infarction, congestive heart failure, atrial fibrillation,

or intervention procedures such as angioplasty, or stenting. A history of stroke was based on self report.

MRI protocol

Patients were scanned on a 3-T MRI (Siemens Magnetom Trio Tim system) with a 32-channel receiver head-coil. The structural imaging protocol included a 3D T1, 2D T2, T2*-weighted and fluid-attenuated inversion recovery (FLAIR) sequences (See eTable 2 for scanner-settings). pCASL images were acquired with labeling duration 1738 ms, post-labeling delay 1500 ms, and gradient-echo echo planar imaging of TR/TE 4000/9 ms, GRAPPA factor 3, 3.0 x 3.0 x 5.0 mm^3 voxels. ASL scans consisted of 23 pairs of images (control – labeled). CBF values were calculated voxel wise from the mean differences of these two images. For each patient, the ASL protocol was carried out twice with a 1-hour interval; CBF was averaged over the two sessions, unless one of the scan sessions was disregarded due to insufficient quality.

MRI ratings

The following MRI markers were rated: Microbleeds were assessed using the Brain Observer MicroBleed Scale [17]. The presence of large (i.e. >5 mm) cortical infarcts, subcortical infarcts (i.e. subcortical infarct and/or lacunar infarcts) and white matter hyperintensities (WMHs) were rated according to the STRIVE criteria [18]. Infarct and WMH volumes were manually segmented on FLAIR and T1-weighted images using an in-house developed tool incorporated in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany). Gray matter (GM), white matter, and CSF were obtained from the T1-weighted image using the unified tissue segmentation approach [19] implemented in Statistical Parametric Mapping (SPM) (Wellcome Trust Centre for Neuroimaging, University College London, UK) from which total brain volume and intracranial volume were quantified. The presence of intracranial stenosis on the 3D time of flight MRA was defined as a narrowing exceeding 50% of the luminal diameter of the posterior cerebral, middle cerebral and anterior cerebral artery and the intracranial part of vertebral, basilar and internal carotid artery [20].

CMIs were rated by visual inspection by a single experienced rater (SvV). In line with previously validated criteria, CMIs were obliged to be less <5mm in the largest dimension and hypointense on 3D T1-weighted images and hyperintense or isointense on FLAIR and T2-weighted images [2]. Lesions had to be strictly intracortical, perpendicular to the cortical surface and distinct from perivascular spaces. Moreover, a lesion was discarded

as CMI if the area was considered part of a larger infarction. There was a good to excellent intra-rater agreement (ICC=0.97; Dice's similarity coefficient=0.65) on the CMI ratings of this cohort. We have previously published the clinical correlates of CMIs in this specific cohort [14]. A 3D representation of all CMIs in the cohort was configured by registering the lesions to the MNI152 standard brain (Figure 1).We used an in-house developed watershed atlas as overlay. This atlas was based on the average territorial ASL maps from individuals with steno-occlusive disease [21]. The watershed areas were defined as overlapping perfusion territories across subjects.

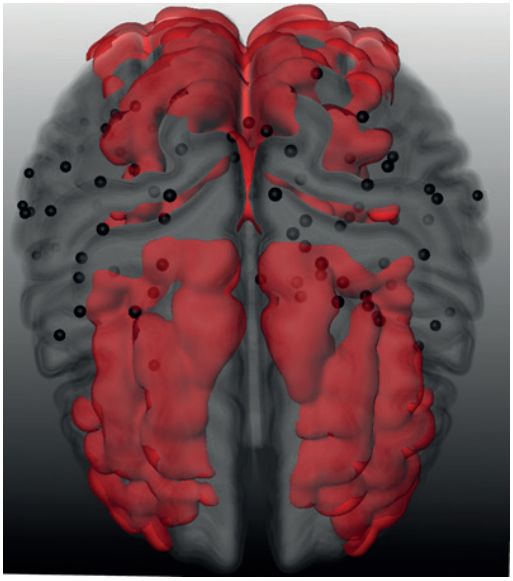


Figure 1: 3D representation of cerebral cortical microinfarcts with watershed overlay. Superior view of a 3D representation of cerebral cortical microinfarcts (black dots) in the cohort projected on an MNI-standard brain. The red overlay signifies the cortical watershed regions.

ASL

ASL post-processing was performed with the *ExploreASL* toolbox, an in-house developed toolbox based on SPM and Matlab7.12.0 (MathWorks, Nattick, MA) [22]. Post-processing included motion correction and rigid body registration of the CBF map to a GM map and normalized into common space through the T1-weighted images using Diffeomorphic Anatomical Registration analysis using Exponentiated Lie algebra (DARTEL) [23] using CAT12 [24]. From the CBF map two ASL parameters were acquired within a pGM>0.7 GM region-of-interest: CBF and Spatial CoV. CBF reflects perfusion in mL blood/100g tissue/minute. To avoid systematic bias in patients with more severe atrophy, partial

volume corrections was applied to the CBF (CBF_{pvc}) using local linear regression within a 3D kernel based on the CAT12 tissue partial volume maps [25]. Spatial CoV is a novel ASL parameter particularly suitable for individuals with severely prolonged ATT, such as patients with a vascular brain disease in which CBF sometimes cannot be readily measured. It serves as a proxy of ATT and is calculated by dividing the SD of the CBF by the mean CBF. Additional details regarding spatial CoV have been published previously [13].

Global CBF and spatial CoV were obtained using a GM mask of anterior brain circulation only (i.e. the internal carotid artery flow territory, not the posterior circulation as supplied by the vertebrobasilar system), as acquisition parameters were not favorable for the longer arrival time in the posterior circulation. Mean cortical GM CBF was calculated from the intersection of the watershed ROI obtained from a watershed atlas [21]- shown in Figure 1- and the subject-wise GM mask. A voxel based analysis of the cortical GM CBF differences between patients with and without CMIs was performed in SPM. CBF maps were smoothed with a 6mm full width at half maximum Gaussian kernel.

Individual CMI ROIs composed of a 2cm diameter sphere around the CMIs. The diameter was set at 2 cm, as a trade-off between spatial specificity requiring the smallest possible diameter and SNR/resolution of ASL, which puts boundaries to the minimum diameter that can be used. Within the sphere only the cortical GM was considered for partial volume corrected CBF assessment. A comparative ROI was created on the same anatomical location in the contralateral hemisphere. CMI ROIs were excluded from analysis if they overlapped (either in the ipsi- or contralateral hemisphere) with another ROI or larger infarct, resulting in a total of 67 CMI ROIs in 35 patients. Figure 5 provides a visual representation of the ROI analysis. Visual quality assessment of the ASL scans was performed by two independent raters (DF and HM) based on visual evaluation. Scans were classified according to 1 of 4 categories (Figure 2): (1) *Unusable* was applied to scans that were incomplete, had labeling errors or severe motion artifacts (n=53). (2) *Angiogram* for scans with predominantly vascular contrast and no or minimal tissue perfusion (n=33) [13](3) *Acceptable* scans which had minor vascular contrast or motion artefacts and reasonable tissue perfusion (n=58) or (4) *good* (n=90). Patients with unusable scans were excluded from analysis. All other patients were analyzed, but the 33 patients with *angiogram*-classified ASL scans were not included in the CBF analysis, as the lack of tissue perfusion causes unreliable interpretation [13].

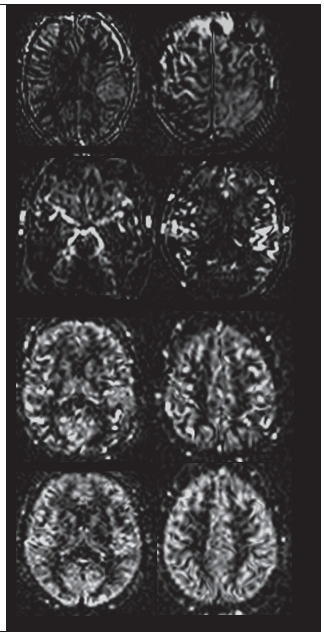
N	ASL quality	Defined by	Example scans
53 (23%)	Unusable	Incomplete protocol, labeling errors or severe motion artifacts	
33 (14%)	Angiogram	Predominantly vascular contrast, no or minimal tissue perfusion	
58 (25%)	Acceptable	Minor vascular contrast or movement artefacts, reasonable tissue perfusion	
90 (38%)	Good	Scans without vascular contrast	

Figure 2: Quality assessment method of the ASL scans, including quality criteria and example images.

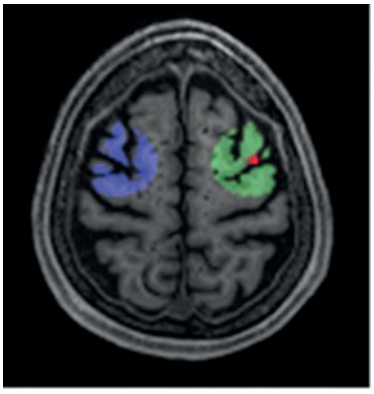


Figure 5: Local CBF analysis using CMI ROIs. Cerebral blood flow (CBF) within a Region-of-Interest (ROI), composed of a 2cm diameter sphere (green area) around the cortical microinfarcts (CMIs) (red dot), was compared to the CBF within an ROI on the same anatomical location in the contralateral hemisphere (purple area).

Statistical analysis

Differences in baseline characteristics between patients with and without CMIs were compared with an independent t-test (for continuous normally distributed data), χ-square test (for proportions) and Mann-Whitney U test (for continuous, non-normally distributed data). WMH volume and spatial CoV data was log transformed. The relation between CMI presence (independent variable) and CBF, CBF_{PCV} and spatial CoV (dependent variable) was assessed using linear regression in model 1. In model 2 age and sex were entered as covariates. In model 3 we performed additional explorative analyses where individual and combined vascular risk factors were added as covariates to model 2 and in model 4 where individual and combined neuro-imaging markers were added as covariates to model 2. Models are presented with b-values representing the mean difference (and 95% confidence interval) in cerebral perfusion between patients with and without CMIs. Within the patients with CMIs, Spearman's rank correlation was used to determine the relationship between the total number of CMIs per patient to the CBF, CBF_{PCV} and spatial CoV. For the voxel-based analysis, significant clusters of voxels were identified using a primary cluster-forming threshold of $p<.001$, family wise corrected for cluster sizes corresponding to $p < 0.05$. For the CMI ROI analysis, CBF was compared between the ROIs in the ipsi- and contralateral hemispheres in a paired (within subjects) t-test. All data was analyzed using IBM SPSS statistics (version 25) and a p-value < .05 was considered statistically significant.

RESULTS

Demographics and CMI occurrence

Of the 234 patients, 53 (23%) were excluded due to insufficient quality of the ASL scan. The excluded patients were on average older and had more severe cognitive impairment (i.e. a lower MMSE and a higher proportion of patients with diagnosis dementia) and a higher CMI burden (eTable 1). Of the remaining 181 patients included in the spatial CoV analyses, 148 patients – without angiogram like ASL scans – were suitable for CBF analyses. The mean age of these 181 patients was 72 years (SD 9), 51% were male and the majority (80%) of Chinese ethnicity. Of these patients, 41% was diagnosed with dementia, 43% with CIND and 16% with NCI. The 33 patients who were unsuitable for the CBF analysis due to angiogram qualified ASL scans had a higher proportion of males and a higher CM burden (eTable 1).

A total of 179 CMIs were detected in 50 (28%) of the 181 patients. The number of CMIs per patient ranged between 1-43 and 23 patients (46%) had multiple CMIs. Patients with CMIs were proportionally often male (p=.063) and had a higher burden of vascular risk factors and cerebrovascular disease, (Table 1). Figure 1 displays a 3D spatial topographical representation of all CMIs in the cohort. Visual inspection suggests a clustering of CMIs in fronto-parietal areas without evident predilection for the cortical watershed regions.

Table 1: Baseline characteristics of patients with and without CMIs

	CMI absent (N=131)	CMI Present (N=50)	*P*- value
Age, mean (SD), years	71.5 (9.3)	72.6 (8.8)	.464
No. (%) males	61 (47)	31 (62)	.063
Ethnicity, No. (%) Chinese	107 (82)	37 (74)	.251
Education, median (IQR) (4 levels)	2 (1-3)	2 (1-3)	.357
Vascular risk factors			
Hypertension, No. (%)	92 (70)	43 (86)	.029
Hypercholesterolemia, No. (%)	82 (63)	44 (88)	.001
Diabetes, No. (%)	46 (35)	25 (50)	.067
Current smoking, No. (%)	11 (8)	7 (14)	.260
Obesity (4 missing), No. (%)	4 (3)	5 (10)	.062
History of stroke, No. (%)	44 (34)	25 (50)	.042
Cardiac disease, No. (%)	19 (15)	20 (40)	<.001
Cognitive performance			
CDR median (IQR)	0.5 (0-1)	0.75 (0.5-1)	.238
MMSE, mean (SD)	22.1 (5.8)	20.6 (5.8)	.129
Clinical diagnosis, No. (%)			.135
NCI	25 (19)	4 (8)	
CIND	56 (43)	21 (42)	
Dementia	50 (38)	25 (50)	
Neuro-imaging markers			
Total brain volume, mean (SD), % of TIV	64.8 (5.7)	62.1 (6.2)	.006
WMH volume, median (IQR), mL	8.3 (3.3-19.8)	12.1 (5.4-29.7)	.010[a]
Presence of cortical infarcts >5mm, No. (%)	8 (6)	17 (34)	<.001
Presence of subcortical infarcts, No. (%)	32 (24)	22 (44)	.010
Presence of microbleeds, No. (%)	66 (50)	35 (70)	.017
Presence intracranial stenosis (4 missing)	23 (18)	15 (31)	.053

Abbreviations: CMI=Cerebral cortical microinfarct, CDR=clinical dementia rating scale, MMSE=mini-mental state examination, NCI=no cognitive impairment, CIND=Cognitive impairment - no dementia, TIV=total intracranial volume, WMH=White matter hyperintensity. Obesity was defined as a body mass index >25.

[a] WMH volume was entered into the analysis after a logarithmic transformation of the data.

Perfusion in patients with and without CMIs

CMI presence was associated with a 12% reduced cortical CBF (effect size d=0.49, p=.016), a 9% reduced partial volume corrected CBF (CBF_{pvc}) (effect size d=0.41, p=.044) and a 22% increased spatial CoV (effect size d=0.46, p=.006) in the anterior circulation (Figure 3; Table 2 model 1). When corrected for age and sex, results remained similar for CBF (p=.038) and spatial CoV (p=.039), but attenuated for CBF_{pvc} (p=.078) (Table 2 model 2). The individual and combined vascular risk factors had a marginal effect on the relationship between CMI presence and cerebral perfusion (Table 2 model 3). Similarly, the individual neuro-imaging markers, including intracranial stenosis, also had a marginal effect on the relationship between CMIs presence and cerebral perfusion, with the exception of total brain volume and WMHs that both attenuated the strength of the association between CMIs and perfusion measures (Table 2 model 4). Moreover, in the model with all neuro-imaging markers combined the relationship between CMI and perfusion measures disappeared. Within the patients with

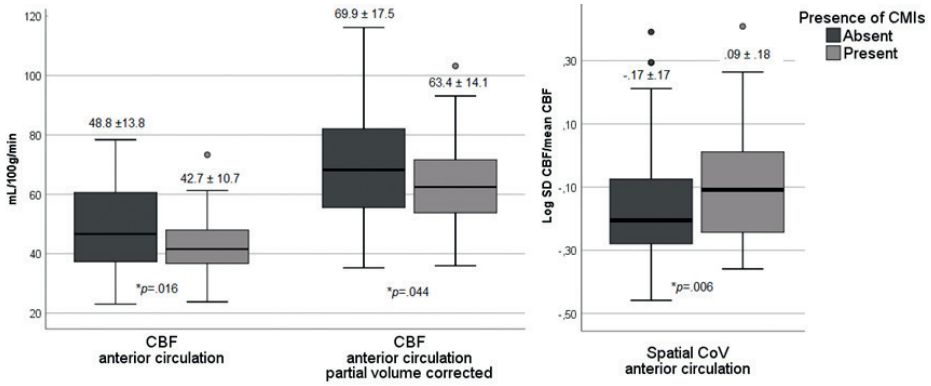


Figure 3: Cerebral perfusion in patients with and without cerebral cortical microinfarcts (CMIs). Boxplots of the mean and standard deviation of the cerebral blow flow (CBF), partial volume corrected CBF and spatial coefficient of variation (CoV) in the anterior circulation in patients with and without CMIs. P-values are reported on the uncorrected differences between patients with and without CMIs.

CMIs, a higher number of CMIs per patient was modestly correlated to a higher spatial CoV (Rho=.27, p=.057), but not to CBF (Rho=-.06, p=.752) or CBF_{pvc} (Rho=-.07, p=.699). With respect to the perfusion in the watershed regions, the difference in CBF (beta=-.17, p<.05) and CBF_{pvc} (beta=-.16, p=.058) between patients with and without CMIs in these regions was of similar magnitude as the whole anterior brain circulation. To determine the spatial pattern of hypoperfusion in patients with CMIs compared to patients without

CMIs, a voxel based analysis of CBF signal was performed, which showed a relatively homogeneous pattern of hypoperfusion over the whole cortex (Figure 4) .

Table 2: Association between CMI presence and cerebral perfusion adjusted for covariates

	CBF (N=148) B [95% CI]	*p*	CBFpvc (N=148) B [95% CI]	*p*	Spatial CoV (N=181) B [95% CI]	*p*
Model 1: CMI presence	-6.1 [-11.1; -1.2]	.016	-6.5 [-12.9; -.2]	.044	.08 [.02; .14]	.006
Model 2: CMI + age, sex	-5.0 [-9.7; -.2]	.038	-5.6 [-11.9; .6]	.078	.06 [.003; .11]	.039
Model 3: CMI + age, sex +						
Hypertension	-4.6 [-9.3; .1]	.054	-5.1 [-11.4; 1.1]	.106	.05 [-.005; .10]	.079
Hypercholesterolemia	-4.4 [-9.3; .4]	.073	-4.8 [11.2; 1.6]	.143	.05 [-.006; .10]	.082
Diabetes	-4.5 [-9.3; .2]	.061	-5.3 [-11.7; 1.0]	.098	.06 [.003; .11]	.040
Current smoking	-5.2 [-9.9; -.4]]	.033	5.8 [-12.1; .5]	.072	.05 [.001; .11]	.045
Obesity	-5.0 [-9.9; -.1]	.044	-6.2 [-12.7; .4]	.064	.06 [.004; .11]	.034
History of stroke	-5.7 [-10.5; -1.0]	.018	-4.9 [-11.2; 1.4]	.126	.05 [.001; .11]	.047
Cardiac disease	-4.5 [-9.2; .3]	.065	-6.4 [-12.7; -.1]	.048	.05 [-.01; .10]	.122
All vascular risk factors	-4.8 [-9.8; .3]	.066	-5.7 [-12.5; 1.0]	.096	.05 [-.005; .11]	.075
Model 4: CMI + age, sex +						
Total brain volume	-3.7 [-8.2; .8]	.110	-4.5 [-10.7; 1.7]	.154	.04 [-.01; .10]	.104
WMH volume [a]	-3.1 [-7.8; 1.7]	.200	-3.6 [-10.0; 2.8]	.265	.03 [-.02; .09]	.210
Presence of cortical infarcts	-5.0 [-10.0; -.1]	.045	-5.6 [-12.2; .9]	.090	.05 [-.003; .11]	.064
Presence of subcortical infarcts	-4.6 [-9.5; .2]	.059	-5.0 [-11.4; 1.4]	.122	.05 [-.001; .11]	.048
Presence of microbleeds	-4.2 [-8.9; .6]	.086	-4.4 [-10.7; 1.9]	.171	.05 [-.004; .10]	.069
Presence intracranial stenosis	-4.4 [-9.2; .4]	.073	-5.0 [-11.4; 1.5]	.128	.05 [-.003; .10]	.064
All imaging markers	-1.1 [-6.0; 3.9]	.670	-1.2 [-8.1; 5.6]	.727	.02 [-.04; .07]	.581

Abbreviations: CBF=Cerebral blood flow; CBFpvc= Partial volume corrected cerebral blood flow; Spatial CoV=Spatial coefficient of variation of the cerebral blood flow; CMI=Cerebral cortical microinfarct; WMH=White matter hyperintensity.
Data presented as B: mean difference between patients with and without CMIs and the 95% confidence interval. Model 1: unadjusted; Model 2: adjusted for age and sex; model 3: adjusted for age, sex and each individual vascular risk factor added separately, model 4: adjusted for age, sex and each individual neuro-imaging marker added separately.
[a] WMH volume was entered into the analysis after a logarithmic transformation of the data.

Perfusion around CMIs

Mean CBF in 2cm cortical GM ROIs around the CMIs (CBF=73.1 mL/100g tissue/minute, SD 26.7) was not different from the CBF of the ROI in the contralateral reference regions (CBF=73.0 mL/100g tissue/minute, SD 28.6) (p=.97).

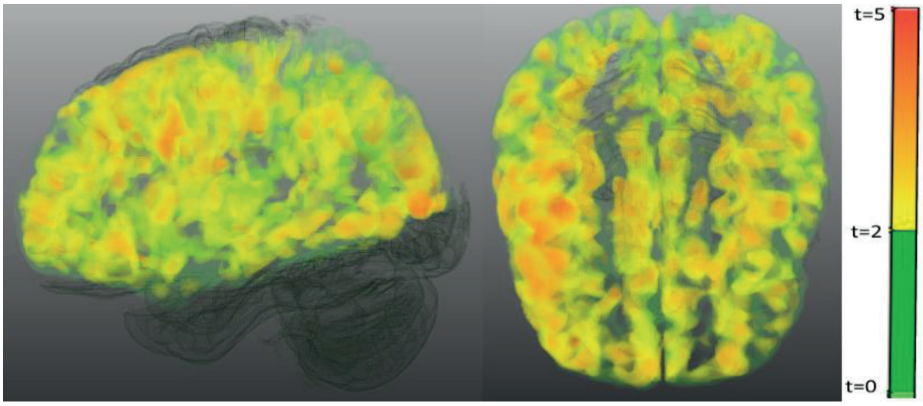


Figure 4: Voxel based analysis of the differences in cerebral blood flow between the group of patients with cortical microinfarct compared to the group of patients without cortical microinfarcts.
Significant clusters (cluster threshold of p<.001, family wise corrected to < 0.05) are shown in yellow to red, non-significant clusters are shown in green.

DISCUSSION

We found that presence of CMIs in memory clinic patients was associated with reduced cerebral perfusion throughout the anterior circulation, but that perfusion in the cortex directly surrounding CMIs was not specifically affected.

The two ASL parameters studied (i.e. CBF and spatial CoV) congruently showed that cerebral perfusion was affected in patients with CMIs. Previous studies had already provided circumstantial evidence of a link between CMIs and perfusion. Post-mortem subcortical CMIs were found to relate to ante-mortem blood pressure decline on serial blood pressure assessments, possibly translating in reduced cerebral perfusion pressure [26]. Furthermore, both post-mortem [5–7] and MRI studies [8,9] have suggested that CMIs might have a predilection for the cortical watershed areas. This hypothesis could, however, not be confirmed in the current study. Although clustered in the fronto-parietal cortex, we observed no evident predilection of CMIs in the watershed areas, as defined by a previously established ASL atlas. These different findings might be attributed to the

study population, as both CMI occurrence and etiology are known to vary in different contexts. Additionally, previous neuropathological studies [5–7] allow for detection of much smaller (i.e. <1mm) CMIs, that could have a different topographical localization. Future research is encouraged to further explore this on ultra-high field MRI, which allows for detection of intermediate size CMIs.

In line with our observations in CMIs, the presence of other manifestations of SVD on MRI has been linked to reductions in cerebral perfusion. There is, for example, a growing body of – mostly cross sectional – studies reporting a relation between WMHs and reduced perfusion [27]. Of note, perfusion is also found to be locally reduced within WMH lesions [28]and longitudinal studies suggested that hypoperfusion probably precedes the formation of WMHs [29,30]. The presence of microbleeds is also reported to be related to globally reduced perfusion [31]. Although we are not aware of studies exploring perfusion directly around the microbleeds, the previous study indicates that hypoperfusion is not restricted to the preferential parietal-occipital location of cortical microbleeds in CAA.

Although perfusion may be reduced at the lesion sites, particular for WMHs, the consistent finding for the different manifestations of SVDs is that they predominantly associate with globally reduced cerebral perfusion. The question is therefore what mechanisms tie these small focal lesions to reduced global perfusion. First, there could be a causal link, where hypoperfusion directly induces CMIs. In that scenario, CMIs may occur at sites that reach a critical perfusion threshold or that are exposed to a second pathophysiological mechanism. Disturbances more proximal in the vascular tree, such as cardiac pump dysfunction [4] or stenosis or occlusion of the cervical and intracranial arteries [14,32], could certainly contribute towards reaching a critical perfusion threshold. In this study we found a marginal effect of intracranial stenosis on the relation between CMIs presence and perfusion. Unfortunately, no data on extracranial stenosis or cardiac pump function was available in this cohort. Evidence for an interaction with a second pathophysiological mechanism comes from a CAA mouse model study, which showed that mice subjected to chronic hypoperfusion not only showed accelerated deposition of leptomeningeal amyloid, but also a markedly higher CMI burden than CAA mice with normal cerebral perfusion [33].

Another possibility is that both reduced perfusion and CMIs are due to shared etiologies, without being directly causally linked to each other. Such shared etiologies could include

SVD, where restriction of vessel lumen by arteriolosclerosis, CAA, loss of autoregulation, blood–brain barrier leakage and inflammation could affect perfusion as well as induce CMIs [34]. Explorative analysis in this study showed that entering other imaging manifestations of SVDs, especially WMHs, together with CMIs in the statistical models, attenuated the association between CMI presence and perfusion. Even shared vascular risk factors, such as hypertension, could contribute to both independently. However, our exploratory analyses indicated that both individual and combined vascular risk factors only marginally affected the relation the relationship between CMIs and perfusion. It is highly likely that the abovementioned mechanisms coincide and even interact. For example, it has been suggested that reduced arterial flow can prompt the formation of thrombo-emboli due to impaired wash-out [35], providing additional mechanisms CMI development. We therefore encourage future studies to further investigate the underlying mechanisms tying CMIs to cerebral perfusion. This could include zooming in on clinical populations known to exhibit compromised cerebral perfusion, such as patients with heart failure or severe carotid artery stenosis. Moreover, the mediating role of SVDs could be further explored, by assessing interrelations with other imaging manifestations of SVDs, in particular WMH, but also by using novel measures of small vessel function and blood brain barrier function, that may provide more direct indications of the condition of the small vessels in SVD, independent of downstream lesions [36].

The interaction between CMIs and reduced cerebral perfusion clearly carries clinical significance. Over the years a robust relationship has been demonstrated between CMIs and cognitive impairment, yet currently without clear prospect of treatment [3]. The current study highlights the importance of cerebral perfusion as potential therapeutic target.

This study has several strengths and limitations. It involved a heterogeneous memory clinic cohort with a broad range of cognitive symptoms, strengthening results as it reflects daily clinical practice. Although CMIs were rated according to validated criteria, the limited sensitivity of 3-T MRI needs to be acknowledged, as it allows detection of only a fraction of the CMIs visible on 7-T or neuropathological examination [2]. It remains unclear how these smaller CMIs relate to cerebral perfusion. A watershed atlas based on a group-averaged territorial ASL map was used in this study. However, it must be acknowledged that there is a large inter-individual variation in the size and exact location of the watershed regions. Major challenges of the ASL technique include a low signal-to-noise ratio and problematic application in patients with prolonged ATT [11].

As a result, we applied relatively large ROIs of 2 cm around the CMIs, while the area of perfusion restriction could in fact be smaller. Finally, we had to exclude a relatively high (23%) proportion of severely affected patients due to poor ASL scan quality. We partially resolved this issue by using the novel ASL parameter spatial CoV and improve ASL quality by repeating the ASL protocol twice.

Conclusion:

CMI presence was associated with decreased cerebral perfusion in the anterior circulation of memory clinic patients, but perfusion in the region directly surrounding CMIs did not appear selectively affected. Hence, in line with other manifestations of SVDs, CMIs predominantly relate to a global reduction in perfusion. This could be the result of a direct causal relation of hypoperfusion on CMI development or shared underlying mechanisms, such as SVD, proximal vascular pathology and vascular risk factors, or a combination of all. Further unravelling the link between CMIs and perfusion reduction will significantly contribute to understanding of vascular cognitive impairment and has important clinical implications with respect to treatment.

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SUPPLEMENTARY MATERIALS

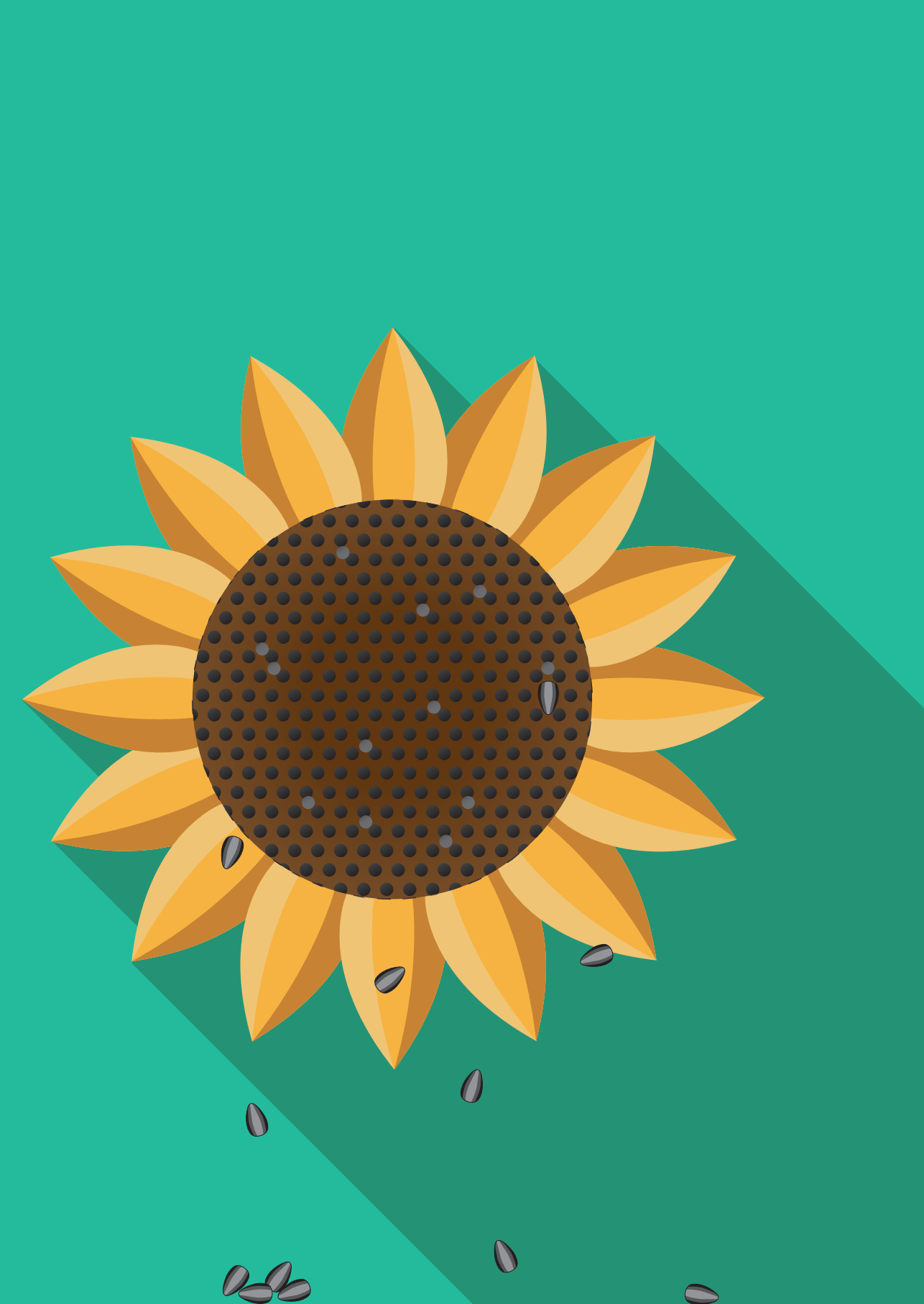
eTable 1: Characteristics of patients included in the CBF analysis, patients with angiogram-classified ASL-scans and excluded patients.

	Study cohort N=181			Excluded N=53	
	CBF analysis	Angiogram	*p*-value	Excluded	*p*-value
N	148	33		53	
Age, mean (SD), years	71.4 (8.8)	73.7 (10.7)	.183	74.8 (8.7)	**.036**
No (%) males	65 (44)	27 (82)	**.000**	22 (42)	.233
Ethnicity, No (%) Chinese	118 (80)	25 (76)	.390	40 (76)	.225
Education, median (IQR) (3 levels)	2 (1-3)	2 (1-3)	.744	2 (1-4)	.420
MMSE, mean (SD)	21.9 (5.8)	20.6 (5.6)	.272	17 (6)	**.000**
Clinical diagnosis, No. (%)			.318		**.000**
NCI	26 (18)	3 (9)		1 (2)	
CIND	64 (43)	13 (39)		10 (19)	
Dementia	58 (39)	17 (52)		42 (79)	
CMI occurrence, No. (%)			**.035**		**.029**
No CMIs	112 (76)	19 (58)		30 (57)	
1 CMI	23 (15)	4 (12)		11 (21)	
≥ 2 CMIs	13 (9) (max 6 CMIs)	10 (30) (max 43 CMIs)		12 (22) (max 10 CMIs)	

Abbreviations: CBF=cerebral blood flow, ASL=arterial spin labeling, IQR=interquartile range, MMSE=mini-mental state examination, NCI=no cognitive impairment, CIND= Cognitive impairment -no dementia, CMI=Cerebral cortical microinfarct. *P*-value of the differences between patients in the CBF analysis (N=148) vs angiogram-classified ASL-scans (N=33); and patients in the CBF analysis (N=148) vs excluded patients due to poor ASL (N=53).

eTable 2: Structural MRI protocol

Sequence	Voxel (mm3)	repetition time (ms)	echo time (ms)	inversion time (ms)	flip angle (°)	Matrix
3D T1	1.0 x 1.0 x 1.0	2300	1.9	900	9	256 x 256
2D T2	1.0 x 1.0 x 3.0	3000	10.1			247 x 256
2D FLAIR	1.0 x 1.0 x 3.0	9000	82	2500		232 x 256
2D T2*	1.0 x 1.0 x 1.5	27	20		15	192 x 256



CHAPTER 4

CEREBRAL CORTICAL MICROINFARCTS IN PATIENTS WITH INTERNAL CAROTID ARTERY OCCLUSION

H. van den Brink*
D.A. Ferro*
J. de Bresser
E.E. Bron
L.P. Onkenhout
L.J. Kappelle
G.J. Biessels
On behalf of the Heart Brain Connection Consortium.
* These authors contributed equally to the manuscript

In preparation

ABSTRACT

Background: Cerebral cortical microinfarcts (CMI) are small ischemic lesions that are associated with cognitive impairment, that probably have multiple etiologies. Cerebral hypoperfusion has been proposed as a causal factor. We studied CMI in patients with internal carotid artery (ICA) occlusion, as a model for cerebral hemodynamic compromise, addressing CMI presence and spatial distribution, also in relation to the condition of the remaining arterial supply.

Methods: We included 95 patients with a complete ICA occlusion (age 66.2±8.3, 22% female) and 125 reference participants (age 65.5±7.4, 47% female) from the Heart-Brain Study. Participants underwent an extensive evaluation including clinical, neuropsychological, and 3T brain MRI assessment. CMI were rated according to standardized criteria.

Results: CMI were more common in patients with an ICA occlusion (54%, median 2, range 1-33) than in the reference group (6%, median 0; range 1-7) (OR 14.3; 95% CI 6.2-33.1; p<.001). Among patients with a unilateral ICA occlusion, CMI were more common ipsilateral to the occlusion than in the contralateral hemisphere (median 2 and 0 respectively; p<.001). In patients with CMI the number of (other) occluded or stenosed cervical arteries was higher (p=.038), and cerebral blood flow tended to be lower (B -6.2 ml/min/100ml; 95% CI -12.4-0.02; p=.05) than in patients without CMI.

Conclusions: CMI are very common in patients with an ICA occlusion, preferably in the hemisphere of the occluded ICA. Moreover, CMI burden was related to the severity of cervical arterial compromise, supporting the etiological role of hemodynamics in the occurrence of CMI.

INTRODUCTION

Cerebral cortical microinfarcts (CMI) are small ischemic lesions that are a common finding in patients with stroke and dementia [1,2]. CMI can be studied histologically, on brain autopsy and recently also with in vivo high-resolution magnetic resonance imaging (MRI) [3]. Studies have shown independent associations between CMI and cognitive impairment, emphasizing their clinical relevance [2].

CMI can have multiple underlying causes that are linked to large vessel disease, small vessel disease and cardiac disease [2]. Hypoperfusion is considered a possible underlying mechanism. A recent study showed a relation between reduced global brain perfusion and CMI in a memory clinic population [4]. However, because of the heterogeneous nature of the etiology of cerebral pathologies inherent to this population, the question is if this observed association could be causal, where low perfusion induces CMI, or just reflects shared risk factors.

In the current study we further investigated hypoperfusion as a possible cause for CMI. We studied CMI in patients with an internal carotid artery (ICA) occlusion, as a model condition for cerebral hemodynamic compromise. An ICA occlusion causes an altered, more vulnerable, hemodynamic brain state. Although only some patients have resting state hypoperfusion, they are clearly at increased risk of hypoperfusion in case of temporary drops in perfusion pressure, thereby also put at increased risk of (temporary) cerebral ischemia [5]. We studied if CMI occur more often in patients with an ICA occlusion than in a reference group. Among patients with a unilateral ICA occlusion, we studied if CMI occurred more often in the ipsilateral hemisphere than in the contralateral hemisphere. Lastly, we examined if occurrence of CMI is associated with the severity of collateral steno-occlusive disease, and with lower cerebral blood flow.

METHODS

Study population

Participants took part in the Heart-Brain Connection study, a Dutch multicenter study focusing on cardiovascular and hemodynamic contributions to cognitive impairment [6]. Data collection took place in four university medical centers (UMCs) in The Netherlands (Leiden UMC, Maastricht UMC, Amsterdam UMC and UMC Utrecht. Reference participants were recruited among spouses of patients and through advertising leaflets.

Generic inclusion criteria for the Heart-Brain Connection study were that both patients and reference participants had to be at least 50 years old, independent in daily life and able to undergo cognitive testing and MRI. Exclusion criteria for all participants were a life expectancy of less than three years, current atrial fibrillation, and neurologic or psychiatric disease affecting cognitive performance other than vascular injury or possible co-occurring Alzheimer's disease. An additional inclusion criterion for the patient group in the Heart-Brain Connection study was a severe stenosis >80% or an occlusion of the ICA, measured either with ultrasound, magnetic resonance angiography or computed tomography angiography. Patients with an ICA occlusion should not have had a brain infarct or transient ischemic attack in the three months prior to inclusion, and no planned carotid surgery or participation in an intervention trial.

For the current study we only selected the patients with a complete ICA occlusion, hence excluding four patients with severe stenosis. In addition, five patients with an ICA occlusion and one reference participant were excluded because the brain MRI was not available, two patients and two reference participants were excluded because of missing MRI sequences and three patients due to insufficient MRI scan quality. This resulted in a study population of 95 patients and 125 reference participants.

The Heart-Brain Connection study was approved by the Medical Ethics Review Committee of the Leiden UMC and local boards of the other UMCs. The study was conducted in accordance with the declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO). Written informed consent was obtained from all participants prior to enrolment in the study.

Clinical characteristics

For the patients with an ICA occlusion we recorded the side of the occlusion and whether the ICA occlusion had been symptomatic, which we defined as an ipsilateral transient ischemic attack (TIA) or ischemic stroke. This was based on medical records. Possible occlusions and stenosis in other cervical arteries (i.e. left and right internal carotid arteries and left and right vertebral arteries) were recorded from medical records as well (i.e. duplex ultrasound, computed tomography angiography and/or magnetic resonance angiography).

Demographics and vascular risk factors were recorded for both patients and reference participants. Hypertension was based on presence in medical history. Current office hypertension was defined as a mean systolic tension >140mm/Hg or diastolic tension >90mm/Hg measured during the research day. Hypercholesterolemia was defined as presence in medical history or medication use. Diabetes mellitus was based on medical history. Smoking was defined as current or previous smoking and obesity was defined as a body mass index over 30.

All participants underwent a standardized neuropsychological test battery. Raw test-scores were corrected for age, sex and education relative to the reference group and a mean composite cognitive z-score was calculated for every patient with an ICA occlusion (for details [6]).

MRI protocol and analysis

Brain MRI was acquired at 3T Philips Ingenia, Philips Achieva and Philips Gemini MRI scanners (Philips, Best, the Netherlands). The brain protocol included a 3D T1-weighted image (TR/TE/TI=8.2/4.5/990 ms; shot interval 3000 ms; flip angle 8°; voxel size 1.0x1.0x1.0 mm^3), fluid-attenuated inversion recovery (FLAIR) image (TR/TE/TI=4800/313/1650 ms; TSE factor 182; voxel size 1.11x1.11x1.11 mm^3), susceptibility-weighted image (SWI) (TR/TE=45/31 ms; flip angle 13°; EPI factor 3; voxel size 0.8x0.8x1.6 mm^3) and phase-contrast flow measurement (TR/TE=12/8.2 ms; flip angle 10°; Venc 200 cm/s; untriggered; 10 averages; voxel size 1.17x1.17x5 mm^3).

CMI were rated according to previously established criteria for 3T MRI [2]. In short, CMI had to be hypointense lesions on T1-weighted MRI with a corresponding hyper- or isointense signal on FLAIR and SWI, <4 mm in diameter, and located strictly intracortical. CMI had to be distinct from perivascular spaces and visible in at least

two planes (i.e. sagittal, transversal, coronal). Lesions neighboring a larger stroke (i.e. <1 cm in the same gyrus) were excluded [2]. CMI were rated by three experienced raters (HvdB, DF, RvdB) with the use of an in-house developed tool in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany). Raters were blind to the clinical condition of the participants, although raters could not be blinded for larger infarcts on the T1-weighted and FLAIR images. Interrater agreement was good (interclass correlation = 0.97 in a random subset of 31 scans).

Infarcts, microbleeds, enlarged perivascular spaces and white matter hyperintensities (WMHs) were rated by a neuroradiologist (JdB) according to the STRIVE-criteria [7]. Small vessel disease (SVD) presence was then summed in a previously developed SVD score for all patients [8]. Total brain volume, grey matter volume and WMH volume were segmented with an automated pipeline (Quantib brain, Rotterdam, the Netherlands), taking into account manually segmented infarcts and other pathologies. Blood flow in the basilar artery and ICAs was quantified (ml/min) based on phase-contrast flow scans. The arteries were manually contoured using the flow analysis tool of Mass software (Division of Image Processing, Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands). We then corrected cerebral blood flow for brain volume (ml/min/100ml) by dividing blood flow by the individual's brain volume and multiplying the result by 100.

Statistics

Differences in baseline characteristics between patients and the reference group were assessed with an independent t-test for age, $\chi 2$ for sex and vascular risk factors, and Mann-Whitney U test for education. Logistic regression was used to determine whether an ICA occlusion (versus reference group) was associated with CMI presence, also when controlling for differences in baseline characteristics between the groups. Total numbers of CMI for patients and the reference group where compared with a Mann-Whitney U test.

Further analyses were only carried out in the group of patients with an ICA occlusion. Differences between patients with and without CMI were assessed with an independent t-test for age and cognition, $\chi 2$ for sex, and Mann-Whitney U test for education. The relations of risk factors and MRI markers with CMI presence were analyzed with logistic regression for continuous data and Mann-Whitney U test or regression for continuous

data. Brain volumetrics were corrected for age, sex and intracranial volume and white matter hyperintensity volume was log transformed additionally.

Analyses were carried out in SPSS Statistics version 25 with the significance threshold set at p<.05.

RESULTS

The characteristics of patients with an ICA occlusion and participants from the reference group are shown in Supplemental table 1. Patients with an ICA occlusion were of similar age as the reference group, but more often male, and lower educated. As expected, the burden of vascular risk factors was higher among patients (Supplemental table 1).

Table 1: Demographics and vascular risk factors for patients with and without CMIs

	ICA occlusion CMI present (n=51)	ICA occlusion CMI absent (n=44)	OR [95% CI]	*p*-value
Characteristics				
Age (y)	67.1 ± 8.4	65.1 ± 8.3	-	.238
Sex (females)	11 (22)	10 (22)	-	.892
Education (7 levels)	5 [4-6]	5 [5-6]	-	.166
Cognitive function[a]	-0.51 ± 0.69	-0.52 ± 0.58	-	.943
Symptomatic occlusion	42 (82)	39 (89)	0.6 [0.2; 1.9]	.392
Vascular risk factors				
Hypertension (medical history)	45 (88)	28 (64)	4.3 [1.5; 12.2]	**.007**
Hypertension (office hypertension)	31 (61)	33 (75)	0.5 [0.2; 1.3]	.143
Hypercholesterolemia	50 (98)	37 (84)	9.5 [1.1; 80.2]	**.039**
Diabetes	18 (35)	10 (23)	1.9 [0.7; 4.6]	.183
Smoking	49 (96)	40 (91)	2.5 [0.4; 14.1]	.315
Obesity	16 (31)	8 (18)	2.1 [0.8; 5.4]	.144

Abbreviations: ICA= internal carotid artery; CMI= cortical microinfarct
Data are presented as mean ± SD, median [25th-75th percentile], or number (percentage).
[a] Cognitive function is measured as a composite z-score, corrected for age, sex, and education.

CMI were present in 54% of the patients with an ICA occlusion, compared to 6% in the reference group (crude OR 17.0; 95% CI 7.5-38.6; p<.001; corrected for sex and education OR 14.3; 95% CI 6.2-33.1; p<.001; additional correction for vascular risk factors OR 6.9; 95% CI 2.4-20.0; p<.001). Among the participants with CMI, 69% of the patients and

25% of the reference participants had multiple CMI (range 2-33 and 2-7 respectively). The total number of CMI per participant was significantly higher in patients than in the reference group (p<.001). The characteristics of patients with CMI compared with patients without CMI are shown in Table 1. Only hypertension and hypercholesterolemia significantly predicted CMI presence in patients.

In patients with an ICA occlusion, the number of CMI was significantly higher in the hemisphere ipsilateral to the occlusion (median 2) than in the contralateral hemisphere (median 0) (p<.001). Figure 1 shows the 3D distribution of CMI relative to an ICA occlusion. CMI were preferentially located in the cranial part of the frontal and parietal lobes. The distribution of CMI in the ipsilateral hemisphere was not different from that in the contralateral hemisphere.

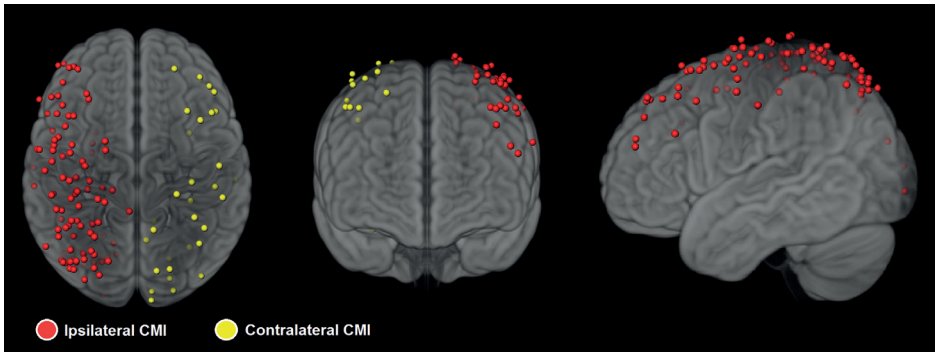


Figure 1: 3D distribution of all CMI in 40 patients with a unilateral ICA occlusion and CMI. For patients with a right ICA occlusion the CMI distribution was flipped to create this image where CMI are presented ipsilateral (red) and contralateral (yellow) to the occlusion. It shows a higher burden in the ipsilateral hemisphere, but no different distribution between hemispheres.

Regarding the severity of the cervical atherosclerotic disease, Figure 2 shows that a higher number of occluded or >50% stenosed cervical arteries (i.e. left and right carotid arteries, and left and right vertebral arteries) was associated with CMI presence (p=.038). In patients with a unilateral ICA occlusion, combined cerebral blood flow tended to be lower in patients with CMI compared to patients without CMI (see figure 3, B -6.2 ml/min/100ml; 95% CI -12.4-0.02; p=.05). This was mostly attributable to lower flow in the contralateral ICA (B -57.7 ml/min; 95% CI -115.1-0.4; p=.048), rather than the basilar artery (B -16.0 ml/min; 95% CI -56.4-24.3; p=.43).

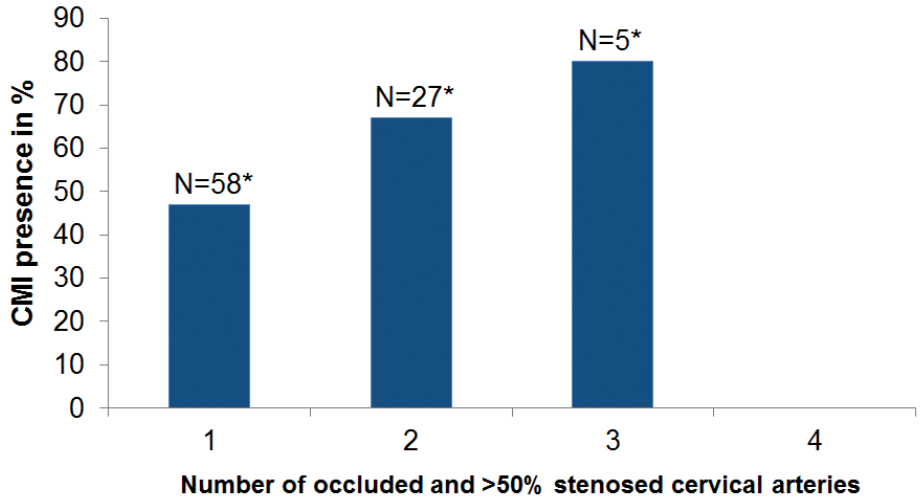


Figure 2: CMI presence in patients with 1-4 occluded or >50% stenosed cervical arteries (i.e. left and right internal carotid arteries and left and right vertebral arteries). None of the patients had occlusions or >50% stenosis in all 4 cervical arteries. *Number of patients with 1, 2 or 3 occluded or >50% stenosed cervical arteries. N=90 because of 5 missings.

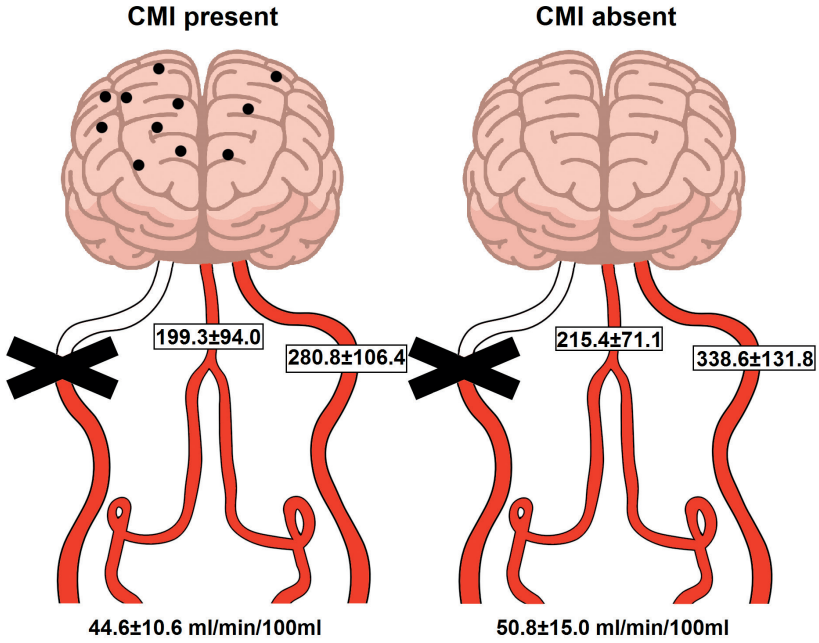


Figure 3: Blood flow (mean ± SD, ml/min/100ml) to the brain through the non-occluded ICA and basilar artery. N=69 (CMI present N=36, CMI absent N=33) because of 11 missings and because 15 patients were excluded from this analysis due to a double-sided ICA occlusion.

Table 2 shows the relations between CMI presence in patients with an ICA occlusion and other MRI-markers of cerebral small vessel and large vessel disease. Lacunar infarcts and microbleeds tended to be more common among patients with CMI, but overall there were no significant associations between CMI presence and any of the other MRI markers. Also, there was no correlation between large artery infarcts or lacunar infarcts ipsilateral to the ICA occlusion and ipsilateral CMI (τb = .13; p=.30).

Table 2: Cerebral MRI-markers in patients with and without CMIs

	ICA occlusion CMI present (n=49)	ICA occlusion CMI absent (n=37)		*p*-value
Brain volumetric			**B [95% CI]**	
Total intracranial volume (ml)	1417.2 ± 141.6	1405.9 ± 123.2	7.9 [-45.8; 61.6]	.771
Total brain volume (ml)[a]	1076.8 ± 110.0	1087.5 ± 114.0	-16.9 [-35.5; 1.7]	.074
Total grey matter volume (ml)[a]	641.4 ± 62.7	640.7 ± 66.5	-1.9 [-14.7; 11.1]	.776
CeVD markers			**OR [95% CI]**	
Large artery infarct	37 (76)	26 (70)	1.3 [0.5; 3.4]	.59
Lacunar infarct	32 (65)	17 (46)	2.2 [0.9; 5.3]	.075
Microbleeds	14 (29)	6 (16)	2.1 [0.7; 6.0]	.184
			B [95% CI]	
WMH volume (ml)[ab]	0.69 [0.3-1.0]	0.67 [0.3-1.5]	-0.1[-0.4.; 0.2]	.395
SVD score	1 [1-2]	1 [0-2]	-	.143

Abbreviations: ICA= internal carotid artery; CMI= cortical microinfarct; CeVD= cerebrovascular disease; WMH= white matter hyperintensity; SVD score= Small vessel disease score, ranging between 0 (no SVD) to 4 (severe SVD).
Data are presented as mean ± SD, median [25th-75th percentile], or number (percentage).
[a] Brain volume analyses corrected for age, sex and intracranial volume; [b]WMH was log transformed.
Brain volumetrics and CeVD markers were not yet available for 9 patients.

DISCUSSION

CMI are very common in patients with an ICA occlusion and occur more frequently in the hemisphere ipsilateral to the occlusion. The severity of collateral compromise, in terms of number of affected vessels and blood flow, is related to CMI occurrence, suggesting that hemodynamics indeed play a role in CMI etiology.

To our knowledge this is the first study to examine CMI in patients with a complete ICA occlusion. CMI burden in terms of occurrence and total number is much higher in

the patients (54%) than in our reference group and control participants from previous studies (6-12%) [9–11]. Earlier studies in patients with ICA stenosis also reported high CMI prevalence. Specifically, CMI prevalence was 26% in a 3T MRI study in patients with a >30% ICA stenosis [12], and 67% in a 7T MRI study in patients with a >50% ICA stenosis [13]. Of note, the prevalence of this last study is not readily comparable due to higher sensitivity of CMI detection on 7T MRI [3].

As indicated, we approached patients with an ICA occlusion as a model condition for hemodynamic compromise. Accordingly, we hypothesized that CMI would be more common in patients with an ICA occlusion than in the reference group, more common in the hemisphere ipsilateral than contralateral to the occlusion, and that the severity of collateral compromise (i.e. worse collateral steno-occlusive disease and worse collateral blood supply) would relate with CMI presence. All hypotheses were met, which seems to support the notion that worse hemodynamics indeed contribute to CMI occurrence. Yet, alternative explanations need to be considered in our patient population with high vascular burden. First of all, vascular risk factors could cause CMI, since we found a relation of CMI presence with hypertension and hypercholesterolemia. However, vascular risk factors cannot explain all CMI occurrence in our cohort, particularly not the differences between CMI occurrence in the hemisphere ipsilateral versus contralateral to the occlusion. Second, patients with an ICA occlusion are strongly affected by large vessel disease which could give rise to microemboli through different mechanisms. Microemboli could for example have originated from large thrombi that caused ischemic stroke. Of note, we excluded CMI that were located in tissue directly adjacent to large artery infarcts. Although we did not find that CMI occurred more often in patients that had been symptomatic (versus asymptomatic) and we did not find a relation of CMI with large artery infarcts on MRI, microemboli could also have originated from pre-occlusive stenosis in earlier disease stages. Indeed it has previously been reported that vulnerable ICA plaques relate with CMI. This poses a limitation that we currently cannot correct for, but it should be noted that this mechanism cannot explain that *current* cerebral blood flow tended to be lower in patients with CMI compared to patients without CMI. We encourage future research to study acute CMI on diffusion imaging in clinically stable patients with an ICA occlusion. If acute CMI are observed in the hemisphere of the occluded ICA, this would provide further support for hemodynamics as cause of CMI, because ipsilateral microemboli from large artery disease are not expected in stable disease stages in patients with a complete occlusion [14]. The causal pathway could be direct, with temporary hypoperfusion directly causing small ischemia in the most

distal and smallest vessels, but could for example also be through embolisms that result from washout disturbances in insufficiently perfused arteries [15]. We did not observe that CMI primarily occurred in watershed areas which may be considered an argument against a purely hemodynamic etiology. However, hypoperfusion in the small branches of the main cerebral arteries may still be responsible for the occurrence of CMI.

The findings of this study may be of potential clinical relevance to patients with an ICA occlusion, or patients that are in other ways vulnerable to hemodynamic compromise. In other settings, acute CMI have been associated with increased risk of poor clinical outcome after two years [16], and with (vascular) cognitive impairment [2]. Since patients with an ICA occlusion are known to be at risk of cognitive impairment [17], the prognostic relevance of CMI in this setting should be topic of future research. Moreover, CMI might be a marker of future stroke risk, even in patients with apparently stable ICA occlusion, but this should be explored in longitudinal studies.

An important strength of this study is the relatively large sample size of patients with an ICA occlusion that were in a stable disease phase. Also, due to the high CMI occurrence in the cohort, we had enough power to investigate the relation of CMI with vascular risk factors and measures of collateral compromise. As yet mentioned, a limitation of our study is that we cannot exclude microemboli from large vessel disease as possible partly cause of CMI in our cohort. We therefore encourage future research to study acute CMI in patients with a clinically stable ICA occlusion. Another limitation of our study is that we could only study the largest CMI with 3T MRI. Yet, the smallest CMI might have a different distribution and pathophysiology wherefore our findings cannot be generalized to CMI detected on 7T MRI or brain autopsy.

Conclusion

In this study we explored hypoperfusion as possible cause for CMI, in patients with an ICA occlusion as a model condition of hemodynamic compromise. We found that CMI are very common in patients with an ICA occlusion, even more so in the hemisphere of the occluded ICA. Moreover, CMI presence related with the severity of collateral compromise. These findings support a role for hemodynamics in the pathophysiology of CMI.

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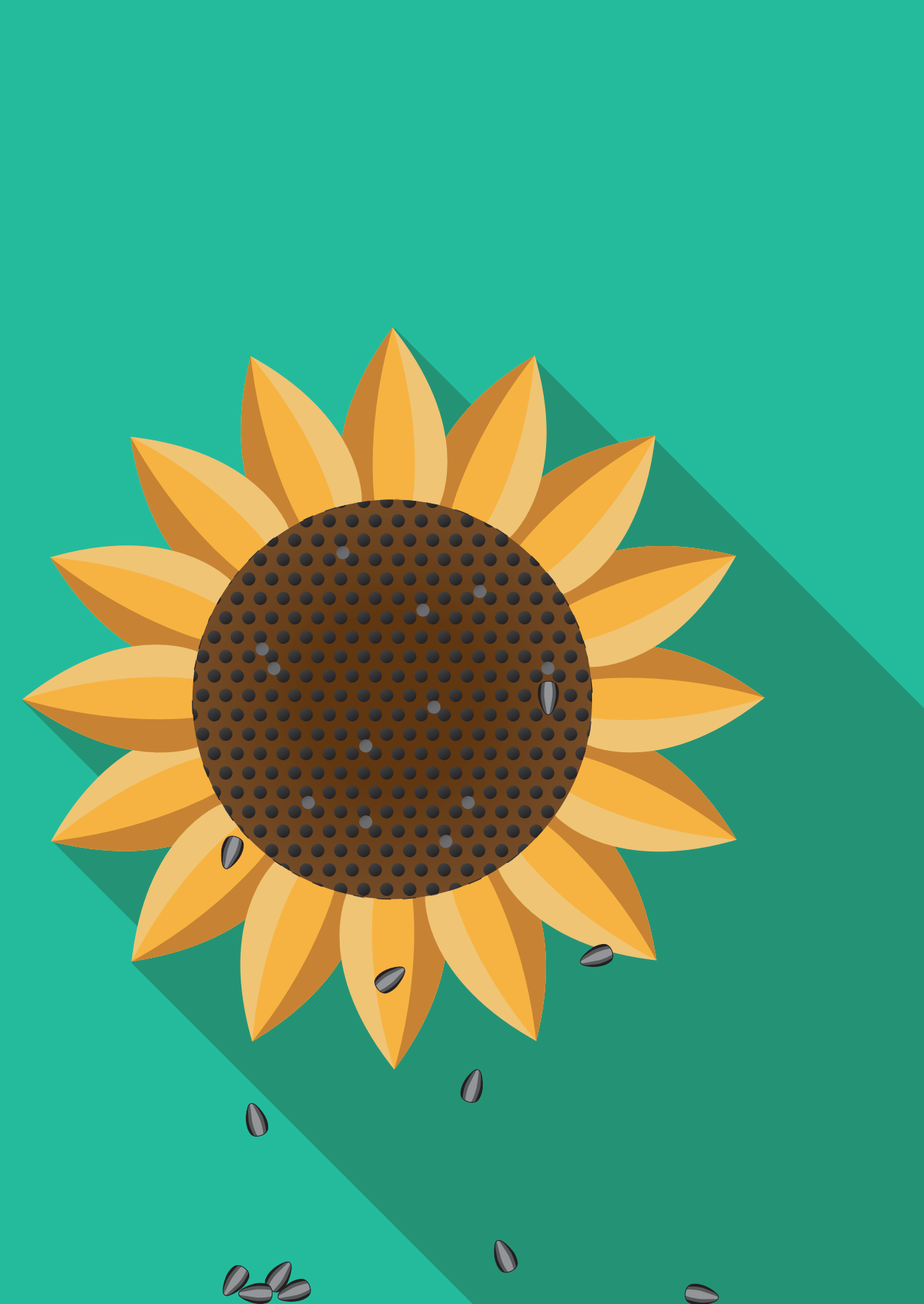
SUPPLEMENT

Supplemental table 1: Demographics and vascular risk factors of patients and controls

	ICA occlusion (n=95)	Reference (n=125)	*p*-value
Demographics			
Age (y)	66.2 ± 8.3	65.5 ± 7.4	.552
Sex (female)	21 (22)	59 (47)	**<.001**
Education (7 levels)	5 [4-6]	6 [5-6]	**.010**
Vascular risk factors			
Hypertension (medical history)	73 (77)	34 (27)	**<.001**
Hypertension (office hypertension) [a]	64 (67)	61 (49)	**.006**
Hypercholesterolemia	87 (92)	38 (30)	**<.001**
Diabetes	28 (30)	2 (2)	**<.001**
Smoking	89 (94)	70 (56)	**<.001**
Obesity	24 (25)	19 (15)	.062

Abbreviations: ICA= internal carotid artery. Data are presented as mean ± SD, median [25th-75th percentile], or number (percentage).
[a] Office hypertension: systolic tension >140mmg/Hg or diastolic tension >90mm/Hg.



CHAPTER 5

CEREBRAL CORTICAL MICROINFARCTS: A NOVEL MRI-MARKER OF VASCULAR BRAIN INJURY IN PATIENTS WITH HEART FAILURE

D.A. Ferro
H. van den Brink
R. P. Amier
M.A. van Buchem
J. de Bresser
E.E. Bron
H.P. Brunner-La Rocca
A.M. Hooghiemstra
N.G.H.M. Marcks
A.C. van Rossum
& G.J. Biessels
On behalf of the Heart-Brain Connection Consortium.

Submitted for publication

ABSTRACT

Background: Patients with heart failure (HF) are at risk for vascular brain injury. Cerebral cortical microinfarcts (CMIs) are a novel MRI marker of vascular brain injury. This study aims to determine the occurrence of CMIs in patient with HF and their clinical correlates, including haemodynamic status.

Methods: From the Heart-Brain Study, a multicenter prospective cohort study, 154 patients with clinically stable HF without concurrent atrial fibrillation (mean age 69.5±10.1, 32% female) and 124 reference participants without HF (mean age 65.6±7.4, 47% females) were evaluated for CMIs on 3T MRI. CMI presence in HF was tested for associations with vascular risk profile, cardiac function and history, MRI markers of vascular brain injury and cognitive profile.

Results: CMI occurrence was higher in patient with HF (17%) than reference participants (7%); after correction for age and sex OR 2.5 [95% CI 1.1–6.0] p=.032; after additional correction for vascular risk factors OR 2.7 [1.0–7.1] p=.052. In patients with HF, CMI presence was associated with office hypertension (OR 2.7 [1.2–6.5] p=.021) and a lower cardiac index (B=-0.29 [-.55–-.04] p=.023 independent of vascular risk factors), but not with cause or duration of HF. Presence of CMIs was not associated with cognitive performance in patients with HF.

Conclusions: CMIs are a common occurrence in patients with HF and related to an adverse vascular risk factor profile and severity of cardiac dysfunction. CMIs thus represent a novel marker of vascular brain injury in these patients.

INTRODUCTION

Heart failure (HF) is associated with an increased risk for vascular brain injury [1]. Patients with HF are reported to have a 2- to 3-fold increased risk of symptomatic stroke [2,3], while clinically "silent" vascular brain lesions – increasingly observed on MRI – are even more prevalent [4,5].

Recently, cerebral cortical microinfarcts (CMIs) have attracted attention as a novel marker of vascular brain injury. CMIs are small ischemic lesions detectable on neuropathological evaluation and MRI [6]. They are associated with vascular risk factors and manifestations of cerebral small vessel disease and thromboembolic stroke [6]. CMIs have shown to predict accelerated cognitive decline in memory clinic [7] and stroke patients [8] emphasizing their potential clinical value.

The occurrence of CMIs in patients with HF has not yet been explored. Yet, compromised cerebral hemodynamics and hypoperfusion - both known symptoms of HF- are confirmed risk factor for CMIs [9]. Moreover, CMIs were shown to relate to biomarkers of (sub)clinical cardiac disease in memory clinic patients [10].

Therefore this study investigated the occurrence of CMIs in patients with HF compared to reference participants without HF. Among patients with HF, we explored the relation between CMIs presence and vascular risk profile, measures of cardiac functions and cardiac history, MRI markers of vascular brain injury and cognitive performance.

METHODS

Population

The Heart-Brain Study (HBS) is a multicenter prospective cohort study that recruited patients with HF and reference participants between May 2014 and December 2017 through four academic medical centers (UMCs) in the Netherlands: Leiden UMC (LUMC), Maastricht UMC (MUMC), Utrecht UMC (UMCU) and VU UMC (VUMC) [11]. HF patients and reference participants were eligible to participate if they were 50 years or older, able to undergo cognitive testing and independent in daily life (operationalized as being capable to come to the hospital and undergo the study protocol, including the MRI). Additional enrollment criteria for patients with HF was a HF diagnosis according to the European cardiology society (ESC) guidelines [12], including (1) typical symptoms (breathlessness at rest or on exercise, fatigue, tiredness, ankle swellings), signs (tachycardia, tachypnea, pulmonary rales, pleural effusion, raised jugular venous pressure, peripheral edema, hepatomegaly) and (2) objective evidence of a structural or functional abnormality of the heart at rest on routine echocardiography. Moreover, patients with HF had to be clinically stable for at least 6 months in order to participate. Additional enrollment criterion for reference participants was the absence of a diagnosis of heart failure (based on medical history). Exclusion criteria for both HF patients and reference participants were (1) current atrial fibrillation, (2) current premature ventricular contractions (PVCs) exceeding 10% of total number of heartbeats, (3) a life-threatening disease other than HF with life-expectancy less than three years, (4) clinical evidence of a neurodegenerative disease other than vascular cognitive impairment of Alzheimer's disease (such as frontotemporal dementia, Lewy Body disease, or hypokinetic rigid syndrome), (5) other neurological or psychiatric diagnosis that affects cognitive performance or testing, such as severe traumatic brain injury or substance abuse. HF patients were recruited from cardiology outpatient clinics from the LUMC, MUMC, VUMC and general practices in the region of South Holland, the Netherlands. The reference participants were selected by active recruitment among spouses of patients and through advertising leaflets in the hospital and by advertisements in local newspapers through the UMCU VUMC, LUMC and MUMC.

This study was approved by the Medical Ethics Review Committee of the LUMC and local boards of the participating UMCs. The study was performed in accordance with the declaration of Helsinki and the Medical Research Involving Human Subjects Act

(WMO). Written informed consent was obtained from all participants prior to research related procedures.

For both HF patients and reference participants a core clinical dataset was collected, including vascular risk factors, detailed neurologic, cardiac and medical history including medication use. In addition, all subjects attended an examination day that included neuropsychological tests, cardiac and brain MRI, and blood samples.

For the current study we included all HF patients and reference participants who successfully underwent brain MRI. Of the 162 HF patients and 128 reference participants, 2 HF patients were excluded due to poor scan quality, and 6 HF patients and 3 reference participants due to lacking of MRI sequences. In addition, 1 reference participant appeared to meet the ESC criteria of HF after inclusion and was excluded from the current analysis. This resulted in a study population of 154 HF patients and 124 reference participants.

Vascular risk factors

The following vascular risk factors were recorded for both HF patients and reference participants. Hypertension was defined as presence in the medical history. Current office hypertension was defined as a mean systolic tension >140mg/Hg or diastolic tension >90mm/Hg measured on the research day. Hypercholesterolemia was defined as presence in the medical history or use of cholesterol lowering medication. Diabetes was defined as presence in the medical history. Body surface area (BSA) was calculated in m^2 according to the following formula: $BSA = 0.007184 \times W^{0.425} \times H^{0.725}$, in which W refers to the weight in kg and H to the height in cm [13]. Obesity was defined as a body mass index over 30. Smoking was defined as current or previous smoking. Vascular claudication was defined as presence in the medical history. History of stroke was defined as previous clinical ischemic of hemorrhagic stroke. For patients with HF use of antiplatelet, direct or oral anticoagulant medication was recorded.

MRI protocol

Cardiac and brain MRI were acquired at 3T on Ingenia or Achieva scanners (Philips, Best, the Netherlands). The cardiac protocol included short-axis multislice cine steady-state free precision (TR 3.1 ms; TE 1.55; flip angle 45°; 40 heart phases; 67 phase percentage; breath-hold; number of slices dependent on size of LV (range 12–16 slices), resolution 1.5 X 1.6 X 8.0 mm^3). The brain MRI protocol included 3D T1-weighted images

(TR 8.2 ms; TE 4.5 ms; shot interval 3,000 ms; flip angle 8°; inversion delay 990 ms, resolution 1.0 X 1.0 X 1.0 mm^3), fluid-attenuated inversion recovery (FLAIR) images (TR 4,800 ms; TE 313 ms; TI 1,650 ms; TSE factor 182, resolution 1.11x1.11x1.11 mm^3) and susceptibility-weighted imaging (SWI) (3D gradient echo; TR 45 ms; TE 31 ms; flip angle 13°; EPI factor 3, resolution 0.8 X 0.8 X 1.6 mm^3). ASL scans were acquired using a pseudo-continuous arterial spin labeling (pCASL) sequence with a label duration of 1.800 ms and a postlabeling delay of 1.800 ms (resolution 3 x 3 x 7mm^3).

Cardiac parameters

For both HF and reference participants the following parameters were derived from cardiac MRI using a semi-automatic contour detection with manual correction by an experienced reader: Left ventricular-end diastolic volume (LV-EDV) and left ventricular end-systolic volume (LV-ESV) in L. Left ventricular stroke volume (LV-SV) in L was calculated according to the formula: *LV-EDV– LV-EDV*. Cardiac output was calculated in L/min according to the formula: *LV-SV x heart rate* (heart rate was derived from the DICOM header) and cardiac index was expressed in L/min/m^2 according to the formula: *LV-SV x pulse rate / BSA*. Left ventricular ejection fraction (LVEF) was calculated according to the formula: *LV-SV/ LV-EDV x 100%*. LVEF was used to categorize patients with HF according to the ESC guidelines [12] into HF- reduced ejection fraction (LVEF <40%), HF- mid-range ejection fraction (LVEF 40-49%) and HF-preserved ejection fraction (LVEF >50%).

For both HF patients and reference participants a history of paroxysmal AF, myocardial infarction, percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) was recorded. Additionally, for HF patients, cause and duration of heart failure, presence of valvular heart disease and cardiac arrhythmias were recorded.

Cerebral MRI-markers including CMIs

The following cerebral MRI-markers were rated by an experienced neuroradiologist according to the STRIVE-criteria [14]: Microbleeds, infarcts (cortical and non-lacunar subcortical infarcts; lacunar infarcts), enlarged perivascular spaces and white matter hyperintensities (WMHs) according to the Fazekas scale [15]. Cerebral small vessel disease score was constructed for each patient according to a previously developed scale [16]. Brain volumetrics, including gray matter, white matter and WMH volume were calculated using an automated pipeline (Quantib brain, Rotterdam, the Netherlands) after manual segmentation of infarcts and other pathologies. Cerebral blood flow (CBF)

of the normal appearing gray matter (GM) was quantified in mL/100 mg/min. Post-processing included motion-correction and partial volume correction [17].

CMIs were rated by visual inspection according to established criteria on 3T MRI [6]. In short, CMIs had to be less than 4 mm, strictly intracortical and hypointense on T1-weighted imaging, cavitated, hyperintense or isointense on FLAIR and iso- or hyperintense on SWI. CMIs had to be visible in at least two planes (eg, sagittal, transversal, coronal) and distinct from enlarged perivascular spaces, arterioles, venules and microbleeds. Lesions neighboring a larger infarct (i.e., <1cm in the same gyrus) were disregarded [6,18]. Ratings were performed in an in-house developed tool in MeVisLab (MeVis medical solutions, Bremen, Germany) by three experienced raters, blinded to the clinical condition of the subjects. Interrater agreement was very good (interclass correlation 0.97 in a subset of 31 scans).

Blood samples

Blood samples were drawn to determine hemoglobin (mmol/L), hematocrit (L/L) and C-reactive protein (CRP) mg/L using standard methods.

Cognitive testing

The educational level was rated according to the 7-point Verhage criteria [19]. Subjects underwent standardized neuropsychological testing, including a Dutch version of the mini-mental state examination (MMSE) [20] and a extensive neuropsychological test battery covering four cognitive domains including memory, language, attention/psychomotor speed and executive functioning [11]. Neuropsychological tests used per cognitive domain are documented in appendix 1. A composite z-score of all cognitive domains was created, corrected for age, sex and education and with reference participants as reference. Additionally, patients were classified as 1) cognitively normal (no cognitive domains impaired), 2) minor cognitive impairment (one domain impaired) or 3) major cognitive impairment (more than one domain impaired). Of note, a cognitive domain was considered impaired when the score was –1.5 SD below the mean age, sex and education-adjusted mean z-score.

Statistical analysis

Differences between HF patients and reference participants, and HF patients with and without CMIs were compared with linear regression (for normally distributed data), logistic regression (for proportions) and Mann-Whitney U tests (for non-parametric

data). Occurrence rate of CMI between HF and reference participants was subsequently corrected for age, sex and vascular risk factors in a logistic regression model. Brain volumetrics were corrected for age, sex and intracranial volume. For the cognitive tests results were standardized as age, sex and education-adjusted z-scores. An exploratory mediation analysis was performed using the PROCESS v3.3 macro (http://processmacro.org/index.html) [21] in SPSS to test the mediation effect of CBF in the relationship between cardiac index and CMI presence with 5000 bootstrapping samples and 95% confidence interval (CI). We also performed exploratory analyses on reference participants with and without CMIs using linear and logistic regression. All statistical analyses were carried out using IBM SPSS v 25 and a p-value <0.05 was considered statistically significant.

The data that support the findings of this study are available from the corresponding author upon reasonable request, within the privacy legislation of the Netherlands and after permission of the Heart-Brain Connection (HBC) steering committee.

RESULTS

Baseline characteristics and occurrence of CMIs in patients with HF and reference participants

Patients with HF were older, proportionally less often female and had more vascular risk factors than reference participants (all except current office hypertension, $p<.05$, Table 1). Of the 154 patients with HF, 26 (17%) presented with at least one 1 CMI of whom 11 with multiple CMIs (max 5 CMIs per person). Of the 124 reference participants, 8 (7%) presented with at least 1 CMIs of whom 2 with multiple CMIs (max 7 CMIs per person). CMI occurrence was higher in patients with HF than reference participants after correction for age and sex (OR 2.5; 95% CI 1.1–6.0; $p=.032$; Model 1; Table 2) and after additional correction for vascular risk factors (OR 2.7 [1.0–7.1]; $p=.052$; Model 4; Table 2). Additionally, the total number of CMIs per person was also higher in HF patients (median 1, interquartile range [1–3] than reference participants (median 1, interquartile range [1–1.5], $p=.008$).

Table 1: Baseline characteristics and CMI presence in patients in HF and references

	HF patients (n=154)	References (n=124)	*p*
Baseline characteristics			
Age (y)	69.5 ±10.1	65.6 ±7.4	.001
Sex (female)	49 (32%)	58 (47%)	.011
Education (7 levels)	5 [4-6]	6 [5-6]	.007
Vascular risk factors			
Hypertension (medical history)	79 (52%)	30 (24%)	.000
Current office hypertension [a]	57 (37%)	60 (48%)	.062
Hypercholesterolemia	100 (66%)	37 (30%)	.000
Diabetes	27 (18%)	2 (2%)	.000
Smoking	107 (70%)	69 (56%)	.014
Obesity	37 (24%)	18 (15%)	.042

Abbreviation: HF= Heart failure; [a] Current office hypertension: systolic tension >140mg/Hg or diastolic tension >90mm/Hg. Data are presented as mean ± SD, median (25th-75th percentile), or number (percentage).
One missing in HF group for education and vascular risk factors, except hypertension (history) and obesity (2 missing).

Table 2: Odds ratio of CMI presence in patients with HF compared to reference participants adjusted for covariates

	OR of CMI presence [95 % CI] in HF patients compared to references	*p*
Model 1: Unadjusted	2.9 [1.3–6.8]	.011
Model 2: Adjusted for age & sex	2.5 [1.1–6.0]	.032
Model 3: Adjusted for age, sex and individual vascular risk factors		
Adjusted education	2.7 [1.1–6.4]	.024
Adjusted hypertension (medical history)	2.4 [1.0–5.8]	.047
Adjusted current office hypertension [a]	2.7 [1.2–5.9]	.012
Adjusted hypercholesterolemia	2.2 [0.9–5.4]	.075
Adjusted diabetes	2.3 [1.0–5.5]	.062
Adjusted current or previous smoking	2.5 [1.1–5.9]	.035
Adjusted obesity	2.3 [1.0–5.5]	.056
Model 4: Adjusted all vascular risk factors combined	2.7 [1.0–7.1]	.052

CMI= cerebral cortical microinfarct; HF= Heart failure; [a] Current office hypertension: systolic tension >140mg/Hg or diastolic tension >90mm/Hg. Data presented as the odds ratio with 95% confidence interval of CMI presence in patients with heart failure compared to reference participants, both unadjusted (model 1), adjusted for age and sex (model 2) and adjusted for individual vascular risk factors (mode 3) and combined vascular risk factors (model 4).

Clinical correlates of CMIs presence in patients with HF

Among the patients with HF, presence of CMIs was not associated with age, sex, use of antiplatelet medication or (direct) anticoagulants, or vascular risk factors, expect for current office hypertension (Table 3). Regarding cardiac function, CMI presence was associated with an 11% lower cardiac index and a 10% lower in pulse rate (both $p<.05$ after correction for age, sex and vascular risk factors), while a trend was observed for an 8% lower cardiac output (after correction for age, sex and vascular risk factors $p=.080$). No relationship was observed between presence of CMIs and LVEF. HF patients with CMIs tended to more often have a medical history of PCI compared to HF patients without CMIs ($p=.058$).

Table 3: Demographics, vascular risk factors, cardiac function and history in HF patients with and without CMIs

	HF CMI present (n=26)	HF CMI absent (n=128)	Odds ratio [95% CI]	*p*
Baseline characteristics				
Age (y)	71.3 ± 8.4	69.1 ± 10.3		.306
Sex (females)	7 (27%)	42 (33%)	0.8 [0.3–1.9]	.557
Body surface area (m^2)	2.0 ± 0.2	1.9 ± 0.2		.312
Education (7 levels)	5 [4-6.5]	5 [4-6]		.204
Vascular risk factors				
Hypertension (history)	16 (62%)	63 (50%)	1.6 [0.7–3.8]	.286
Current office hypertension	15 (58%)	42 (33%)	2.7 [1.2–6.5]	**.021**
Hypercholesterolemia	20 (77%)	80 (63%)	2.0 [0.7–5.2]	.179
Diabetes	6 (23%)	21 (17%)	1.5 [0.5–4.2]	.428
Smoking	20 (77%)	87 (69%)	1.5 [0.6–1.1]	.396
Obesity	8 (31%)	29 (23%)	1.5 [0.6–3.8]	.404
Vascular claudication	2 (8%)	6 (5%)	1.7 [0.3–8.9]	.540
History of stroke	3 (12%)	5 (4%)	3.2 [0.7–4.3]	.530
Medication				
Antiplatelet	17 (65%)	64 (50%)	1.8 [0.8–4.5]	.156
(Direct) oral anticoagulants	7 (27%)	41 (32%)	0.8 [0.3–2.0]	.609
Cardiac function				
Cause of HF [a]			1.4 [0.6–3.5]	.374
Non-ischemic	8 (31%)	54 (42%)		
Ischemic	16 (62%)	66 (52%)		
Unknown	2 (7%)	7 (6%)		
Duration of HF [b]			1.6 [0.7–3.8]	.273
≤5 years	12 (46%)	73 (58%)		
>5 years	14 (54%)	53 (42%)		
ESC HF criteria (n=147)				

HF-reduced EF	13 (54%)	50 (41%)	1.7 [0.7–4.2]	.224
HF-mid-range EF	9 (38%)	47 (38%)	1.0 [0.4–2.4]	.948
HF preserved EF	2 (8%)	26 (21%)	0.4 [0.1–1.6]	.161
			B [95% CI]	
LVEF (%) (n=147)	40.5 ± 7.1	43.0 ± 10.0	-2.5 [-6.7–1.8]	.253
Cardiac index (L/min/m^2)	2.49 ±.46	2.79 ±.59	-.29 [-.55–-.04]	**.023**
Cardiac output (L/min)	4.95 ± 1.22	5.40 ±1.23	-.45 [-.9 –.10]	*.105*
Pulse rate (beats/min)	58.5 ± 8.8	64.9 ± 12.6	-6.4 [-11.7–-1.1]	**.018**
Cardiac history			**OR [95% CI]**	
Myocardial infarction	16 (64%)	60 (48%)	2.0 [0.8–4.8]	.139
Valvular heart disease	2 (8%)	11 (9%)	0.9 [0.2–4.2]	.872
Cardiac arrythmia	8 (32%)	32 (26%)	1.4 [0.5–3.5]	.510
Paroxysmal AF	4 (16%)	19 (15%)	1.1 [0.3–3.4]	.919
PCI	13 (50%)	38 (30%)	2.3 [1.0–5.4]	*.058*
CABG	5 (19%)	25 (20%)	1.0 [0.3–2.8]	.958

Abbreviation: HF= Heart failure; CMI= cortical microinfarct; ESC HF criteria=European society of cardiology Heart failure criteria; HF reduced EF= Heart failure with reduced ejection fraction (LVEF <40%); HF-mid-range EF= Heart failure with midrange ejection fraction (LVEF 40-49%); HF preserved ejection fraction (LVEF >50%); LVEF= Left ventricular ejection fraction; AF= atrial fibrillation. PCI= percutaneous coronary intervention; CABG= coronary artery bypass grafting.
Data are presented as mean ± SD, median (25th–75th percentile), or number (percentage). OR or B with corresponding 95% confidence intervals represent differences between HF patients with and without CMIs. [a] Ischemic compared to non-ischemic HF on CMI presence [b] >5 years of HF vs ≤ 5 years of HF on CMI presence. One missing for education, cause of HF, cardiac valve disease, CABG and vascular risk factors (except hypertension (history) and obesity) 2 missing: hypertension (history) and obesity, duration of HF; 3 missing: PTA, myocardial infarction; 4 missing: cardiac arrhythmia. 7 missing: cardiac MRI derived measures (EF, cardiac index, pulse rate, cardiac output).

With respect to cerebral MRI-markers (Table 4), CMI presence in HF patients was associated with a higher occurrence of larger cortical infarcts (>5mm, p=.012) and a marginally decreased TBV (after adjustment for age, sex and TIV, p=.068), while no significant association was observed with MRI-markers of cerebral small vessel disease (e.g. WMH volume, lacunar infarcts, microbleeds or cerebral small vessel disease score). CMI presence in HF patients was accompanied with a marginally lower CBF of the GM, but this difference was not statistically significant. The relationship between CMI presence and cardiac index was not significantly mediated by CBF (Figure 1 for mediation analysis).

Scores on the MMSE, composite z-score of the cognitive domains and the composite score of all cognitive domains (age, sex and education adjusted) was not associated with

presence of CMIs in HF patients (Table 5). The proportion of patients with cognitive impairment also did not differ according to CMI presence.

Table 4: Cerebral MRI-markers in HF patients with and without CMIs

	HF CMI present (n=26)	HF CMI absent (n=120)	B [95% CI]	*p*
TIV (ml)	1414.2 ± 115.7	1387.6 ± 136.0	19.9 [-28.3; 68.0]	.417
TBV volume (ml)[a]	1090.7 ± 103.4	1092.3 ± 117.7	-15.8 [-32.8; 1.2]	.068
GM volume (ml) [a]	650.1 ± 51.4	649.4 ± 66.9	-7.0 [-20.4; 6.3]	.300
WMH volume (ml) [ab]	2.2 [1.2 – 5.2]	1.6 [0.4 - 4.5]	.28 [-.28; .84]	.331
CBF GM (ml/100g/min) [c]	49.9 ± 10.7	54.8 ± 11.4	-4.9 [-10.1; .4]	.124
			OR [95% CI]	
Cortical infarcts and non-lacunar subcortical infarcts	8 (31%)	12 (10%)	3.9 [1.3; 11.3]	**.012**
Lacunar infarcts	11 (42%)	31 (26%)	1.9 [0.8; 4.7]	.153
Microbleeds	4 (15%)	31 (26%)	0.4 [0.1; 1.2]	.108
SVD score	1 [0-2]	0 [0-1]	-	.603

Abbreviation: HF= Heart failure; CMI= cortical microinfarct; GM= gray matter; TIV; total intracranial volume; TBV= total brain volume; WMH= white matter hyperintensity; CBF=cerebral blood flow; GM= gray matter; SVD score= Small vessel disease score. Data are presented as mean ± SD, median (25th-75th percentile), or number (percentage). [a] Brain volumetrics were corrected for age, sex and intracranial volume. [b]WMH was log transformed. [b] CBF was corrected for age, sex and partial volume. Differences between HF patients with and without CMI presented with OR or B and corresponding 95% confidence intervals. Eight missings for all cerebral MRI-markers.
Blood biomarkers, including hemoglobin (4 missing), hematocrit and CRP (both 5 missing) were not related to CMI presence in HF patients (data not shown, $p>0.05$).

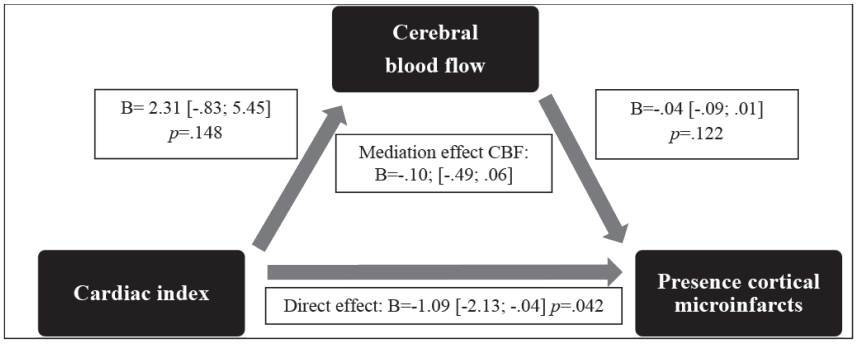


Figure 1: Mediation analysis of cerebral blood flow (CBF) in the relationship between cardiac function and cortical microinfarct (CMI) presence.

Table 5: Cognitive performance and proportion of patient with global cognitive impairment in HF patients with and without CMIs

	HF CMI present (n=26)	HF CMI absent (n=127)	P
MMSE	28.9 ±1.0	28.5 ±1.5	.149
Composite of all cognitive domains	-0.04 ± 0.46	-0.22 ± 0.58	.133
Memory	0.02 ± 0.83	-0.29 ± 1.27	.237
Language	-0.06 ± 0.45	-0.32 ± 0.95	.186
Attention/speed	-0.22 ± 0.85	-0.28 ± 0.80	.729
Executive functioning	-0.11 ± 0.53	0 ± 0.83	.528
Global cognitive impairment			.239
No	24 (92%)	101 (80%)	
Minor	1 (4%)	21 (17%)	
Major	1 (4%)	4 (3%)	

Abbreviation: HF= Heart failure; CMI= cortical microinfarct; MMSE= mini-mental state examination. Composite of cognitive scores are the mean z-score for all cognitive domains corrected for age, sex and gender in relation to the references. Global cognitive impairment: No = no impaired cognitive domains, Minor = one impaired cognitive domain; Major= more than one impaired cognitive domain. One missing for MMSE, two missings for composite cognitive scores and global cognitive impairment.

Cardiac correlates of CMIs in reference participants

Presence of CMIs in reference participants was associated with sex (13% vs 49% females in CMI present vs absent, p=.045), but not with vascular risk factors. There was no relationship between CMI presence in and cardiac function (cardiac index B=-.10 [-.47–.26] p=.578 and LVEF: B=-2.1 [-6.0–1.8] p=.281). None of the reference participants with CMIs had a history of AF, myocardial infarction or any cardiac interventions (no statistical tests performed).

DISCUSSION

We found that CMIs commonly occur in patients with HF. Among patients with HF, presence of CMIs was associated with hypertension and severity of cardiac-pump dysfunction, but did not relate to cognitive impairment. These results show that vulnerability for vascular brain injury in patients with HF extends to CMIs.

In this study of patients with HF without current AF, we observed a CMI occurrence of 17%, which is markedly higher than the CMI occurrence in reference participants in this study (7%) and healthy controls from previous studies (6–12%) [22–24]. The

occurrence rate in HF patients seems to be comparable to that reported for patients with a primary vascular brain disease, such as patients with acute stroke (10–15%) [8,25] and vascular cognitive impairment (20%) [22]. These findings emphasize that patients with HF should indeed be considered a population at considerable risk for vascular brain injury, including CMIs.

The question is what underlying causes contribute to the high CMI occurrence in HF patients. The first cause to consider is shared vascular risk factors. Risk factors, such as hypertension, hypercholesterolemia and diabetes, have been related to CMIs [6], but are also common in patients in HF [12]. Similarly, vascular risk factors are known contributors to risk of (silent) macroscopic infarcts in HF patients [26]. Although adjustment for vascular risk factors in this study did not attenuate the effect size of the relation between CMIs and HF, effects of previous exposure or current treatment of vascular risk factors can evidently not be ruled out.

Another possibility is that the condition HF is causally related to CMIs. Two pathophysiological mechanisms have been proposed that can lead to brain infarction in the context of HF. Firstly, thromboembolism, the risk of which is known to be elevated by a marked reduced cardiac output in HF combined with stasis of blood in the cardiac chamber and the pro-thrombotic state associated with HF [27]. Secondly, cerebral hypoperfusion, primarily related to low cardiac output. Especially in the presence of small vessel disease, when autoregulation of the small vessels may be impaired, the brain is likely to be more vulnerable to bouts of hypoperfusion [28]. Both thromboembolism and hypoperfusion have shown to contribute to the development of CMIs in the context of conditions other than HF [6,9].

A key finding in the current study is the relation between CMIs and a markedly reduced cardiac index indeed supports the presence of thromboembolic and cerebral hypoperfusion pathways. Moreover, we found that CMIs presence was related to large cortical infarcts on MRI, which also suggests a thromboembolic origin. Notably, we found no significant association between CMIs and LVEF, despite the strong physiological link between LVEF and cardiac output/index. This discrepancy could possibly be explained by the lack of standardization for BSA for LVEF. However, we found that cerebral perfusion (CBF) was not an obvious mediator in our exploratory mediation analysis on the cardiac index – CMI relationship. Although it might be argued that this is to some extent due to the lack of powering of this study for these relatively small effect sizes. Another option

is that the relation between CMI does not solely dependent on the cardiac output status. It would certainly be of interest to observe the clinical correlates of CMIs in HF patients with preserved EF, in which other principal pathophysiological processes might play a role. Future studies are encouraged to explore this issue in selected population of HF patients with preserved EF and taking into account other variables of interest such as venous congestion.

An important clinical issue is the prognostic value of CMIs in patients with HF. We found that CMI presence in HF patients was not related to worse cognitive functioning. This is unexpected finding in the light of previous research, showing a relatively consistent, though sometimes modest relationship between CMIs and cognitive impairment across different populations [6,29]. It might be explained by the fact that HF patients in the current study were all in a clinically stable phase of HF. Additionally, the HF patients had a relatively low burden of concurrent vascular brain injury and on the whole relatively preserved cognition (as they scored only 0.2 SD z-score below reference participants), limiting the threshold to detect subtle differences in cognitive functioning. Another potential clinical implication of CMIs is the risk future stroke, as acute microinfarcts have shown to be associated with a 3-fold increased risk of poor clinical outcome after 2 years [30]. Future studies addressing the prognostic value of CMIs on stroke and cognition in HF patients are therefore recommended.

Strengths of this study are the well-defined cohort of HF patients and reference participants, the elaborate cerebral and cardiac MRI protocol and extensive cognitive testing. Moreover, by actively excluding patients with AF upon enrollment (the rationale of which was possible interference of AF with the cardiac MR protocol) we reduced the potential distortion of results by thromboembolic stroke. It must be acknowledged that since only patients with stable HF were eligible for inclusion this could have resulted in a bias of relatively healthy HF patients, thereby underestimating the found effects. Moreover, no data was available on clinical course including previous bouts of acute HF or AF, while it is likely that such episodes could have significantly contributed to structural brain injury. As expected, CMIs occurred in a minority of subjects, especially in reference participants. We could therefore not further stratify results according to causes of HF and the limited statistical power may contribute to the fact that we failed to find an association between CMIs and manifestations of cerebral small vessel disease despite OR over 1.5. Although detection of CMIs on 3T MRI is practical on a large scale, it is well established that smaller CMIs (visible on 7 Tesla MRI or neuropathological

evaluation) escape the detection limit, while these truly microscopic lesions may certainly also contribute to the cognitive decline [29].

Conclusion

We found that CMIs were common in patients with HF and CMIs were related to vascular risk factor profile and severity of cardiac dysfunction. This study thus identifies CMIs as a novel marker of vascular brain injury in these patients.

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APPENDIX

Specification of neuropsychological tests used per cognitive domain:

Cognitive domain	Neuropsychological test	Test variable included
Memory	Rey Auditory Verbal Learning Test[1]	Total immediate recall Delayed recall Recognition
	Visual Association Test, short version[2]	Part A
Language	Visual Association Test	Part A
	Fluency (animals) [3]	Number correct in 1 minute
Attention/psycho-motor speed	Trail Making Test [4]	Part A
	Stroop Color-Word Test[5]	Mean of cart 1 and 2
	Letter-Digit Substitution Test[6]	Number correct in 90 sec
	Digit span[7]	Forward
Executive functioning	Trail Making Test	Part B/Part A
	Stroop Color-Word Test	Interference (card 3 /([card 1 + card 2] / 2)
	Digit span	Backwards

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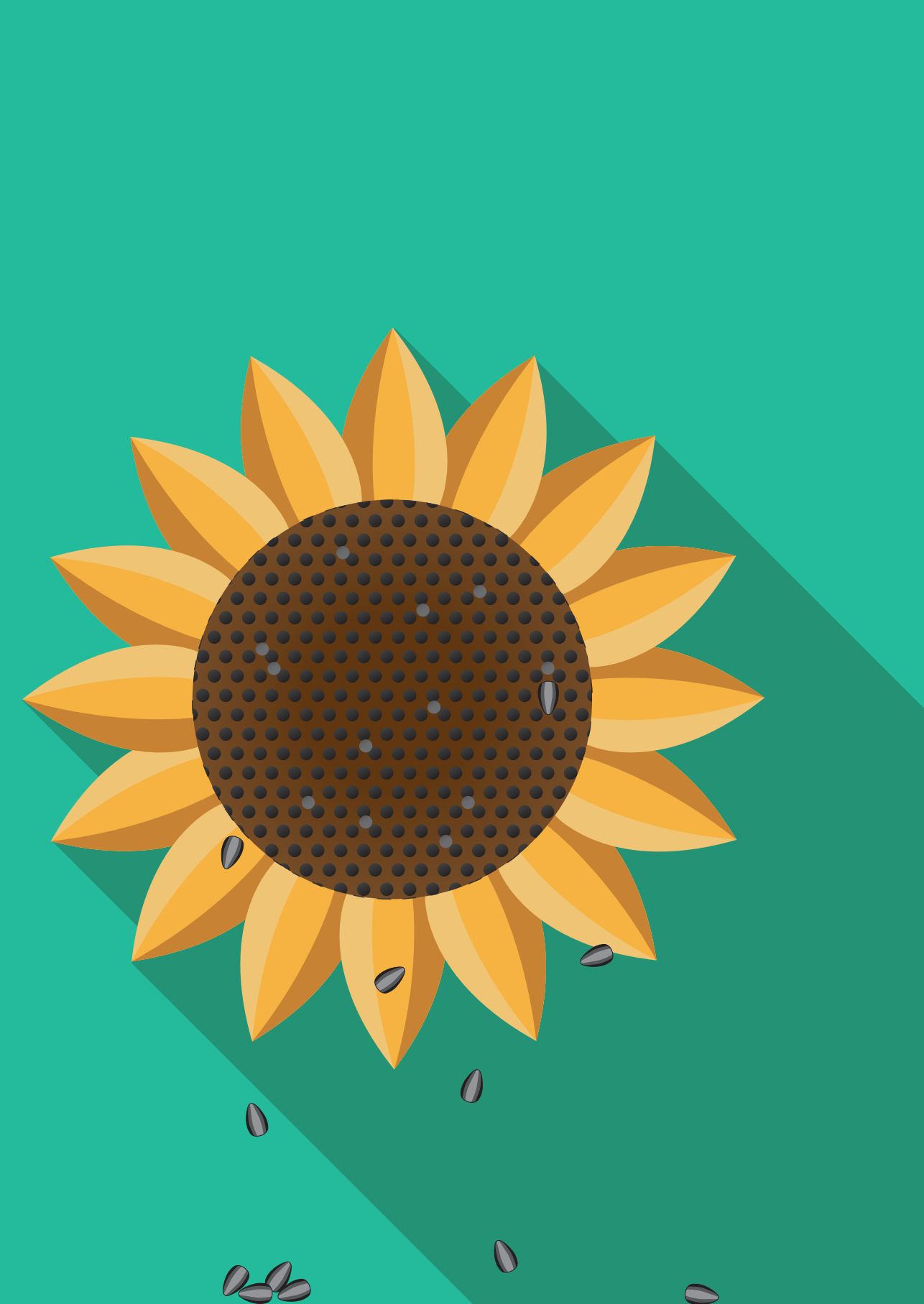
PART II

IDENTIFYING PERILESIONAL INJURY ASSOCIATED WITH CEREBRAL MICROINFARCTS



CHAPTER 6

CEREBRAL CORTICAL MICROINFARCTS ARE ASSOCIATED WITH PERILESIONAL CORTICAL ATROPHY

Doeschka Ferro
Hugo Kuijf
Saima Hilal
Susanne van Veluw
Daniëlle van Veldhuizen
Narayanaswamy Venketasubramanian
Tan Boon Yeow
Geert Jan Biessels
Christopher Chen

submitted for publication

ABSTRACT

Objective: Cerebral cortical microinfarcts (CMIs) are a novel MRI-marker of cerebrovascular disease (CeVD) that predicts accelerated cognitive decline. Presence of CMIs is associated with global cortical atrophy, although the mechanism linking the two is unclear. We examined whether CMI presence in memory clinic patients is related to local cortical atrophy around the actual site of the CMI.

Methods: In 238 memory clinic patients (mean age 72.5 SD 9.1 years) cortical thickness, gray matter volume, CMIs and conventional MRI-markers of CeVD were assessed on 3 T MRI. All patients underwent cognitive testing. Cortical thickness was compared *globally* between patients with and without CMIs, *regionally* between brain regions with CMIs and the contralateral region without CMIs, and *locally* within a 50mm radius of CMIs. Cortical thickness was analyzed as mediator in the relation between CMI and cognitive performance.

Results: Patients with ≥1 CMIs (n=75) had a 2.1% lower global cortical thickness (B=-.049mm [95% confidence interval .091; -.007] p=.022) and a 3.0% lower total gray matter volume (B=-18.1ml [-28.5; -7.7] p=.001) compared to patients without CMIs, after correction for age, sex, education and intracranial volume. Brain regions with CMIs had a lower cortical thickness than contralateral regions without CMIs (B=-.048mm [-.071; -.026] p<.001), especially in regions with multiple CMIs (B=-.12mm [-.17; -.06] p<.001). Cortical thickness was lower in the immediate surrounding area of a CMI, especially within a 20 mm radius (F=10.0, p=.002). Global cortical thickness was a significant mediator in the relationship between CMI presence and cognitive performance (B=-.12 [-.22; -.01] p=.025).

Conclusion: We found marked cortical atrophy the immediate surrounding area of CMIs, suggesting a perilesional effect in a surrounding cortical area many times larger than the CMI-core. Our findings support the notion that CMIs affect brain structure and function beyond the lesion site.

INTRODUCTION

Cerebral cortical microinfarcts (CMI) are small ischemic lesions that can be detected on neuropathological evaluation and MRI [1]. CMIs are common in patients with dementia and cerebrovascular disease (CeVD) [1] and are associated with accelerated cognitive decline [2].

Several studies have observed that CMI presence is associated with global cortical atrophy [3–6]. However, the underlying mechanism of this relationship is not fully understood. Even when considering estimates from neuropathological studies that total brain CMI counts may be in the 100s to 1000s in individual subjects [7], due to the tiny volume (typical diameter on histology is less than a mm [8]), the total summed volume of all these lesions together would still generally not exceed 1 ml. By contrast total brain volume has been reported to be around 20-30 ml lower in patients with CMI, compared to those without [5,6]. The question is what explains this mismatch. Recently, it has been suggested that CMIs could be linked to local cortical atrophy in mice [1,9]. This is thought to be due to the disruptive effect CMIs exert on surrounding cortex, resulting in perilesional tissue damage [1,9,10].

In this study we examined the relation between CMIs and cortical atrophy in memory clinic patients and specifically if local perilesional atrophy can be detected around the actual site of the CMIs. Additionally, we examined the role of global cortical atrophy in CMI-associated cognitive impairment.

METHOD

Population

We studied memory clinic patients from the National University Hospital and St. Luke's Hospital in Singapore [5]. Patients were included in the study if they had received one of the following diagnoses. (1) No cognitive impairment (NCI): patients without objective cognitive impairment on formal neuropsychological tests or functional loss. (2) Cognitive impairment – no dementia with (VCIND) (2a) or without (CIND) (2b) a history of stroke: patients who were impaired in at least one cognitive domain on neuropsychological testing but did not meet the DSM (4th edition) criteria for dementia. Ischemic stroke was assessed based on medical history and confirmed by neuroimaging. Patients with dementia received an etiological diagnosis on the basis of internationally established criteria for (3) Alzheimer dementia (AD) [11] and (4) Vascular dementia [12]. Patients with significant neurological comorbidities, loss of functional independence or other diagnoses (e.g. FTD) were excluded from participation. All patients underwent a standardized clinical examination, 3 T MRI and neuro-psychological testing. For the present study we included all patients recruited between December 2010 and September 2013, who had MRI sequences of sufficient quality for analysis as previously described [5]. Ethical approval for this study was obtained from the National Healthcare Group Domain-Specific Review Board (DSRB). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained, in the preferred language of the patients, by bilingual study coordinators before recruitment into the study. Consent for patients lacking capacity was provided by their legal representative, as allowed by the DSRB.

MRI

Patients were scanned on a 3 T MRI (Siemens Magnetom TrioTim system) with a 32-channel receiver head-coil. The MRI protocol consisted a 3D T1-weighted (1.0 X 1.0 X 1.0 mm^3 voxels; repetition time (TR) 2300 ms; echo time (TE) 1.9 ms; inversion time (TI) 900 ms; flip angle 9?; matrix 256 X 256), a 2D multislice T2-weighted (1.0 X 1.0 X 3.0 mm^3 voxels; TR 3000 ms; TE 10.1 ms; matrix 247 X 256), a 2D multislice FLAIR (1.0 X 1.0 X 3.0 mm^3; TR 9000 ms; TE 82 ms; TI 2500 ms; matrix 232 X 256), and a 2D multislice T2*-weighted image (1.0 X 1.0 X 1.5 mm^3 voxels; TR 27 ms; TE 20 ms; flip angle 15°; matrix 192 X 256). The following MRI markers were assessed: Microbleeds according to the Brain Observer MicroBleed Scale [13], presence of large (i.e. >5 mm) cortical infarcts, subcortical infarcts (i.e. subcortical infarct and/or lacunar infarcts) and

white matter hyperintensities (WMHs) according to the STRIVE criteria [14]. Infarct and WMH volumes were manually segmented on FLAIR and T1-weighted images using an in-house developed tool incorporated in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany). Gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) segmentations were obtained from the T1-weighted image using the CAT12 toolbox (Gaser, Dahnke, 2016) implemented in Statistical Parametric Mapping (SPM) (Wellcome Trust Centre for Neuroimaging, University College London, UK) from which total GM volume, total brain volume, and intracranial volume (ICV) were quantified.

Cortical thickness was measured using a surface-based method by calculating the distance between the GM/WM boundary and the GM/CSF boundary after masking large cortical infarcts using the CAT12 toolbox [16]. *Global cortical thickness* was the cortical thickness averaged over the whole cortical surface per subject. *Regional cortical thickness* was determined using brain regions of the Hammers atlas [17,18], a 3D atlas including 68 brain regions. Cortical thickness in Hammers regions with CMIs was compared to the anatomically corresponding Hammers region in the contralateral hemisphere without CMIs within the same subject. Only Hammers regions without CMIs in the contralateral Hammers were included in the analysis. *Local cortical thickness* was assessed within discrete intervals within a 50 mm radius around individual CMIs, starting from a 3mm distance of the CMI center point to exclude the CMI-core. Of note CMIs within <15 mm distance from a large infarct or other CMI were excluded from this analysis.

CMIs were rated by visual inspection according to validated criteria, as described previously [5]. Briefly, CMIs had to be hypointense on T1 weighted imaging and hyperintense or isointense on FLAIR and T2-weighted and less <5 mm in the largest dimension [19]. Location of CMIs was also registered to the Hammers atlas, to identify whether a region had one, multiple, or no CMIs.

Analysis

Differences in baseline characteristics between patients with and without CMIs were compared with an independent t-test (for continuous normally distributed data), χ-square test (for proportions) and Mann-Whitney U test (for continuous, non-normally distributed data). WMH volume was log transformed. Global mean cortical thickness was compared between patients with and without CMIs using linear regression. The model was subsequently corrected for age, sex, education and ICV, and additionally

for MRI-markers of CeVD. Spearman's rank correlation was used to assess the relation between total number of CMIs per subject and cortical thickness.

Mean regional cortical thickness in regions with CMIs was compared to the anatomically corresponding Hammers region in the contralateral hemisphere within the same subject using a repeated-measure ANOVA. To assess the interaction effect of one versus multiple CMIs in a region compared to the contralateral Hammers region on cortical thickness a mixed-design ANOVA was used. Local cortical thickness was analyzed in 6 discrete intervals in a 50 mm radius of the individual CMIs, and the corresponding mean regional cortical thickness using repeated measures ANOVA and compared to each other in post-hoc contrasts. Mediation analysis was performed with the PROCESS v3 (Hayes, 2018). All data was analyzed using IBM SPSS statistics (version 25) and a p-value $< .05$ was considered statistically significant.

RESULTS

Patient and CMI characteristics

This study included 238 patients with a mean age of 72.5 years (SD 9.1) of whom 117 (49%) were male. A total of 274 CMIs were found in 75 patients (32%), of whom 36 patients had two or more CMIs (range 1-43). CMIs were located in 34 of the 68 Hammers regions, especially in the most cranial parts of the pre- and postcentral brain area's (see Appendix 1 for a 3D representation of the spatial distribution and a frequency table of the number of CMIs per Hammers region). Characteristics of patients with and without CMIs are presented in Table 1. Patients with CMIs were proportionally more often diagnosed with vascular cognitive impairment – no dementia (VCIND) and vascular dementia, and had a higher burden of (vascular) injury on MRI, including (sub) cortical infarcts, microbleeds, WMH volume and total brain volume, compared to patients without CMIs (as reported previously in this cohort [5]).

Global cortical atrophy

Patients with CMIs had a 2.1% lower mean global cortical thickness (mean thickness in patients with one or more CMI(s) 2.38 mm ± .17 mm; in patients without CMIs 2.43 mm ± .16 mm) (p=.022 after correction for age, sex, education and ICV). Moreover patients with CMIs had a 3.0 % lower GM volume (GM volume in patients with CMI present 518 ml ± .62 ml; in patients with CMI absent 534 ml ± .64 ml) (p=.039 after correction for age, sex, education and ICV) (Table 2, model 2). Results attenuated for cortical thickness

after adjustment for microbleeds and even more so WMH, and for GM volume only after adjustment for all CeVD MRI-markers together (Table 2, model 3). Within the group of patients with CMIs, the total number of CMIs per subject was associated with a lower cortical thickness (Rho= -.23, *p*=.049), but not with GM volume (Rho= 0.52, *p*=.660).

Table 1: Baseline table of patients with and without CMIs

	CMI present (N=75)	CMI absent (N=163)	*p*
Demographics			
Age (years)	72.5 ± 9.0	72.5 ± 9.2	.976
Sex (males)	41 (55)	76 (47)	.249
Education (4 levels)	1 [1-2]	1 [1-2]	.226
Ethnicity (Chinese)	55 (73)	130 (80)	.298
Clinical diagnosis			**.002**
NCI	4 (5)	26 (16)	
CIND	5 (7)	29 (18)	
VCIND	23 (31)	32 (20)	
Alzheimer dementia	31 (13)	66 (41)	
Vascular dementia	12 (16)	10 (6)	
Cerebral MRI-Markers			
Presence of cortical infarcts (i.e. >5 mm)	27 (36)	12 (7)	**.000**
Presence of subcortical infarcts	33 (44)	42 (26)	**.000**
Presence of microbleeds	52 (72)	83 (51)	**.003**
WMH volume (mL)	13.5 [5.4-51.7]	9.0 [3.8-21.6]	**.011**[a]
Intracranial volume (mL)	1413 ± 141	1404 ± 129	.636
Total brain volume (mL)	897 ± 108	918 ±114	**.003**[b]
Cortical atrophy markers			
Total gray matter volume (mL)	518.1 ± 61.7	534.3 ± 64.1	**.001**[b]
Global cortical thickness (mm)	2.38 ± .17	2.43 ± .16	**.032**

Abbreviations: CMI=Cerebral cortical microinfarct, MMSE=mini-mental state examination, NCI=no cognitive impairment, CIND=Cognitive impairment - no dementia, VCIND=Vascular cognitive impairment - no dementia, WMH=White matter hyperintensity. Data are presented as mean ± standard deviation, n (percentage) or median [interquartile range].

[a] WMH volume difference between CMI present and absent tested after log transformation. [b] Total brain volume and total gray matter volume difference between CMI present and absent tested after adjusted for age, gender, and intracranial volume.

Regional and local cortical atrophy

For the regional analyses of cortical thickness, a total of 139 regions (with 197 CMIs) from 74 patients were eligible, as 77 regions were excluded due to CMIs in both hemispheres.

Cortical thickness was on average 2.2 % lower in the region with CMIs compared to the reference regions without CMIs in the contralateral hemisphere in the same subject (F=29.7, p<001) (Figure 1A). Results were stratified into regions with a single CMI and regions with multiple CMIs. Cortical thickness was 1.7% lower in regions with a single CMI compared to the reference region in the contralateral hemisphere (Figure 1B), while cortical thickness was 5.6 % lower in regions with multiple CMIs compared to the reference region (Figure 1B) (F =9.7, p=.002).

Table 2: Linear regression model of CMI presence on global cortical thickness and gray matter volume

	Cortical thickness			Gray matter volume		
	B [95% CI]	**Beta**	***p***	**B [95% CI]**	**Beta**	***p***
Model 1: CMI presence (unadjusted)	-.050 [-.095; -.004]	-.140	**.032**	-16.2 [-33.6; 1.3]	-.118	.069
Model 2: CMI presence (adjusted for age, sex, education and ICV)	-.049 [-.091; -.007]	-.138	**.022**	-18.1 [-28.5; -7.7]	-.132	**.001**
Model 3: CMI presence (adjusted for age, sex, education, ICV and additionally for each individual CeVD MRI-markers)						
Large cortical infarcts	-.045 [-.090; .000]	-.127	**.049**	-16.2 [-27.3; -5.0]	-.118	**.005**
Lacunes and subcortical infarcts	-.048 [-.091; -.006]	-.136	**.026**	-17.2 [-27.8; -6.6]	-.126	**.002**
Presence of microbleeds	-.040 [-.083; .003]	-.113	.069	-16.5 [-27.1; -6.0]	-.121	**.002**
WMH volume	-.031 [-.072; .009]	-.089	.128	-15.6 [-25.7; -5.4]	-.115	**.003**
All combined	-.018 [-.062; .027]	-.138	.433	-10.2 [-21.4; 1.0]	-.075	.074

Abbreviations: CMI=Cerebral cortical microinfarct, ICV=intracranial volume; CeVD=Cerebrovascular disease; WMH=White matter hyperintensity. Data presented as B (mean difference between patients with and without CMIs and the 95% confidence interval) and standardized beta. [a] WMH volume was entered into the analysis after a logarithmic transformation of the data.

For the local analysis of cortical thickness, a total of 120 CMIs in 68 patients were eligible, 154 CMIs were excluded due to neighboring CMIs or large infarct within a 15 mm radius of the CMI. There was an evident relationship between radial distance from the CMI and cortical thickness (F=7.4, p=.000) (Figure 2). Post-hoc contrasts showed that cortical thickness within the 3-20mm radius was significantly lower than mean cortical thickness

directly outside the 20mm radius (F=10.0, *p*=.002) and the mean cortical thickness of the whole region (F=12.0, *p*=.001). Cortical thickness in the radial intervals between 20-50mm from the CMI-core were not different from the mean cortical thickness of the whole CMI region (*p*>.05). In order to compare the size of the perilesional area to the area of the CMI-core, we assumed a circle shaped form for both perilesional zone and the CMI. The radius of the perilesional zone was approximately 20mm, therefore we estimated perilesional area (calculated as πr^2, where r= 20) to be approximately 1256 mm^2. The radius of a typical CMI was approximately 1.5 mm, the estimated CMI area would therefore be around 7 mm^2. The perilesional area appears to be at least 150 times greater than the CMI-core.

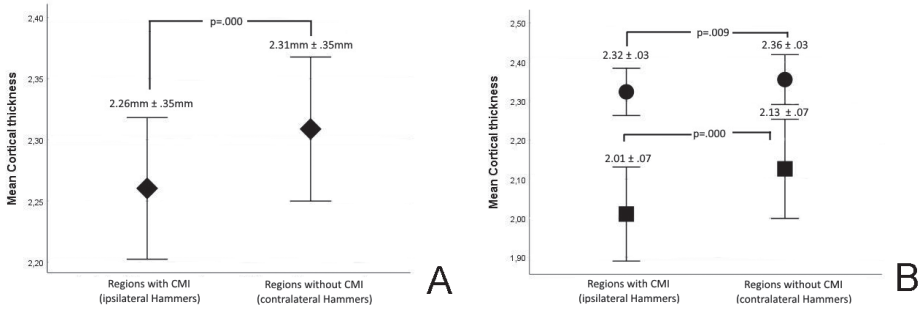


Figure 1A: Mean estimated regional cortical thickness (with 95% confidence intervals) in 139 regions with CMIs (ipsilateral Hammers region) compared to the contralateral region without CMIs within the same subject in a total of 74 patients. Figure 1B: Mean estimated regional cortical thickness stratified according to regions with a single (dot; 111 regions, 71 patients) and multiple CMIs (square, 28 regions, 19 patients) compared to the contralateral region without CMIs within the same subject.

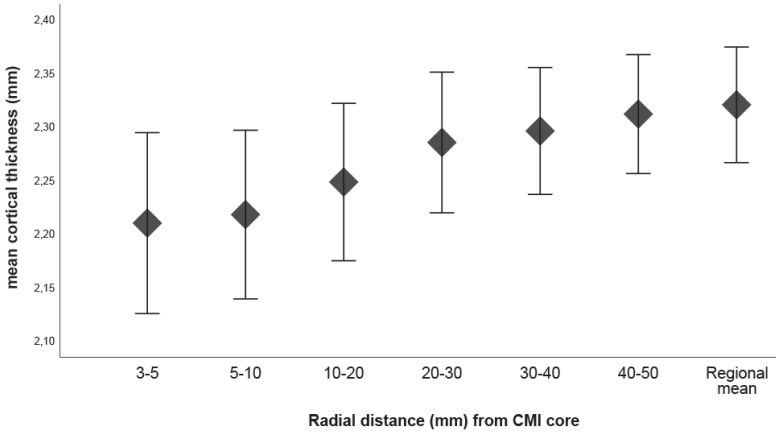


Figure 2: Mean estimated cortical thickness (with 95% CI) within discrete distance intervals from the CMI core (120 CMIs in 68 patients).

Cortical thickness as mediator in the relation between CMIs and cognitive impairment

CMI presence was associated with reduced cognitive performance as measured with the MMSE (Beta=-.24, p=.040) and a trend was observed for composite score of all cognitive domains (Beta= -.20, p=.065) after correction for age, sex, education and ICV. Global cortical thickness significantly mediated the relationship between CMI presence and MMSE (indirect effect B = -.12 CI [-.22; -.01] p=.025) but did not reach statistical significance for the composite score of all cognitive domains (indirect effect B = -.11 [-.23; .02] p=.084].

DISCUSSION

We found that memory clinic patients with CMIs had marked local cortical atrophy around the actual site of the CMIs. This suggests a perilesional effect on surrounding cortex far greater than the CMI- core. Moreover, we observed that CMI-associated cognitive impairment is partially mediated by global cortical thickness. These results are an important step towards the understanding of the structural and functional impact of CMIs.

Previous research has shown microscopic cortical injury beyond the CMI-core [9], with one mouse model study estimating that this perilesional zone was 12-fold greater than the CMI-core itself [9]. In the current study we observed the most pronounced reduction in cortical thickness within a 20 mm radius from the center point of CMI, suggesting a perilesional area at least 150 times the sizes of the CMI-core. Considering this magnitude, it is likely that perilesional damage significantly contributes to the global cortical atrophy associated with CMI presence.

The perilesional zone in mice displays several histopathological alterations, including neuronal death, dendritic spine loss, astrogliosis and blood–brain barrier leakage [9,10,20]. Exactly how injury in the perilesional zone evolves is not yet clear. It has been proposed that CMIs directly incur this damage through mechanisms such as ischemic cortical spreading depression and disrupted cortico-cortical and cortico-subcortical circuits [9]. Of note, in a recent study we did not find evidence for disruption of subcortical white matter connectivity in patients with CMIs using diffusion tractography MRI Future research on the mechanistic pathways of perilesional injury is encouraged

to bridge the gap between the histopathological findings in mice and in vivo studies in humans, for example by using 7 T MRI.

Our results indicate that associated other forms of CeVD may contribute to global cortical atrophy in memory clinic patients with CMIs. CMIs have shown to co-occur with several MRI-manifestations of SVD, including WMHs, lacunar infarcts and microbleeds [1], which in turn have been linked to cortical atrophy independent of CMIs [21–24]. After correction for these SVD-manifestations, especially WMH-lesion volume, CMI presence is no longer a predictor for global cortical thickness, suggesting that concomitant SVD in patients with CMIs co-contributes to the severity of cortical atrophy. Since CMIs have multiple underlying causes beyond SVD, it is likely that these other pathophysiological mechanisms may also add to the development of global cortical atrophy in patients with CMIs, e.g. cerebral hypoperfusion [25], intracranial plaque [5,26] and thromboembolic infarction [27]. Even aging is a plain risk factor for both CMIs and cortical atrophy, highlighting the fact that CMIs should also be considered a marker of underlying cerebrovascular pathology and aging, which independently contribute to cortical atrophy. Yet, none of these factors can explain the within-patient perilesional effects that were observed.

It remains one of the key questions how CMIs – considering their minimal lesion volume – can bring about functional impact, for example on cognition. Our results indicate that part of the CMI-associated cognitive impairment is meditated through cortical atrophy, which in turn is likely to be the result of perilesional damage and underlying cerebrovascular pathology. It would be of interest to observe if local cortical atrophy is associated with a different cognitive profile than global cortical atrophy in patients with CMIs, as previous studies found that CMIs were associated with deficits in "cortical" cognitive domains, including visuo-construction and language [5,28,29]. Moreover, most studies on the functional impact of CMIs have focused on cognition, while other outcomes such as gait and psychiatric symptoms certainly warrant further investigation.

This study has several strengths and limitations. A strength of this study is the relatively large and well defined cohort and the standardized imaging and neuropsychological testing protocol. Moreover, well validated tools were used to perform the cortical thickness measurements [16]. We have made our source codes open access on GitHub allowing other researchers to reproduce our results. It is, however, unclear how well results from this memory clinic generalize to other populations, as causes, burden and

correlates of CMIs differ according to their clinical context. The lack of follow up in this cross-sectional study is a limitation that makes it hard to untangle causality in the relationship between CMIs, cortical atrophy and underlying cerebrovascular pathology. Our study was also limited by spatial resolution of 3 T MRI used to assess CMIs and cortical atrophy. Only a fraction of CMIs found on 7 T are detectable on 3 T and it is unclear whether the association with cortical atrophy also applies to the numerous smaller CMIs that escape detection.

In conclusion, this study found evidence for a perilesional effect of CMIs on surrounding cortical tissue in memory clinic patients. Considering the magnitude of this perilesional zone, it is likely that perilesional damage is a relevant contributor to the global cortical atrophy observed in patients with CMIs. Together with influences of other associated cerebrovascular pathologies, such as SVD, on cortical atrophy, these findings help to better understand the impact of these tiny lesions on overall brain structure and function.

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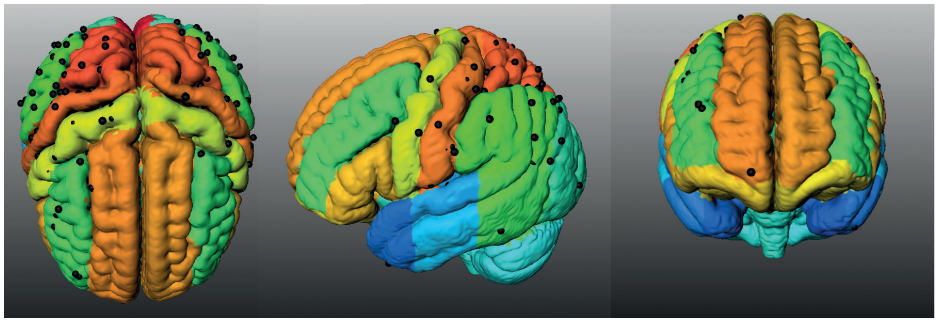
APPENDIX

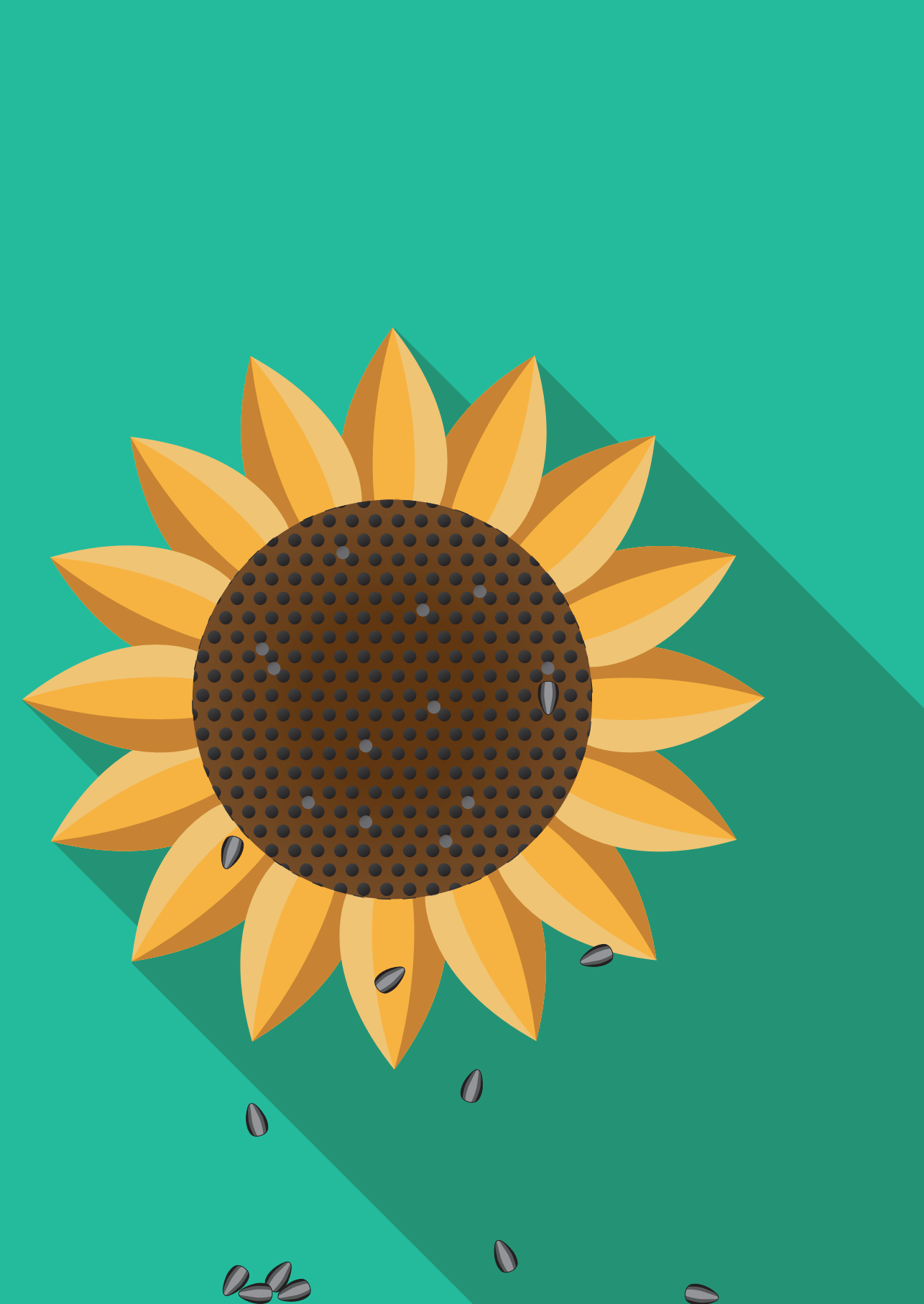


Appendix figure 1: 3D representation of the spatial distribution of cortical microinfarcts (CMIs; represented as black dots) across the brain in transversal, sagittal views and coronal view (left to right). The colored areas represent the Hammers-atlas regions.

eTable 1: Number of CMIs per Hammers region

Hammers Region	Name region	Number of CMIs	Percentage of total CMIs
1	Left Hippocampus	0	0
2	Right Hippocampus	0	0
3	Left Amygdala	0	0
4	Right Amygdala	0	0
5	Left Anterior Medial Temporal Lobe	1	.4
6	Right Anterior Medial Temporal Lobe	1	.4
7	Left Anterior Lateral Temporal Lobe	2	.7
8	Right Anterior Lateral Temporal Lobe	0	0
9	Left Ambient and Parahippocampal Gyri	0	0
10	Right Ambient and Parahippocampal Gyri	0	0
11	Left Superior Temporal Gyrus	2	.7
12	Right Superior Temporal Gyrus	5	1.8
13	Left Inferior Middle Temporal Gyri	2	.7
14	Right Inferior Middle Temporal Gyri	1	.4
15	Left Fusiform Gyrus	0	0
16	Right Fusiform Gyrus	0	0
17	Left Cerebellum	0	0
18	Right Cerebellum	0	0
19	Left Brainstem	0	0
20	Right Brainstem	0	0
21	Left Insula	0	0
22	Right Insula	0	0
23	Left Lateral Occipital Lobe	6	2.2
24	Right Lateral Occipital Lobe	7	2.6
25	Left Anterior Cinguli Gyrus	2	.7

26	Right Anterior Cinguli Gyrus	3	1.1
27	Left Posterior Cinguli Gyrus	0	0
28	Right Posterior Cinguli Gyrus	0	0
29	Left Middle Frontal Gyrus	18	6.6
30	Right Middle Frontal Gyrus	26	9.5
31	Left Posterior Temporal Lobe	1	.4
32	Right Posterior Temporal Lobe	3	1.1
33	Left Inferior Lateral Parietal Lobe	18	6.6
34	Right Inferior Lateral Parietal Lobe	26	9.5
35	Left Caudate Nucleus	0	0
36	Right Caudate Nucleus	0	0
37	Left Accumbens Nucleus	0	0
38	Right Accumbens Nucleus	0	0
39	Left Putamen	0	0
40	Right Putamen	0	0
41	Left Thalamus	0	0
42	Right Thalamus	0	0
43	Left Pallidum	0	0
44	Right Pallidum	0	0
45	Left Corpus Callosum	0	0
46	Right Corpus Callosum	0	0
47	Left Lateral Temporal Ventricle	0	0
48	Right Lateral Temporal Ventricle	0	0
49	Left Third Ventricle	0	0
50	Right Third Ventricle	0	0
51	Left Precentral Gyrus	23	8.4
52	Right Precentral Gyrus	17	6.2
53	Left Gyrus Rectus	0	0
54	Right Gyrus Rectus	0	0
55	Left Orbito-Frontal Gyri	1	.4
56	Right Orbito-Frontal Gyri	1	.4
57	Left Inferior Frontal Gyrus	1	.4
58	Right Inferior Frontal Gyrus	1	.4
59	Left Superior Frontal Gyrus	6	2.2
60	Right Superior Frontal Gyrus	11	4.0
61	Left Postcentral Gyrus	19	6.9
62	Right Postcentral Gyrus	23	8.4
63	Left Superior Parietal Gyrus	20	7.3
64	Right Superior Parietal Gyrus	25	9.1
65	Left Lingual Gyrus	1	.4
66	Right Lingual Gyrus	0	0
67	Left Cuneus	1	.4
68	Right Cuneus	0	0



CHAPTER 7

CORTICAL MICROINFARCTS AND WHITE MATTER CONNECTIVITY IN MEMORY CLINIC PATIENTS

Doeschka Ferro*
Rutger Heinen*
Bruno de Brito Robalo
Hugo Kuijf
Geert Jan Biessels
Yael Reijmer
On behalf of the Utrecht VCI study group.
* these authors contributed equally to this manuscript.

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ABSTRACT

Background and purpose: Cerebral microinfarcts (CMIs) are associated with cognitive impairment and dementia. CMIs might affect cognitive performance through disruption of cerebral networks. We investigated in memory clinic patients whether cortical CMIs are clustered in specific brain regions and if presence of cortical CMIs is associated with reduced white matter (WM) connectivity in tracts projecting to these regions.

Methods: 164 memory clinic patients with vascular brain injury with a mean age of 72 ± 11 years (54 % male) were included. All underwent 3 tesla MRI, including a diffusion MRI and cognitive testing. Cortical CMIs were rated according to established criteria and their spatial location was marked. Diffusion imaging-based tractography was used to reconstruct WM connections and voxel based analysis (VBA) to assess integrity of WM directly below the cortex. WM connectivity and integrity were compared between patients with and without cortical CMIs for the whole brain and regions with a high CMI burden.

Results: 30 patients (18%) had at least 1 cortical CMI [range 1- 46]. More than 70% of the cortical CMIs were located in the superior frontal, middle frontal and pre- and postcentral brain regions (covering 16% of the cortical surface). In these high CMI burden regions, presence of cortical CMIs was not associated with WM connectivity after correction for conventional neuroimaging markers of vascular injury. WM connectivity in the whole brain and WM voxels directly underneath the cortical surface did not differ between patients with and without cortical CMIs.

Conclusion: Cortical CMIs displayed a strong local clustering in highly interconnected frontal, pre- and postcentral brain regions. Nevertheless, WM connections projecting to these regions were not disproportionally impaired in patients with compared to patients without cortical CMIs. Alternative mechanisms, such as focal disturbances in cortical structure and functioning, may better explain CMI associated cognitive impairment.

INTRODUCTION

Cerebral microinfarcts (CMIs) are small (<5mm) ischemic lesions that are increasingly recognized as a clinically relevant marker in stroke and dementia [1]. Besides post-mortem detection at autopsy, CMIs can now also be detected in vivo on MRI as *chronic cortical* CMIs on T1-weighted MRI and *acute* CMIs on diffusion-weighted MRI [2]

Both pathology and MRI studies have found a consistent association between CMI presence and cognitive impairment, also after adjustments for the presence of co-occurring Alzheimer's disease [3] and conventional neuroimaging markers of vascular injury [4–7]. Although these findings suggest that CMIs play a causative role in the process of cognitive decline, the exact mechanism by which CMIs and cognitive impairment are linked is not yet clear.

Several manifestations of cerebral small vessel disease (SVD), such as white matter hyperintensities (WMHs), lacunes and cerebral microbleeds have been suggested to affect cognitive functioning by disruption of the WM network [8–12]. It appears that the severity and location of these SVD lesions determine their impact on the brain network and consequently cognition [12,13]. Disruption of WM connectivity may also play a role in the relation between cortical CMIs and cognitive impairment. We hypothesized that cortical CMIs exert their effect on the brain network by secondary degeneration of connecting WM pathways. A small study with cerebral amyloid angiopathy (CAA) patients showed that acute subcortical CMIs were indeed associated with changes in the surrounding local WM microstructural integrity [14]. Whether similar effects on WM connectivity occur in relation to chronic cortical CMIs is unknown.

We have previously reported that presence of CMIs in memory clinic patients with vascular brain injury is associated with other neuroimaging markers of vascular injury, a diagnosis of vascular dementia and reduced performance in multiple cognitive domains [4]. In the present study we investigated whether cortical CMIs in this cohort predominantly occur in specific brain regions and if presence of cortical CMIs is associated with impaired WM connectivity in tracts projecting to these regions.

METHODS

Study population

This study involved patients from the TRACE-VCI cohort of the University Medical Center (UMC) Utrecht, an observational prospective cohort study of memory clinic patients with vascular brain injury (i.e. possible VCI) recruited between September 2009 and December 2013 (details described previously [4,15]). Patients were included in the cohort if they presented with cognitive complaints at the memory clinic, and had evidence of vascular brain injury on MRI, operationalized as: 1) WMHs with a Fazekas scale grade ≥ 2 [16]; 2) ≥ 1 lacunar or non-lacunar infarcts; 3) ≥ 1 cerebral microbleeds; 4) ≥ 1 intracerebral haemorrhage(s) or 5) Fazekas scale grade 1 combined with ≥ 2 vascular risk factors [15]. In line with proposed VCI criteria, patients with possible co-existing neurodegenerative disorders (such as Alzheimer's disease) were included in this study cohort, but patients with primary non-vascular or non-neurodegenerative causes of cognitive dysfunction (e.g. brain tumours, depression) were excluded [15]. All patients (n=196) underwent a standardized clinical assessment and 3 tesla brain MRI. Patients were included for the present study if they had complete MRI data, including a diffusion weighted scan (n=177), another 13 patients were excluded due to poor quality of the MRI (n=3) or DTI (n=9, including 2 network outliers) and 1 failure to co-register the AAL-template, resulting in a study population of 164.

Ethical approval was provided by the institutional review board of the UMC Utrecht. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from all participants prior to any research related procedures in the preferred language of the patients. Consent for patients lacking capacity was provided by their legal representative, as allowed by the DSRB

Clinical diagnosis of cognitive impairment

Educational level was rated according to the 7-point Verhage scale [17]. The Clinical Dementia Rating scale (CDR; range: 0-3) was used to assess the severity of cognitive symptoms and functional deficits [18]. The mini-mental state examination (MMSE) in Dutch was used as a global measure of cognitive performance [19].

Severity of cognitive impairment was classified at a multidisciplinary consensus meeting. *No objective cognitive impairment* (NOCI) was defined as cognitive complaints, but

without objective cognitive impairment on neuropsychological testing. *Mild cognitive impairment* (MCI) was defined as complaints or deterioration from prior functioning and objective impairment in at least one cognitive domain, but with no or mild impairment of activities in daily living. *Dementia* was defined as deficits in two or more cognitive domains at neuropsychological testing and who experienced interference of these deficits in daily living. Further etiological diagnoses of dementia were made based on internationally established diagnostic criteria (without knowledge of CSF biomarkers) into *vascular dementia (VaD)* [20], *Alzheimer's disease (AD)* [21] or other (i.e. dementia such as Lewy body, primary progressive aphasia, cortical basal syndrome, unknown etc [15].

MRI

All patients were scanned on a 3 tesla MRI scanner (Philips Achieva or Philips Ingenia [Philips Medical Systems, Best, the Netherlands]). The standardized MRI protocol included a 3D T1-weighted sequence (192 slices, voxel size: 1.00 x 1.00 x 1.00 mm^3, repetition time (TR)/echo time (TE): 7.9/4.5 ms); the following transversal 2D sequences (48 slices, voxel size: 0.96 x 0.96 x 3.00 mm^3): T2-weighted turbo spin echo (TSE; TR/TE: 3198/140 ms), T2*-weighted (TR/TE: 1653/20 ms), and fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time: 11000/125/2800 ms); and diffusion-weighted imaging (DWI; 48 slices, voxel size: 1.72 x 1.72 x 2.50 mm^3, TR/TE: 6600/73 ms, 45 gradient directions with a b-value of 1200 s/mm^2 and one with a b value of 0 s/mm^2 (3 averages)).

Neuroimaging markers

The following neuroimaging markers were rated according to the STRIVE criteria [22] by or under supervision of a neuroradiologist, who was blinded to the clinical condition of the participants: 1) WMHs on the Fazekas scale [16]; 2) Lacunes (presence and number); 3) Cerebral microbleeds (presence and number); 4) Medial temporal lobe atrophy (MTA) using the Scheltens scale averaged for both hemispheres [23].

Brain volume measurements

The following semi-automated workflow was used to obtain brain volumes : 1) automated WMH segmentation of 2D FLAIR images using kNN-TTP [24]; 2) lesion-filling of 3D T1 images using SLF toolbox (http://atc.udg.edu/nic/slfToolbox/index.html) for Statistical Parametric Mapping 12 (SPM Wellcome Department of Cognitive Neurology, Institute of Neurology, Queen Square London) with default settings [25,26]; 3) default settings were used to obtain probabilistic segmentations for gray matter, WM and CSF. Total

brain volume was defined as the sum of the gray and WM volume. Brain volumes were expressed as a percentage of the total intracranial volume.

Rating of cortical CMIs

Cortical CMIs were rated by visual inspection according to previously proposed criteria [2,27]. Cortical CMIs were rated on 3 tesla MRI and were hypointense on T1-weighted imaging, hyper- or isointense on FLAIR or T2-weighted imaging and isointense on T2*-weighted imaging. Lesions had to be strictly intracortical and ≤ 4 mm in the greatest dimension on T1. If the lesions measured substantially larger than 4 mm on T2-weighted imaging or within 1 cm proximity of a larger stroke, it was disregarded as the lesion was considered part of a larger stroke. The lesion had to be visible in two viewing planes of the brain (e.g. sagittal, transversal or coronal plane) and distinct from other structures and lesions such as arteries, veins, enlarged perivascular spaces and cerebral microbleeds. Rating were carried out using MeVisLab (MeVis medical solutions, Bremen, Germany) [28], while the rater was blinded to the clinical condition of the subjects. There was a good intra-rater and interrater (both intra-class correlation coefficient > 0.95) agreement, details regarding the intra- and interrater reliability were published previously [4].

Cortical CMI spatial mapping

Cortical CMI locations from all patients were registered to Montreal Neurological Institute (MNI) space. The automated anatomic labelling (AAL) template [29] was used as overlay on this sample-averaged CMI map. The number of CMIs within each AAL region was determined to assess whether CMIs predominantly occurred in specific brain regions. The AAL regions with a relatively high number of CMI were defined as *high CMI burden regions*, other AAL regions were defined as *low CMI burden regions*. The threshold for high versus low CMI burden regions was arbitrarily set at > 5 CMIs (For a histogram of the CMI numbers per AAL region, see Appendix 1). For 3D rendering of the spatial distribution of cortical CMIs see Figure 1. The volume per AAL region was calculated using automated segmentation using CAT12 after registering the AAL template to the T1 image in patient space.

Diffusion MRI processing and network reconstruction

Diffusion tensor imaging (DTI) scans were preprocessed as previously described [12,30] using ExploreDTI version 4.8.6 (www.exploredti.com) and included subject motion correction, unwarping of eddy current and EPI induced distortions and a robust tensor estimation (including adjustment of the B-matrix) [31–33]. Next, whole brain deterministic

WM tractography was performed using constrained spherical deconvolution (CSD)-based tractography, which is different from standard tensor-based tractography, as it allows reconstruction of crossing fiber pathways [34–36]. Reconstruction of fiber tracts was performed by using uniformly distributed starting seed samples throughout the brain's WM at every voxel with a fiber orientation distribution (FOD) > 0.1 (indicating WM) at a 2x 2 x 2 mm^3 resolution. Fiber reconstruction was terminated if either a deflection in an angle of more than 45 degrees occurred or if a fiber entered a voxel with a FOD of less than 0.1 (indicating no WM). An additional terminating mask was not applied. Brain network nodes were defined using the same AAL template as used for the cortical CMI mapping described above, consisting of 90 cortical and subcortical gray matter regions. The AAL template is a commonly used atlas to define nodes in clinical network studies (8, 9, 11). The atlas has the advantage that the gray matter regions also contain a small portion of WM, which allows streamlines that terminate just before the gray-white matter border to be included in the network, thereby reducing the chance of false negative connections. Nodes were considered to be connected if two end points of a reconstructed fiber bundle lay within those nodes, resulting in a 90 x 90 binary connectivity matrix. This matrix was then weighted by multiplying each connection by the mean fractional anisotropy (FA) or mean diffusivity (MD) of that connection, resulting in two weighted-connectivity matrices for each patient. To reduce partial volume effects in white matter connections a threshold of FA > 0.2 was applied to all the connectivity matrices. See Figure 2 (upper part A-D) for a graphical representation of this workflow.

Measures of Whole brain and regional WM connectivity

The Brain Connectivity Toolbox (www.brain-connectivity-toolbox.net) was used to calculate network properties, including nodal degree (i.e. number of WM connections per node) and nodal strength (here defined as the mean FA or MD of all WM connections to that node) [37]. For this study we used the following constructs: *Whole brain WM connectivity* was assessed by the average FA and MD-weighted nodal strength of all network nodes. *WM connectivity in high and low CMI burden regions* was assessed by the average FA- and MD-weighted nodal strength of the high and low CMI burden regions respectively (see paragraph 2.7, for an overview of regions see Figure 1).

Voxel-based WM diffusion analysis

In addition to the network-based connectivity analyses we also performed a WM voxel-based analysis to assess differences in mean FA and MD. Although we assume that secondary degeneration affects the whole axon running from the cortex to the deep WM,

one may speculate that the WM *directly* underneath the CMI containing cortical (i.e. juxtacortical) surface is primarily affected. As can be seen in Figure 2 (lower part) AAL regions mainly consist of GM, but also contain a small WM section in close proximity to the cortical surface. Therefore, we also calculated the mean FA and MD of the WM voxels within each AAL region (using a WM mask with a WM probability threshold of .75). The FA and MD was averaged across all AAL regions for the high and low CMI burden regions respectively, see Figure 2 (lower part E-G) for a graphical representation).

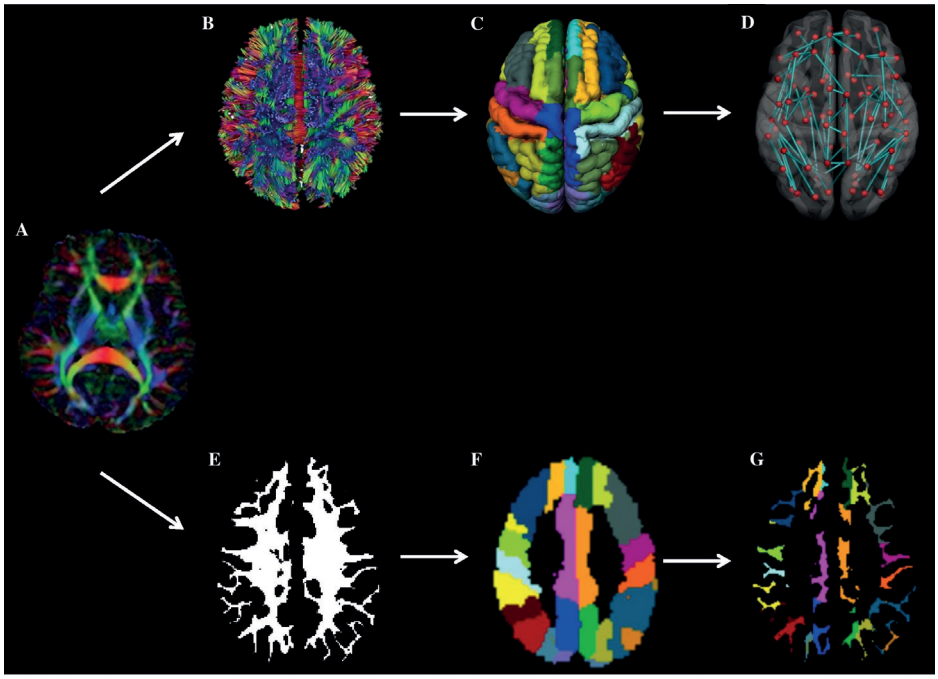

Figure 2: Overview of workflow. In the top panel (network-based approach): from a patients' DTI images (A), WM connections are reconstructed using fiber tractography (B). Next, brain network nodes were defined using the cortical parcellation using the AAL template (C). Subsequently, the structural brain network was reconstructed (D). Weighting of the network was done by multiplying each connection by the mean fractional anisotropy (FA) or mean diffusivity (MD). Finally, the mean FA and MD of connections towards high and low cortical microinfarcts (CMI) burden regions were compared between patients with and without CMIs. In the bottom panel (voxel-based approach), the patient's DTI image (A) is combined with the patient's WM segmentation results (E) and AAL template (F) to assess diffusion properties of the WM voxels in the AAL region (i.e. directly underneath the cortex) (G).

Statistical analysis

Differences in baseline characteristics between patients with and without cortical CMIs were analyzed using independent sample t-tests (for continuous normally distributed

data), χ-square test (for proportions) and Mann-Whitney U test (for continuous, non-normally distributed data). Differences in volume and connectivity strength between brain regions that were identified as high and low CMI burden regions were compared using a paired sample t-test (regardless of CMI presence).

The association between the presence of cortical CMIs (predictor) and FA- and MD-weighted WM connectivity (outcome) was analyzed using linear regression and included sex and age (Model 1) and sex, age and conventional neuroimaging markers (WMH Fazekas scale grade 3, presence of lacunar and non-lacunar infarcts) (Model 2) as covariates. Beta values are reported with 95% confidence interval (CI) and corresponding t-values and degrees of freedom (df). These analyses were carried out separately for whole brain, high and low CMI burden regions. Within the group of patients with cortical CMIs, patients with 1 versus patients with multiple cortical CMIs (predictor) were compared on WM connectivity (outcome) using an independent t-tests and corresponding df. Using a voxel based approach, the association between cortical CMI presence (predictor) and the mean FA and MD of WM voxels in close proximity to the cortex (outcome) was analyzed using linear regression, adjusted for age and sex. A possible interaction effect between cortical CMI presence and clinical diagnosis on WM connectivity was explored in a regression analysis with post hoc Helmert contrasts, where each clinical diagnosis (except the first) was compared to the main effect of all previous diagnoses. Post-hoc power analysis was carried out using G*Power (Heinrich-Heine-University, Dusseldorf, Germany)[38]. All analyses were carried out using IBM SPSS statistics (version 22). A p-value of <.05 was considered significant, p-values were not adjusted for multiple comparisons, as all analyses were planned (not post-hoc).

Data availability statement

Any data on the VCI cohort used in these analyses that is not published within this article is available by request from any qualified investigator.

RESULTS

Baseline characteristics of patients with and without cortical CMIs

The 164 patients had a mean age of 72 (± 11) years and 88 (54%) were male. A total of 134 cortical CMIs were detected in 30 (18%) of the 164 patients. The number of cortical CMIs per patient ranged between 1 and 46, 14 patients had 1 cortical CMI and 16 patients had 2 or more cortical CMIs. Baseline characteristics of patients with and without cortical

CMIs are presented in Table 1. We have previously published the detailed cognitive profile of patients with cortical CMIs in this specific cohort [4]. In short patients with cortical CMIs were more often male, had more non-lacunar infarcts and were more often diagnosed with vascular dementia (all p<.05).

Table 1: Characteristics of patients with and without cortical CMIs

	Cortical CMI absent (N=134)	Cortical CMI Present (N=30)
Demographics		
Age (years)	72±11	71±11
Sex (males)	**67 (50)**	**21 (70)***
Level of education (7 categories)	5 [4-6]	5 [4-6]
Cognitive performance		
MMSE (n=161)	26±3	25±3
CDR	0.5 [0.5-1]	0.5 [0.5-1]
Clinical diagnosis (n=154)		
NOCI	24 (19)	3 (11)
MCI	49 (39)	7 (25)
Alzheimer's dementia	48 (38)	13 (46)
Vascular dementia	**5 (4)**	**5 (18)***
Other[a]	8 (6)	2 (7)
Neuroimaging markers		
Total brain volume (% of TIV)	68±4	67±3
Gray matter volume (% of TIV)	36±2	35±2
WMH (Fazekas scale)	2 [1-2]	2 [1-2]
Presence of non-lacunar infarcts	**26 (19)**	**19 (63)¥**
Presence of lacunar infarcts	43 (32)	12 (40)
Presence of cerebral microbleeds	46 (35)	10 (35)

Abbreviations: CMI=Cortical microinfarct, MMSE = mini-mental state examination, CDR= Clinical dementia rating scale, NOCI = No objective cognitive impairment, MCI= Mild cognitive impairment, TIV = total intracranial volume, WMH = White matter hyperintensities . [a]Other: includes dementia such as Lewy body, primary progressive aphasia, cortical basal syndrome, unknown etc. Data presented as mean ± SD, n (percentages) or median [interquartile range]. * ***p*<.05** ¥ ***p*<.0001**

Characteristics of high and low CMI burden regions

The spatial location of the cortical CMIs was highly clustered, as more than 70% (n=99) of all cortical CMIs were located within 7 AAL regions (*High CMI burden regions*: middle frontal and pre- and postcentral regions of both hemispheres and the right superior frontal region; Figure 1). The other 83 supratentorial brain regions (i.e. *low CMI burden region)* contained the remaining 37 cortical CMIs. The mean volume of the high CMI

burden regions was 68 ± 8.5 ml (16% of total cortical GM volume) compared to 349 ± 41 ml of the low CMI burden regions. Network analyses showed that the high CMI burden regions were more highly connected to the rest of the network than the low CMI burden regions. This was reflected in a higher nodal degree (high burden: 27.2 ± 4.1 vs low burden: 24.0 ± 2.8), higher FA-weighted nodal strength (high burden: .300 ±.020 vs low burden: .293 ±.016) and higher MD-weighted nodal strength (high burden: .940 $\times 10^{-3}$ mm^2/s ± .059 vs low burden: .985$\times 10^{-3}$ mm^2/s ± .059 all comparisons p<.0001).

Association between cortical CMI presence and WM connectivity

The presence of cortical CMIs was not associated with whole brain FA- and MD-weighted WM connectivity (Table 2). Within the group of patients with cortical CMIs, the number of cortical CMIs (cortical CMI=1 vs cortical CMI ≥ 2) also was not related to whole brain FA- ($t_{(df=28)}$=-.71, p=.485) or MD-weighted WM connectivity ($t_{(df=28)}$=.05, p=.964). Regional analyses showed that in the high CMI burden regions, patients with cortical CMIs had marginally higher MD-weighted WM connectivity (reflecting greater WM disruption), although not statistically significant (p=.071) while a similar FA-weighted connectivity was observed (Table 2). These association remained non-significant when conventional neuroimaging markers of vascular injury were entered as covariates in the model (Model 2; Table 2). Within the low CMI burden regions, cortical CMI presence was not associated with FA or MD-weighted WM connectivity (Table 2).

Since not all cortical CMIs were located in the high burden regions, a sensitivity analysis was performed between patients who had CMIs *exclusively* in the high burden regions (n=20) and patients without CMIs, which yielded similar results.

A post-hoc power analysis for CMI presence in high CMI burden regions indicated a power of 0.24 for FA- and 0.44 for MD-weighted connectivity.

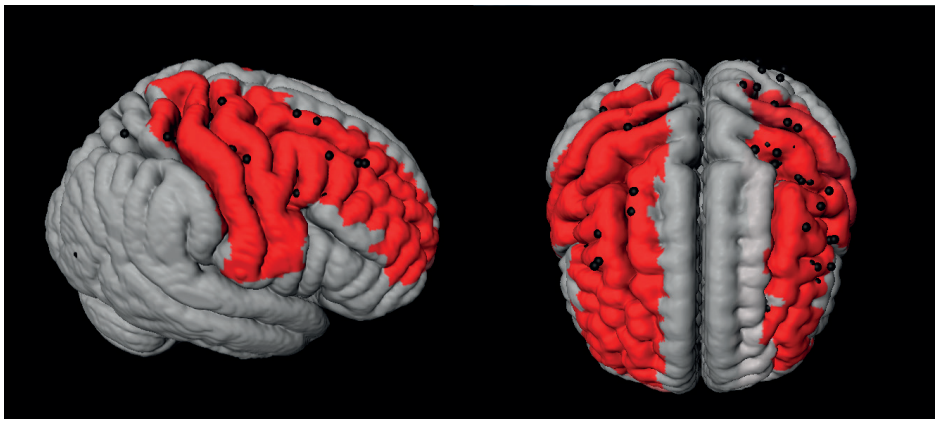

Figure 1: 3D representation of the spatial distribution of cortical microinfarcts (CMIs; represented as black dots) across the brain in the cohort. The red areas represent the Automated Anatomical Labeling (AAL)-atlas regions with a high CMI burden (i.e. the 7 brain regions which contained 75% of all the cortical CMIs).

Voxel-based WM analysis

Limiting our analysis to WM voxels in close proximity to the cortex showed similar results, i.e. the presence of cortical CMIs was not associated with abnormal mean FA and MD in high CMI burden regions (FA: $t_{(df=158)}$=-1.01, p=.314; MD: $t_{(df=158)}$=.753, p=.452) or in low CMI burden regions (FA: $t_{(df=158)}$=-.97, p=.336, MD: $t_{(df=158)}$=1.28 p=.204).

Association between clinical diagnosis, WM connectivity and cortical CMI presence

Clinical diagnosis (NOCI, MCI, AD or VaD) was a significant predictor of whole brain FA- ($F_{(df=4,152)}$= 13.9, p=.005) and MD-weighted WM connectivity ($F_{(df=4,152)}$=10.2, p=.008). Post-hoc analyses revealed that this effect was driven by the patients with the most severe clinical diagnosis, i.e. patients with AD and VaD had abnormal WM connectivity compared to the other groups (Figure 3). No significant interaction was observed between cortical CMI presence and clinical diagnosis on FA- or MD-weighted WM connectivity ($F_{(df=4,152)}$= .42, p=.783) or MD ($F_{(df=4,152)}$=.67, p=.700), indicating that the association between cortical CMI presence and WM connectivity did not differ across the various clinical diagnoses. In a sensitivity analysis of patients without dementia (n= 83) presence of cortical CMIs was also not associated with whole brain FA ($t_{(df=79)}$=.43, p=.667) or MD ($t_{(df=79)}$=-.92, p=.359).

Table 2: Association between cortical CMI presence and whole brain and regional FA- and MD-weighted WM connectivity in high and low CMI burden regions.

	Cortical CMI absent (N=134)	Cortical CMI Present (N=30)	Model 1			Model 2		
			Beta [95% CI]	*t*-value	*p*	Beta [95% CI]	*t*-value	*p*
Whole brain								
FA	.294 ± .017	.290 ± .017	-.093 [-.256;.070]	*-1.19*	.234	-.052 [-.234;.104]	-.69	.490
MD[a]	.979 ± .057	.993 ± .061	.087 [-.047;.228]	*1.27*	.208	.018 [-.108;.138]	.26	.795
High Cortical CMI burden regions								
FA	.301 ± .020	.296 ± .021	-.109 [-.254;.036]	-1.40	.165	-.059 [-.216;.098]	-.78	.440
MD[a]	.936 ± .057	.958 ± .066	.136 [-.013;.285]	1.82	.071	.030 [-.102;.162]	.41	.683
Low Cortical CMI burden regions								
FA	.294 ± .016	.290 ± .016	-.091 [-.228;.068]	-1.16	.247	-.051 [-.204;.102]	-.67	.501
MD[a]	.983 ± .058	1.000 ± .063	.082 [-.050;.208]	1.20	.231	.017 [-.102;.130]	.24	.808

Abbreviations: CMI= Cerebral microinfarct, FA = Fractional anisotropy-weighted WM connectivity, MD = Mean diffusivity-weighted WM connectivity. Lower FA and higher MD indicated impaired WM connectivity.

[a]MD values$\times 10^{-3}$ mm^2/s

Model 1: Covariates age and sex (degrees of freedom=160).

Model 2: Covariates sex, age, WMH Fazekas grade 3, presence of lacunar and non-lacunar infarct (degrees of freedom=157)

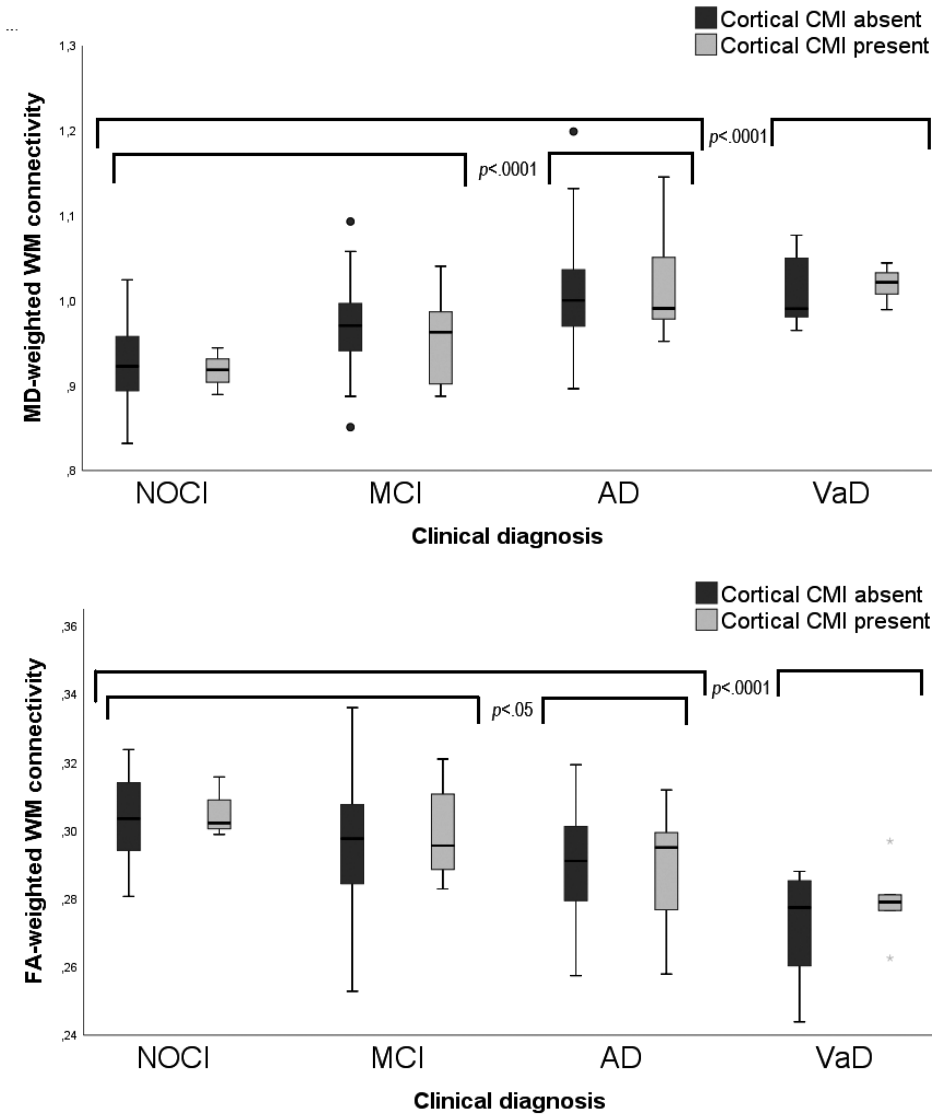

Figure 3: Boxplots of FA- (upper) and MD- (lower) weighted WM connectivity between patients with and without cortical CMIs (labels) stratified for clinical diagnosis (X-axis). Note: MD values×10^{-3} mm^2/s.
Abbreviations: CMI= Cerebral microinfarct, NOCI = No objective cognitive impairment, MCI= Mild cognitive impairment, AD =Alzheimer's disease, VaD= Vascular dementia.

DISCUSSION

This study shows that cortical CMIs in memory clinic patients vascular brain injury display a strong spatial clustering, as more than 70% of the cortical CMIs were located in frontal, precentral, and postcentral brain regions covering only 16% of the cortical surface. These high CMI burden regions proved to be strongly connected with the rest of the network. However, we found no evidence that the actual presence of cortical CMIs was related to disruption of WM connections to either the high CMI burden regions or within the whole brain.

Cortical CMIs showed a strong predilection for the frontal, precentral and postcentral brain regions. A similar pattern of CMIs has been found in memory clinic patients [6], but also in patients with ischemic stroke [39,40], Alzheimer's disease [41] and even in patients with CAA, where vessels are typically affected in the posterior brain regions [42]. This preferential lesion location is likely to be of etiological significance. A similar predilection for frontal, pre- and postcentral brain regions was observed in patients with post-stroke cognitive impairment, where a thromboembolic origin has been suggested [43]. Future research is encouraged to further explore the relation between lesion location and the pathophysiological origin of cortical CMIs using larger study samples.

We hypothesized that cortical CMIs might affect cognitive performance by disruption of cerebral networks. We have previously reported a relationship between cortical CMIs and reduced cognitive performance on multiple domains in this same cohort [4]. In the current study we investigated impaired WM connectivity as possible underlying mechanism. As lesion location could be crucial for its effect on the cerebral network [13], regions with high and low CMI burden were compared. We established no convincing relationship between cortical CMIs and WM connectivity, as the association between cortical CMIs and impaired WM connectivity in high CMI burden regions disappeared after correcting for conventional neuroimaging markers of vascular injury. These findings were in line with our voxel based analysis, showing no local disturbances in the WM directly below the cortical surface of high CMI burden regions. Independent of CMI presence, we did find that patients with dementia, especially VaD, presented with impaired WM connectivity, which corresponds to the known association between network disruption and cognitive deficits [44].

Previous studies in patients with SVD found a disruptive effect of SVD MRI-manifestations, such as WMHs and lacunes, on WM connectivity [8–10,12,14,45–47]. Our study is the first to assess the effect of cortical CMIs and did not observe an effect on WM connectivity. This contrasting finding could be explained by the fact that these subcortical manifestations of SVD have a more direct impact on WM integrity, while cortical CMIs are thought to exert their effect indirectly through secondary degeneration. The limited size of the cortical CMIs could also account for the lack of association, as for macroscopic cortical infarcts the size of the lesion is directly correlated to the extent of the axonal injury [48]. Considering the average lesion volume of cortical CMIs on 3-T MRI is max 0.1 ml, their effect on WM connectivity could indeed be modest and not of major clinical relevance.

Since cortical CMIs were not related to WM connectivity, other underlying mechanisms should be considered to explain how cortical CMIs affect cognitive impairment. Our earlier work showed that the cortical CMIs were mainly associated with deficits in "cortical" cognitive domains, including visuoconstruction and language [4,6] suggesting that cortical CMIs potentially affect cognition by disruption of local cortical processes. This notion is supported by a mouse study, that found diminished neural activity and neurovascular coupling in the cortical tissue surrounding the CMI [49]. An alternative explanation is that cortical CMIs are a marker of more widespread vascular brain damage that affects cognitive performance [1,2]. As cortical CMIs smaller than 1mm escape detection on 3 tesla MRI, larger visible cortical CMIs probably only represent the tips of the iceberg. Moreover, it is important to clarify the etiological underpinning of both the detectable as well as these smaller cortical CMIs in order to develop therapeutic strategies that counter cognitive decline.

The strength of our study includes the use of high quality imaging and clinical data of this memory clinic cohort and the systematic approach in cortical CMI rating. Moreover, this study utilized two different DTI approaches to assess the relation with cortical CMIs; a network-based analysis and a voxel-based analysis. However, this study also has some limitations. Firstly, the sample size of cortical CMI cases in our cohort was small, since MRI detectable cortical CMIs occur only in approximately a quarter of memory clinic patients [6]. Based on our post-hoc power analysis for the observed effect sizes in our study, it would be recommended to replicate results in a larger cohort. Another possible limitation concerns the heterogeneity of the cohort, which includes memory clinic patients with different etiologies, severity of cognitive dysfunction and with large

variation in cortical CMI burden. Although this reflects daily clinical practice, it may have reduced our sensitivity to detect abnormalities in WM connectivity due to cortical CMIs.

Conclusion: We showed that cortical CMIs in memory clinic patients displayed a strong local clustering in frontal and central brain regions, which warrants further investigations into their etiology. Nevertheless, the WM connections projecting to these regions were not impaired in patients with cortical CMIs. This does not support the hypothesis that cortical CMIs affect the brain's integrity through disturbance of WM connections, although further studies, also in larger cohorts with high burden of cortical CMIs, are recommended to confirm our observations.

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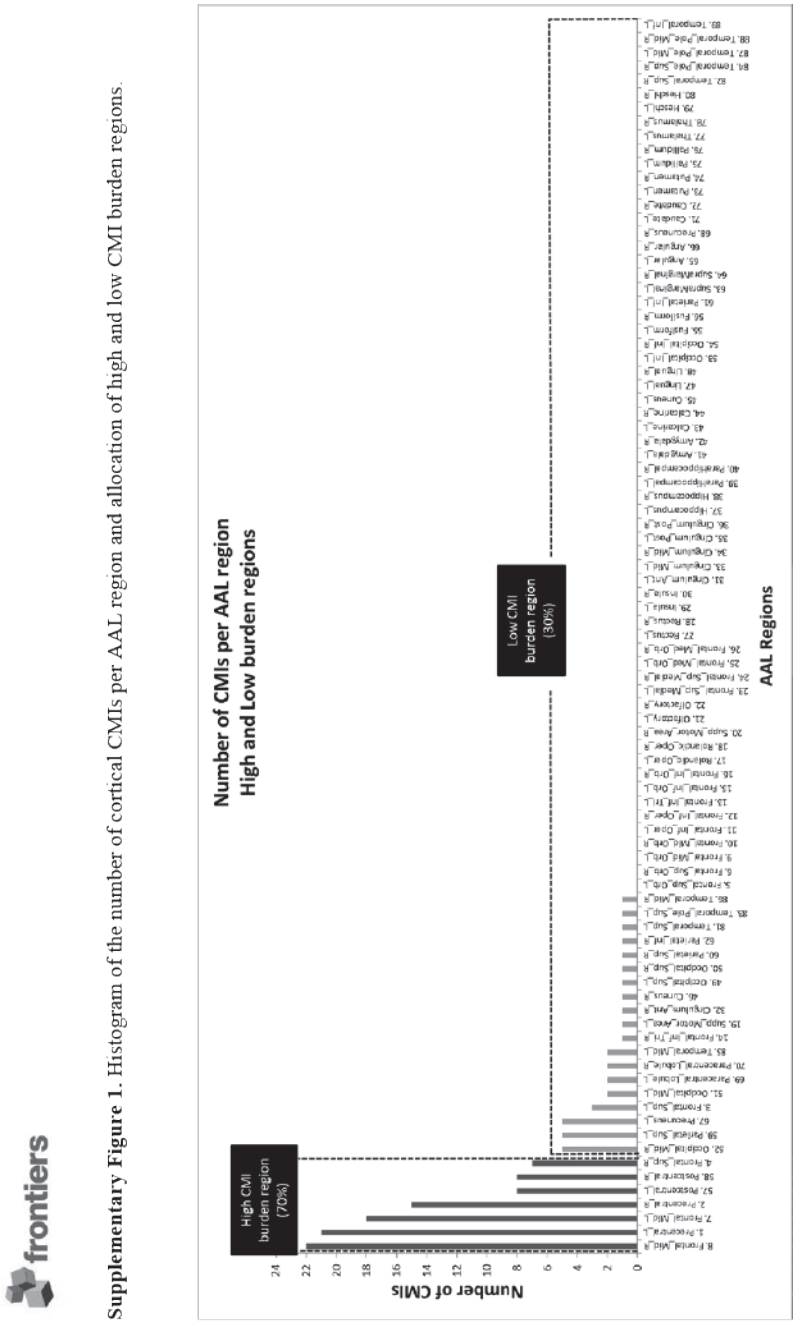
SUPPLEMENT

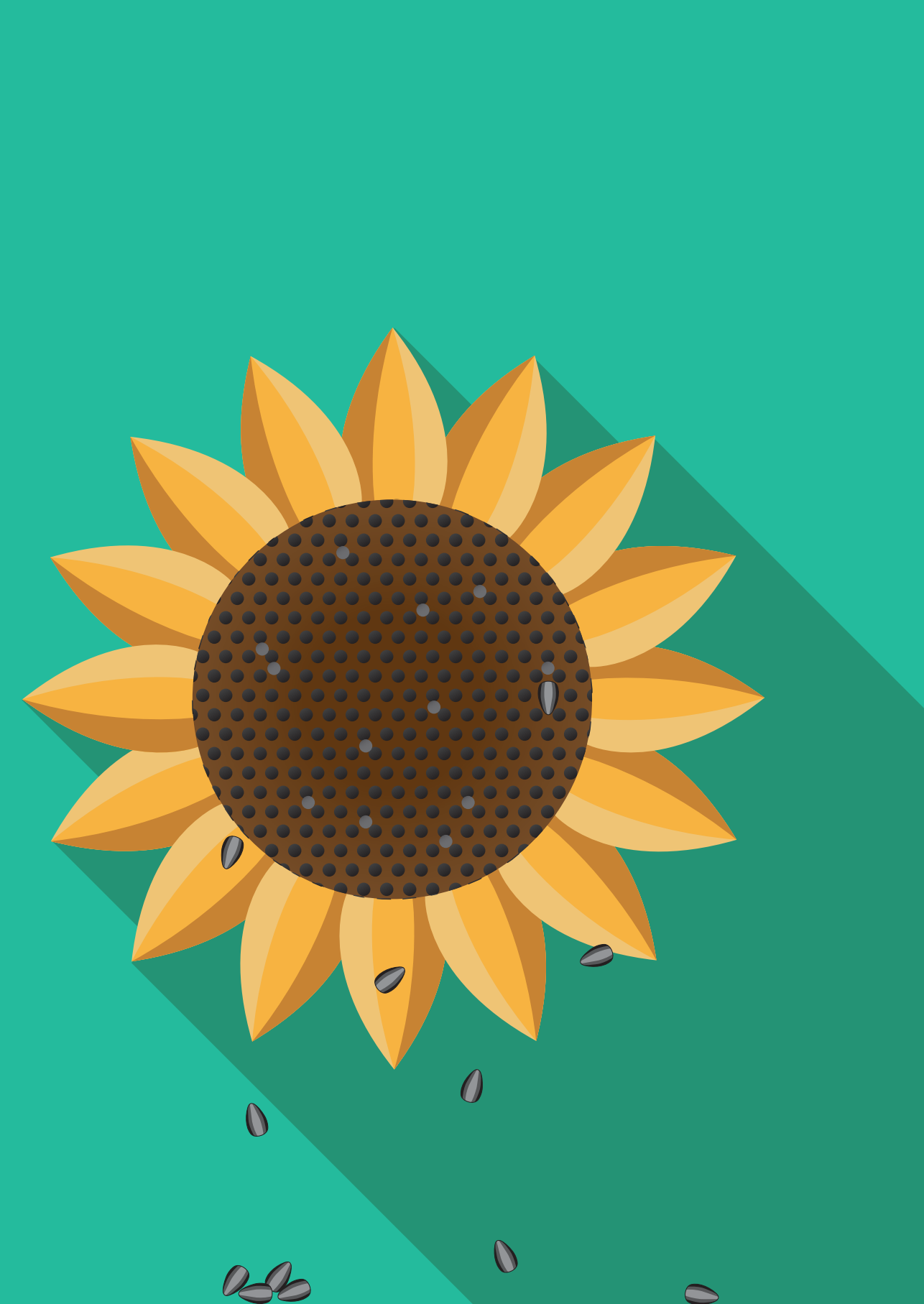


Supplementary Figure 1. Histogram of the number of cortical CMIs per AAL region and allocation of high and low CMI burden regions.

Supplementary Figure 1. Histogram of the number of cortical CMIs per AAL region and allocation of high and low CMI burden regions.

PART III

FUNCTIONAL IMPACT OF CEREBRAL MICROINFARCTS



CHAPTER 8

CLINICAL RELEVANCE OF ACUTE CEREBRAL MICROINFARCTS IN VASCULAR COGNITIVE IMPAIRMENT

Doeschka Ferro*
Hilde van den Brink*
Lieza Exalto
Jooske Boomsma
Frederik Barkhof
Niels Prins
Wiesje van der Flier
Geert Jan Biessels
On behalf of the TRACE-VCI study group.
* these authors contributed equally to this manuscript.

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ABSTRACT

Objective: To determine the occurrence of acute cerebral microinfarcts (ACMIs) in memory clinic patients and relate their presence to vascular risk and cognitive profile, CSF and neuroimaging markers, and clinical outcome.

Methods: The TRACE-VCI study is a memory clinic cohort of patients with vascular brain injury on MRI (i.e. possible vascular cognitive impairment (VCI)). We included 783 patients (mean age 67.6 ±8.5, 46% female) with available 3 tesla diffusion-weighted imaging (DWI). ACMIs were defined as supratentorial DWI hyperintensities <5mm with a corresponding hypo/isointense ADC signal and iso/hyperintense T2*-weighted signal.

Results: 23 ACMIs were found in 16 of the 783 patients (2.0%). Patients with ACMIs did not differ in vascular risk or cognitive profile, but were more often diagnosed with vascular dementia (odds ratio (OR) 5.1; 95% CI 1.4-18.9, p=.014). ACMI presence was associated with lower levels of amyloid beta (p<.004) and with vascular imaging markers (lacunar infarcts; OR 3.5; 1.3-9.6, p=.015, non-lacunar infarcts; OR 4.1; 1.4-12.5, p=.012, severe white matter hyperintensities; OR 4.8; 1.7-13.8, p=.004, microbleeds; OR 18.9; 2.5-144.0, p=.0001). After a median follow up of 2.1 years, the risk of poor clinical outcome (composite of marked cognitive decline, major vascular event, death and institutionalization) was increased among patients with ACMIs (HR 3.0; 1.4-6.0, p=.005).

Conclusion: In patients with possible VCI, ACMI presence was associated with a high burden of cerebrovascular disease of both small and large vessel etiology and poor clinical outcome. ACMIs may thus be a novel marker of *active* vascular brain injury in these patients.

INTRODUCTION

Cerebral microinfarcts are small ischemic lesions that are increasingly recognized as an important contributor to cognitive decline and dementia [1]. Until recently microinfarcts were only considered to be detectable on brain autopsy, but they can now be visualized in vivo on MRI [2,3]. *Acute cerebral microinfarcts* (ACMIs) are detectable on diffusion-weighted MRI (DWI) for up to two weeks after their onset[1]. Despite this narrow window, ACMI detection provides valuable information on microinfarct occurrence and may help identify individuals at risk for ongoing vascular brain injury.

ACMIs, previously referred to as small DWI hyperintense lesions, have been reported in patients with cerebral amyloid angiopathy (CAA) [4,5], hemorrhagic stroke [6–11] and memory clinic patients [12,13]. These studies -with modest sample sizes- demonstrated an association between ACMIs and other small vessel disease (SVD) imaging markers, but not a link with a specific vascular risk profile. The relation with CSF biomarkers has not yet been investigated, despite the known association between microinfarcts and CAA. Studies assessing the correlation between ACMIs and cognitive performance have yielded inconclusive results[12,13].

Follow up studies of patients with acute hemorrhagic stroke found that ACMIs were associated with increased disability after 3 months [8,14] and stroke and vascular death after 3 years [7]. However, no studies have focused on the long-term cognitive consequences of ACMIs.

This study investigated the occurrence of ACMIs in a memory clinic cohort with possible vascular cognitive impairment (VCI) and their association with vascular risk and cognitive profile, CSF and neuroimaging markers and clinical outcome.

METHOD

Population

This study involved patients from the TRACE-VCI study, an observational prospective cohort study of 861 consecutive memory clinic patients with vascular brain injury on MRI (i.e. possible VCI) [15]. Patients were included from VU Medical Center (n=665) and the University Medical Center Utrecht (n=196) between September 2009 and December 2013. All patients had evidence of vascular brain injury on MRI, operationalized as the presence of at least one of the following neuroimaging markers (note that multiple criteria could apply to a single patient): a) white matter hyperintensities (WMH) Fazekas scale grade ≥2 (46% of included patients) [16] b) Fazekas scale 1 and two vascular risk factors (e.g. hypertension, diabetes etc) (36%) c) ≥1 lacunar infarcts (22%) d) ≥1 non-lacunar infarct (10%) e) ≥1 cerebral microbleed (43%) f) ≥1 intracerebral hemorrhage (2%). Presence of co-existing neurodegenerative disorders (such as Alzheimer dementia) was accepted in line with earlier proposed VCI criteria [17]. Patients with primary non-vascular/non-degenerative causes of cognitive dysfunction (such as traumatic brain injury, inflammatory disease and brain tumors), psychiatric disease (other than depression) and patients with monogenetic (non-) vascular causes were excluded from the cohort. Extensive details on the inclusion and exclusion criteria of the cohort have been previously published [15]. For the present study only the patients with available DWI and ADC sequences on 3.0 Tesla MRI were selected (n=788). A further 4 patients were excluded due to insufficient scan quality and one patient because revision of the MRI revealed a tectum glioma. This resulted in a study population of 783 patients. All study patients underwent a standardized, one-day assessment, including clinical interview, physical examination, neuropsychological testing and MRI.

Standard protocol approvals, registrations and patient consents

All study patients provided written informed consent prior to research related procedures. Ethical approval was granted by the institutional review board of the two hospitals. The study was conducted in adherence to the Declaration of Helsinki.

Vascular risk factors

Hypertension was defined as a blood pressure above 140/90 mmHg, use of antihypertensive medication or reported in medical history. *Hypercholesterolemia* was identified based on medical history or use of cholesterol lowering medication. *Diabetes mellitus* was identified based on medical history or use of appropriate medication.

Obesity was defined as a body mass index (BMI) ≥ 30, calculated as weight in kilograms divided by height in meters squared. *History of stroke* was based on a history of clinical hemorrhagic or ischemic stroke. *History of atrial fibrillation* was based on a history of paroxysmal and permanent atrial fibrillation and *history of ischemic cardiac disease* was based on a history of myocardial infarction, surgery, or endovascular treatment for coronary artery disease.

Level of cognitive impairment and clinical diagnosis

Patients were divided in three levels of cognitive impairment; *Dementia* was diagnosed if there was a deficit in two or more cognitive domains at neuropsychological testing and interference with daily living. Further etiological diagnoses of dementia were made based on internationally established diagnostic criteria (without knowledge of CFS biomarkers) into *vascular dementia* [18], *Alzheimer's disease (AD)* [19], other neurodegenerative (e.g. Lewy body, frontotemporal dementia) or unknown origin. *Mild cognitive impairment* (MCI) was defined as complaints or deterioration from prior functioning and objective impairment in at least one cognitive domain, with no or mild impairment of activities in daily living. Patients with complaints but without objective cognitive impairment on neuropsychological testing were classified as "*No objective cognitive impairment*" (NOCI).

Neuropsychological assessment and level of education.

The education level of patients was rated according to the 7-point Verhage criteria [20]. The Clinical Dementia Rating (CDR; 0-3) was used to assess the severity of cognitive symptoms and associated functional deficits [21]. A Dutch version of the mini-mental state examination (MMSE) was used as a measure of global cognitive performance [22]. An extensive neuropsychological test battery was carried out covering the following domains: *working memory*, *memory*, *attention and executive functioning*, *perception and construction* and *processing speed*. Detailed information on the tests comprising these cognitive domains has been previously reported [15]. Domain scores per patient were created by averaging individual z-scores per subtest and then standardized again into z-scores.

MRI acquisition and neuroimaging markers

All participants underwent an elaborate brain MRI protocol that included at least 3D T1, 2D T2 and T2*-weighted/susceptibility-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, as well as DWI and ADC. Details on the MRI sequence parameters were

previously published [15]. Brain volumetric measurements were carried out using automated segmentation using Computational Anatomy Toolbox - CAT12 [23]. The automated CAT 12 segmentations for *total brain, grey and WMH volume* were subsequently corrected for by hand segmentation of stroke lesion volume (e.g. lacunes, non-lacunar infarcts, hemorrhages). Neuroimaging markers were analyzed by visual inspection. Microbleeds, lacunar and non-lacunar infarcts were rated according to the STRIVE criteria [24], WHM were rated according to the modified Fazekas scale [16] and medial temporal lobe atrophy (MTA) according to the Scheltens scale (scores for both hemispheres averaged) [25].

ACMI rating

ACMIs were rated in accordance with criteria proposed by a group of international collaborators [1] as hyperintense lesions on DWI <5mm along the longest diameter with a corresponding hypo- or isointense signal on ADC and hyper- or isointense signal on T2*-weighted image. Following these criteria ACMIs within the first week (hyperintens DWI; ADC hypointensity) and thereafter up to approximately two weeks (hyperintens DWI; isointense ADC) were detected [26]. An example of two ACMIs is displayed in figure 1. ACMI screening procedure started with assessment of the DWI sequence of the supratentorial brain in the axial followed by the sagittal plane. In case a hyperintense DWI lesion was found, ADC and T2*-weighted images were examined as well to confirm the finding. The total number of ACMIs per patient, as well as size and location was recorded. Location was classified as either cortical, subcortical white matter or deep grey matter (basal ganglia, thalamus). All scans were rated using MeVisLab (MeVis medical solutions, Bremen, Germany) by one trained rater (H.B.) blinded to the clinical condition of the patient. There was a good intrarater agreement (test set 50 scans; k =.85; Dice's similarity index (DSC) =.86) and interrater agreement (test set 50 scans; k =.83; DSC=.80 to a second trained rater (D.F.)).

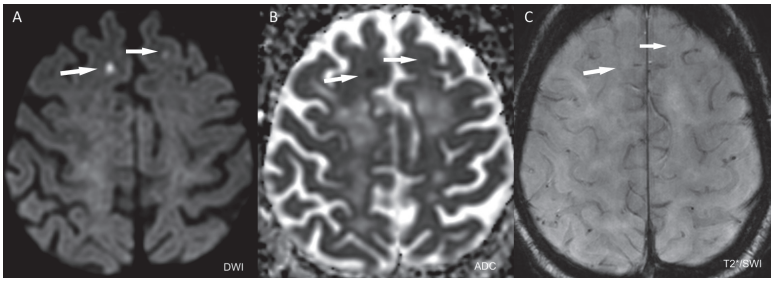

Figure 1: Example of two ACMIs in a single patient. Legend: Two ACMIs (arrows left and right frontal lobe) in one patient. (A) There is a hyperintens signal on DWI, (B) corresponding hypointens signal on ADC and (C) isointens (i.e. not distinctly visible) signal on T2*-weighted image (right).

CSF biomarkers

Lumbar puncture to collect CSF was carried out in a subset of the patients at the physician's and patient's discretion. CSF concentrations of amyloid beta 1-42 (Aβ) (n=499), tau (Tau) and total tau phosphorylated at threonine 181 (p-Tau) (n=492) were determined [27].

Follow up data

Follow up data was collected approximately 2 years after baseline inclusion. According to the predefined TRACE-VCI protocol, only patients with a MMSE score≥20 or a CDR≤1 at baseline were eligible for follow up (n=648; i.e. 83% baseline population). Data was collected from patients and care-givers during a visit at the outpatient clinic. Patients who were not able to attend the clinic where contacted by phone. If patients or relatives could not be reached, the general practitioner or doctor of the nursing home was contacted, but only if patients had provided informed consent at baseline visit. 14 Patients were lost to follow up and 5 patients had withdrawn their consent, resulting in a follow up population of 629 patients. Information was collected regarding death (including vascular cause), stroke, cardiovascular events and institutionalization (due to either somatic or cognitive decline). Cognitive symptoms were rated using the CDR [21]. *Poor clinical outcome* was defined as a composite of (1) marked cognitive decline (operationalized as change in CDR of ≥1 and/or institutionalization due to cognitive dysfunction, (2) occurrence of a major vascular event (stroke (TIA excluded) or myocardial infarctions), (3) death and (4) institutionalization due to reasons other than cognitive decline.

Statistical analysis

Differences between patients with and without ACMIs were analyzed with independent T-tests for normally distributed data (age, log transformed CSF biomarkers), χ-square test for categorical data (sex, vascular risk factors) and Mann-Whitney U tests for non-normally distributed continuous data (education, CDR, microbleed count, MTA). Binary logistic regression was used to analyze the odds (with 95% confidence interval) of ACMI presence given the clinical diagnosis (odds ratio (OR) reflects "severity steps" in clinical diagnosis) and presence of imaging markers. Linear regression was used to analyze the association between ACMI presence and cognitive functions (adjusted for age, sex and education) and brain volumetrics (adjusted for intracranial volume).

Follow up data was analyzed using Cox proportional hazard model, to assess the risk of ACMI presence on the time to event for the composite *poor clinical outcome*,

and separately for each component (death, stroke, cardiovascular events and institutionalization). Results are presented with and without adjustment for age, sex and level of cognitive impairment and also for other vascular imaging markers. *Change in CDR* was analyzed using a Mann-Whitney U test. IBM SPSS statistics (version 22) was used for data analysis, a p-value < .05 was considered significant.

Data availability statement

Any data on the TRACE-VCI cohort used in these analyses that is not published within this article is available by request from any qualified investigator.

RESULTS

ACMI occurrence in the cohort

A total of 23 ACMIs was found in 16 of the 783 patients (2%). Another 6 larger DWI positive lesions were found, but disregarded due to size (between 6-10mm). Thirteen patients had a single ACMI and 3 patients had multiple ACMIs (range 2-5). The majority of the ACMIs was located in the subcortical white matter (n= 16, 70%), 4 ACMIs (17%) were located in the cortical grey matter and 3 ACMIs (13%) were located in the deep grey matter.

Demographics and vascular risk factors in patients with and without ACMIs

The mean age of patients in the study population was 67.6 (SD 8.5) years and 46% was female. No differences in demographics or vascular risk factors were observed between patients with ACMIs compared to patients without ACMIs (Table 1).

Cognitive performance in patients with and without ACMIs

Patients with and without ACMIs did not differ with respect to clinical diagnosis, severity of cognitive impairment or cognitive profile (i.e. scores on the five domains) (Table 2). However, patients with ACMIs were more likely to have received the etiological diagnosis vascular dementia (4% ACMI absent vs 8% in the ACMI present, OR 5.1 [1.4-18.9], p=.014), but not Alzheimer's disease (35% ACMI absent vs 25% ACMI present, OR 0.6 [.2-2.0], p=.418).

Table 1: Demographics and vascular risk factors in patients with and without ACMIs

	No ACMI (n = 767)	ACMI Present (n=16)	p
Demographics			
Age (y)	67.5 ±8.5	69.4 ±9.6	.386
Sex (female)	352 (46)	6 (38)	.505
Education	5 [1-7]	4.5 [2-7]	.310
Vascular risk factors			
Hypertension	646 (84)	14 (88)	.722
Hypercholesterolemia	342 (45)	6 (38)	.573
Diabetes mellitus	139 (18)	2 (13)	.565
Current smoking (n=775)	156 (21)	2 (13)	.435
Obesity (n=773)	158 (21)	3 (19)	.836
History of reported stroke	68 (9)	3 (19)	.186
History of ischemic heart disease	55 (7)	1 (6)	.862
Atrial fibrillation (n=777)	31 (4)	1 (6)	.667

Abbreviations: ACMI=Acute cerebral microinfarct. Data presented as group mean ±SD, n (%) and median [range]. All comparisons between groups with and without ACMIs were performed with a χ-square test, except age (T-test) and education (Mann-Whitney U).

Table 2: Clinical diagnosis and cognitive performance in patients with and without ACMIs.

	No ACMI (n=767)	ACMI Present (n=16)	OR (95% CI)	*p*
Clinical diagnosis			1.2 [.61-2.18]	.450
NOCI	178 (23)	2 (13)		
MCI	195 (25)	6 (38)		
Dementia	394 (51)	8 (50)		
Cognitive functions			**Mean estimated differences [95% CI]**	
CDR	0.5 [0-3]	0.5 [0-2]		.542
MMSE (n=779)	24.3 ±4.8	25.1 ±4.9	-1.12 [-3.46; 1.21]	.346
Memory (n=778)	.002 ±1.006	-.077 ±.676	-.08 [-.57; .40]	.733
Working memory (n=761)	.004 ±1.002	-.189 ±.899	.12 [-.35; .58]	.627
Processing speed (n=761)	.000 ±1.008	-.020 ±472	.13 [-.62; .35]	.588
AEF (n=771)	.005 ±1.002	-.264 ±.885	.14 [-.35; .64]	.568
PC (n=645)	-.002 ±1.004	.105 ±.807	-.17 [-.70; .36]	.521

Abbreviations: ACMI=Acute cerebral microinfarct; OR=odds ratio; NOCI=No objective cognitive impairment; MCI=mild cognitive impairment; CDR=Clinical Dementia Rating; AEF=attention and executive functioning; PC=perception and construction.
Data are presented as mean ±SD, n (%) or median [range].
Statistical testing. Clinical diagnosis: Logistic regression with the odds of ACMI presence given diagnosis (odds ratio reflects "severity steps" in clinical diagnosis). Cognitive functions: Linear regression with estimated mean differences adjusted for age and sex of groups with and without ACMIs. CDR compared between group with and without ACMIs with Mann-Whitney U.

Neuroimaging markers in patients with and without ACMIs

The presence of ACMIs was associated with WMH (both WMH volume and presence of Fazekas score 3), presence of lacunar and non-lacunar infarcts and microbleeds (all $p<.05$, Table 2). In patients with ACMIs compared to those without ACMIs, microbleeds were present both in a higher proportion of cases and in larger numbers per case (both $p=.0001$). Moreover the spatial distribution of microbleeds was different in patients with ACMIs, with relatively more microbleeds in deep and mixed (i.e. both deep and lobar) locations than in patients without ACMIs (both $p<.05$). No association was found between ACMIs and total brain volume, grey matter volume or MTA (table 2). Regarding the CSF biomarkers, the presence of ACMIs was associated with a lower level of CSF Aβ ($p=.011$), with marginally higher levels of Tau or p-Tau (p=.065 and p=.062, respectively, Table 3).

Table 3: Neuroimaging and CSF biomarkers patients with and without ACMIs

	No ACMI (n = 767)	ACMI Present (n=16)	Mean estimated difference [95% CI]	*p*
Brain volumetrics				
Total brain volume (ml) (n=768)	1034 ±116	1031 ±100	8.9 [-23.8; 41.6]	.592
Total grey matter volume (ml) (n=768)	565 ±76	557 ±60	10.0 [-23.3; 43.2]	.557
WMH volume (ml) (n=768)	11.8 ±15.3	30.0 ±27.5	na*	**.0001**
Imaging markers			**OR [95% CI]**	
WMH (Fazekas 3)	88 (12)	6 (38)	4.8 [1.7; 13.8]	**.004**
Non-lacunar infarcts	71 (9)	5 (31)	4.1 [1.4; 12.5]	**.012**
Lacunar infarcts	163 (21)	8 (50)	3.5 [1.3; 9.6]	**.015**
Microbleeds number (n=776)	0 [0-500]	12 [0-200]		**.0001**
All microbleed presence (n=776)	330 (43)	15 (94)	18.9 [2.5; 144.0]	**.005**
Strictly lobar	214 (65)	6 (40)	1.5 [.5 ;4.1]	.467
Strictly deep	42 (13)	3 (20)	3.9 [1.1; 14.3]	**.040**
Mixed	73 (22)	6 (40)	5.5 [2.0; 15.7]	**.001**
MTA (n=758)	1 [0-4]	1 [0-2.5]		.928
CSF biomarkers				
Aβ ng/L (n=499)	721.3 ±303.2	470.8 ±174.3	na*	**.011**
Tau ng/L (n=492)	471.4 ±329.5	715.6 ±445.3	na*	.065
p-Tau ng/L (n=492)	63.7 ±34.1	96.6 ±69.1	na*	.062

Abbreviations: ACMI=Acute cerebral microinfarct; OR=odds ratio; WMH=White matter hyperintensities; MTA =medial temporal lobe atrophy; Aβ= amyloid beta; p-Tau=phosphorylated tau. Data are presented as group mean ±SD, n (%) and median [range]. Statistical testing: Brain volumetrics and CSF biomarkers: Lineair regression between group with and without ACMIs. * WMH volume and CSF biomarkers log transformed. Imaging markers: Logistic regression with the odds of the imaging marker given ACMI presence. MTA and Microbleed number analyzed with Mann-Whitney U test. Bold text indicates $p<.05$.

Table 4: Events during follow up for patients with and without ACMIs

	ACMI absent (n=615)	ACMI present (n=14)	Model 1 HR [95% CI]	*p*	Model 2 HR [95% CI]	*p*	Model 3 HR [95% CI]	*p*
Poor clinical outcome*	148 (24)	7 (50)	3.0 [1.4-6.0]	**.005**	2.8 [1.3-6.0]	**.010**	2.6 [1.2-5.8]	**.017**
Death (n=629)	58 (9)	2 (14)	1.9 [.5-7.7]	.379	1.5 [.4-6.1]	.600	1.1 [.3-4.8]	.879
Stroke (n=612)	16 (3)	3 (21)	9.3 [2.7-31.9]	**.0001**	6.6 [1.8-23.5]	**.004**	7.5 [1.8-31.3]	**.006**
Ischemic	14 (2)	3 (21)						
Hemorrhage	2 (.3)	0						
Cardiovascular event (n=599)	12 (2)	0		(..)		(..)		(..)
Institutionalization (n=609)	51 (9)	4 (29)	3.9 [1.4-10.8]	**.009**	4.9 [1.7-14.2]	**.003**	3.6 [1.2-10.6]	**.019**
Due to cognitive decline	34 (6)	3 (21)						
Change CDR* (n=535)	0 [-1 ;2]	.5 [0 ;2]		*.128*				

Abbreviations: ACMI=Acute cerebral microinfarct; OR=odds ratio; CDR=Clinical Dementia Rating. *Poor clinical outcome defined as cognitive decline, major vascular events (including stroke), death or institutionalization. Data are presented as n (%) and median [range]. (..) insufficient data to perform statistical analysis. HR was calculated with Cox proportional hazard model. Model 1: unadjusted; Model 2: adjusted for age, sex and level of cognitive impairment at baseline; Model 3: adjusted for vascular imaging markers at baseline (presence of Fazekas 3, microbleeds lacunar and non-lacunar infarcts). Change in CDR was analyzed with a Mann-Whitney U test. Bold text indicates $p<.05$

Follow up

Follow up data was obtained from 629 of 648 eligible (97%) patients (n=615 (97%) of patients without ACMIs; n=14 (100%) from patients with ACMIs) after a median follow up of 2.1 years [range 0.2-3.0]. Patients with ACMIs had a higher risk of *poor clinical outcome*, also after adjusting for age, sex and cognitive impairment at baseline and additionally for vascular imaging markers (Table 4, all $p<.02$). ACMI presence also increased the risk of stroke (mainly driven by the occurrence of ischemic stroke) and institutionalization (both $p<.01$, also in fully adjusted models) but not death (Table 4). The increase in CDR at follow up (i.e. more cognitive symptoms) tended to be higher for patients with ACMIs at baseline, ($p=.128$, Table 4).

DISCUSSION

In this large cohort of memory clinic patients with possible VCI, ACMIs were detected in 2% of patients and located predominantly in the subcortical white matter. The presence of ACMIs was associated with a high burden of both small and large vessel disease on MRI and the diagnosis of vascular dementia. Moreover, patients who presented with ACMIs at baseline had 3-fold increased risk of poor clinical outcome after two years.

This study extends an emerging literature from previous studies, few of them in a memory clinic setting, on the occurrence and clinical correlates of ACMIs. ACMI occurrence was in agreement with previously smaller studies assessing ACMIs in a memory clinic setting (incidences 3-4%) [12,13], whereas higher occurrence rates have been described in patients with CAA (15-35%) [4,5] and hemorrhagic stroke (12-44%) [6–10]. In contrast, in a population based cohort of elderly persons ACMIs were more rare (0-0.3%) [28]. Of note, even low occurrence rates of ACMIs may reflect substantial disease burden. A mathematical model, which considered the limited spatial and temporal resolution of ACMI detection on DWI, estimated that detecting one ACMI suggests an annual incidence of several hundreds of microinfarcts [13].Thus in contrast to chronic microinfarcts that represent cumulative damage over time, ACMIs provide a measure of very recent and thus "active" vascular brain injury that may predict further vascular injury.

We found an association between ACMIs and CSF biomarkers. Previously, elevated CSF tau without changes in Aß, has been observed for months after acute clinical ischemic stroke [29] Yet, given the tiny volume of the affected tissue in ACMI, we expect that the

currently observed CSF changes more likely reflect the underlying pathology of ACMIs rather than the tissue injury due to the ACMIs themselves. The reduced – i.e. more abnormal - CSF Aβ levels in patients with ACMIs, may thus suggest a relation with more widespread CAA or other AD pathology [30]. A link with prototypical AD pathologies – i.e. plaques and tangles - appears less likely, as no association was found between ACMIs and the clinical diagnosis AD and the CSF biomarkers pattern was not typical for AD. The CSF pattern we found, with reduced Aβ and slightly elevated Tau and p-Tau, has recently been reported in a meta-analysis of patients with clinically diagnosed CAA [31]. In CAA primarily Aβ 40, but Aβ 42 also to a lesser extent, accumulates in the walls of leptomeningeal and cortical vessels and leads to structural vessel wall changes [32]. These CAA-laden vessels cause both hemorrhagic and ischemic stroke, including lobar microbleeds and microinfarcts [33]. Paradoxically, we found that ACMIs were associated specifically with deep and mixed microbleeds, rather than lobar microbleeds which are considered to be CAA-related. Also, the question arises how CAA, which predominantly occurs in leptomeningeal and cortical vessels, can be linked to the preferential subcortical localization of ACMIs. A further challenge to the interpretation of the CSF pattern is that different underlying pathologies frequently coexist. How these pathologies interact and contribute biomarker profile certainly warrants further investigation.

No relationship was established between ACMI presence and conventional vascular risk factors, which has been a relatively common finding throughout ACMI literature [1]. The observed association of ACMIs with imaging markers of both SVD (i.e. microbleeds, lacunar infarcts and WMHs) and large vessel disease (i.e. non-lacunar infarcts) is also in line with currently evolving literature on MRI detected microinfarcts [1]. A remarkably strong relationship was observed between ACMIs and microbleeds, which appeared both in higher numbers and relatively more often in deep and mixed locations. These locations suggest that multiple etiologies contribute to ACMIs, as occurrence of deep microbleeds is considered to be driven by hypertensive arteriolopathy rather than CAA related pathology [34]. The association with non-lacunar infarcts is also in line with this multiple etiology concept.

ACMIs have shown to evolve into chronic lesions, appearing on follow up imaging as WMH [35], cavitated lesions and cortical microinfarcts [5]. It has been proposed that ACMIs damage network integrity and therefore result in worsening of cognitive performance [36]. No correlation was found between ACMI presence and cognitive impairment at baseline. However, patients with ACMIs were four times as likely to be

institutionalized at follow-up, mostly due to cognitive decline, and tended to have a faster deterioration of their CDR score. These findings support the notion that ACMIs should be considered a risk factor for cognitive deterioration.

Follow up data from this study showed that ACMI presence at baseline predicted *poor clinical outcome*, future stroke and institutionalization. One previous study of acute hemorrhagic stroke patients with adequate follow up established a similar result with respect to recurring stroke and vascular death [7]. These findings emphasize the important clinical implications of ACMIs as a marker of active vascular disease. Future studies should explore if ACMI may guide targeted prevention strategies.

Strength of this study is the large cohort with high quality MRI, cognitive data, CSF biomarker availability and follow up in the large majority of eligible participants. Limitations concern the limited spatial and temporal resolution of ACMI detection on DWI, which provides a "snapshot" of ischemic injury from the past two weeks. Longitudinal studies with frequent repeat MRIs and cognitive testing could provide more detail on these lesions, their evolution over time and cumulative effect on cognitive performance. Another issue is the relatively arbitrary proposed cut off of 5mm [1], as this and many other studies found DWI lesions measuring up to 10mm. Whether these slightly larger lesions represent a different cerebrovascular pathology is unclear. Finally, it is still unclear whether our findings are generalizable to chronic microinfarcts and other types of (non)vascular cohorts. It may be argued that the acute stage of a microinfarct should have the same risk factors and clinical correlates as its chronic stage. Yet, the DWI lesion does represent an acute stage in the process, whereas the chronic lesions may reflect events that have occurred even decades ago. Moreover, not all ACMIs evolve into a lesion that is detectable as a chronic [5]. With regard to generalizability to other cohorts, it should be noted that the etiology, burden comorbidities of ACMI may markedly differ between cohorts [1] which may be relevant to their clinical correlates.

This study found that ACMIs in memory clinic patients with possible VCI are associated with an increased burden of mixed vascular brain pathology, especially SVD and reduced levels of CSF Aβ. Our findings highlight that ACMIs, despite being relatively rare, are an important imaging marker of active

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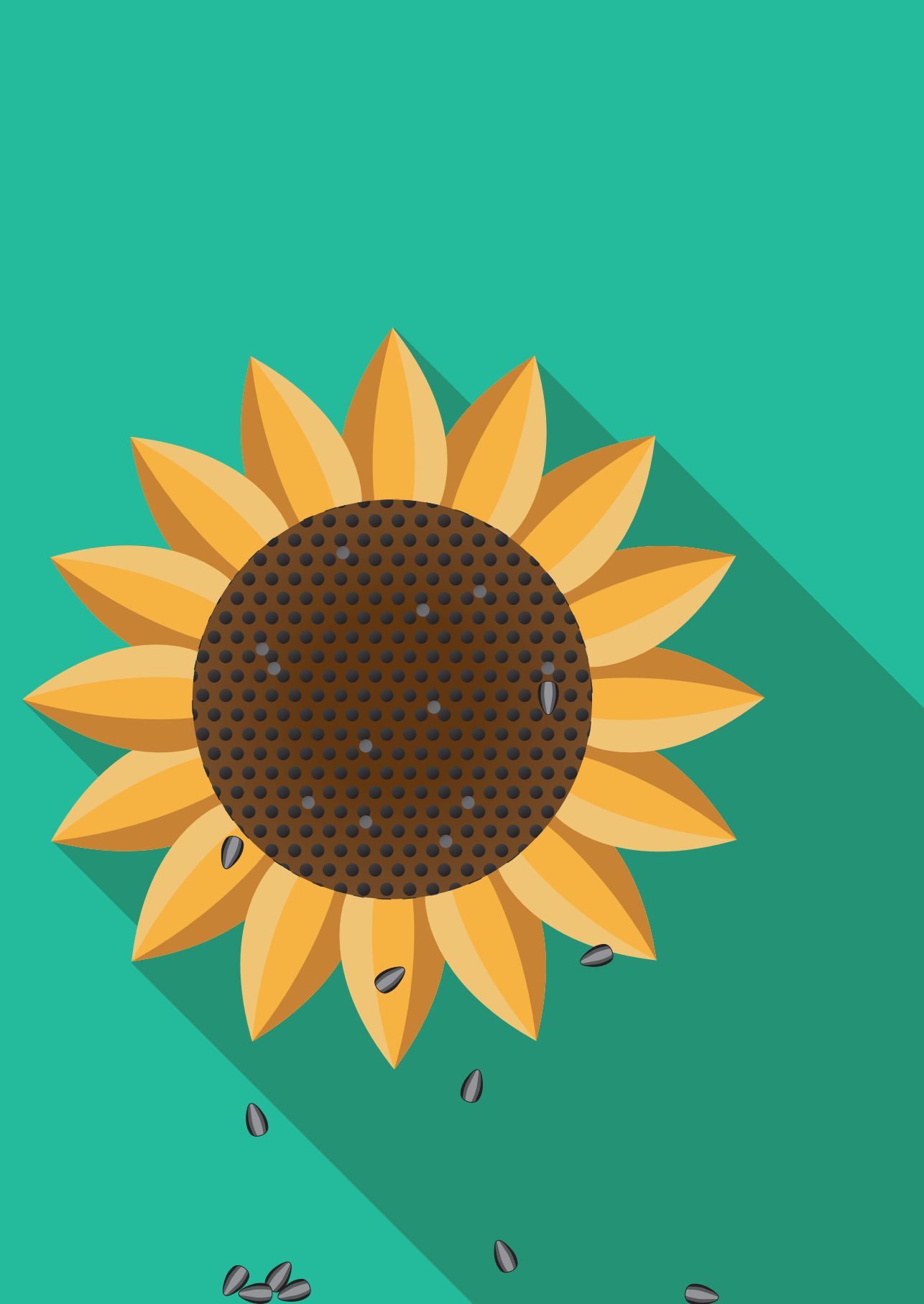

CHAPTER 9

GENERAL DISCUSSION

The aim of this thesis was to identify causes and consequences of cerebral microinfarct on 3 tesla MRI. Within this chapter, I will discuss the main findings of this thesis, relate them to existing literature and provide suggestions for future research. I will finish with a discussion on the implications of this research for clinical practice.

Modalities of microinfarct detection: integrate findings

Microinfarct detection has undergone a rapid development in the last decade. Detection evolved from post-mortem histopathological examination to in vivo detection of chronic cortical microinfarcts on structural MRI and acute cerebral microinfarcts on diffusion-weighted MRI (DWI) [1]. This thesis utilized these novel detection methods to study microinfarct in vivo by observing their risk factors, causes and consequences in different populations at risk.

The developments in microinfarct detection techniques not only led to a surge of in vivo MRI-studies, but also to an increased research effort into neuropathological microinfarct studies in humans and animals [1]. As discussed in the introduction, these detection modalities differ fundamentally in their spatial and temporal resolution and whether or not they allow whole brain evaluation. To illustrate some of these differences: The lowest detection limit ranges from 50 μ in neuropathology studies to 1 or 2 mm in MRI studies; the temporal window for detection of acute microinfarcts on DWI is very short (up two weeks) compared to the detection of chronic microinfarcts with MRI or neuropathology; and chronic microinfarcts on structural MRI can only be detected in the cortex, whereas acute microinfarcts on DWI are detectable throughout the brain. It is therefore evident that findings from these modalities cannot be compared to each other one-on-one. The outstanding challenge is to start bridging the gap between these modalities and *integrate* findings within a broad microinfarct-framework. As such work on white matter hyperintensities (WMHs) on brain MRI provides an example of how successful integration of multimodal research findings can propagate biomarker development [2]. In additional to conventional detection on MRI, histopathology and animal studies, epidemiological cohort studies and advanced MRI-techniques, such as diffusion tensor imaging, contributed to our knowledge on WMHs and helped to establish its value as biomarker in the clinical setting today [2]. I will therefore place the main findings of this thesis in a broader context of multimodal microinfarct research to explore the potential value of microinfarcts as biomarker.

Risk factors and mechanisms of cerebral microinfarcts

Neuropathology studies show that - as the name suggests - microinfarcts are indeed a lesion based on ischemic infarction [3]. In recent years there has been increasing literature suggesting that microinfarcts have multiple cerebrovascular causes, including small vessel disease (SVD), microemboli and cerebral hypoperfusion [1].

Since risk factors for other manifestations of cerebrovascular disease have been well characterized, we used these vascular risk factors as a starting point to observe specific risk factors for microinfarcts. Although it was not the main focus of this thesis, we found an association between microinfarcts and hypertension (Chapter 4 and 5), hypercholesterolemia (Chapter 4) and obesity (Chapter 5) in patients with heart failure and internal carotid artery occlusion. These findings are in agreement with results from neuropathological studies [4] and in vivo MRI-studies in memory clinic patients [5] and a population-based cohort [6]. Another observation of interest was that microinfarcts occurred more often in males than females (Chapter 2 and 5). Sex differences in vascular disease are becoming increasing apparent, with higher rates of vascular risk factors and stroke being reported in males [7]. Further research is encouraged to explore how this sex difference in microinfarct occurrence is to be interpreted.

SVD is an important cause of microinfarct and refers to "a group of pathological processes with various etiologies that affect the small arteries, arterioles, venules, and capillaries of the brain"[8]. Two common forms of SVD, hypertension-related SVD and cerebral amyloid angiopathy (CAA), have been recognized as underlying pathology of microinfarcts. Hypertensive-SVD is histologically characterized by arteriolosclerosis of the perforating small vessels to the white matter and the deep gray nuclei [8]. It is common in elderly and considered an important contributor to cognitive decline [8]. Evidence for involvement of hypertensive-SVD in microinfarcts comes from consistent associations between microinfarcts and SVD-manifestations, such as lacunar infarcts and WMHs, observed in neuropathology studies [4] and in vivo MRI-studies (Chapter 2 and 8 of this thesis) [5,9,10]. CAA is characterized by β-amyloid in the walls of cortical blood vessels and its clinical manifestations include cognitive decline and intracerebral hemorrhage [11]. The fast majority of CAA-cases are sporadic, though genetic variant also exist, such as hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) [12]. Microinfarcts are common on neuropathological examination [4], in vivo structural imaging [13,14] and diffusion imaging [15,16] in patients with CAA. Moreover, they are linked to the other MRI-manifestations of CAA, including lobal microbleeds and

cortical superficial siderosis in patients with CAA [13,14] and memory clinic patients (Chapter 8 this thesis) [5]. Although the characteristics of hypertensive-SVD and CAA are relatively well defined, it is less clear what pathophysiological mechanism leads to cortical microinfarction. Intuitively, it seems easier to understand how this might work in CAA with typical cortical vessel alterations. Indeed in CAA, microinfarcts were found in the proximity of amyloid-positive vessels with prominent lumen narrowing, suggesting a pathophysiological mechanism of occlusion or hypoperfusion[17]. A similar mechanism has been supposed in hypertensive-SVD, although less is known about the actual relation between affect vessels and (sub)cortical microinfarcts. Other pathophysiological mechanisms have been proposed in SVD, as for example to explain the subcortical manifestations in CAA, which include WMHs and white matter integrity disruption [11]. One of the ideas is that impaired vascular reactivity, due to vessel wall amyloid deposition, may contribute to the ischemic injury in CAA by cause a mismatch between perfusion and metabolic demand [11]. One study actually investigated the in vivo relation between microinfarcts and fMRI BOLD reactivity, but did not find an association [13].

Other research modalities helped to untangle pathophysiological mechanisms of SVD causing microinfarcts. Several mouse-models have been developed to mimic SVD-related vessel occlusion in a controlled setting. These include microinfarct-inducible models, such as injection of microemboli and laser-induced occlusion of tiny cortical arterioles [18]. There are also models of spontaneous microinfarct development, for example the Tg-SwDI mouse model of CAA [18]. These mouse-models are highly informative on a number of matters: 1) Both induced and spontaneous microinfarcts in mice have a strikingly similar appearance as spontaneous microinfarcts humans, suggesting that microinfarcts can result from occlusion of arterioles or other factors that cause ischemia in the territory of these tiny vessels; 2) They highlight the importance of other mechanisms that occur in SVD, such as impaired vascular autoregulation, endothial dysfunction, blood–brain barrier damage and local subclinical inflammation [8]; 3) That besides SVD, other pathophysiological mechanisms probably underlie the formation of microinfarcts, including microemboli and hypoperfusion and that it is likely that these mechanisms interact. I will elaborate on the role of microemboli and hypoperfusion in the next paragraphs. These aforementioned studies allowed us to observe the SVD-related parenchyma changes, yet less is known about in vivo function of the small vessel themselves. Over the past years, our group has developed techniques to assess small vessel function using ultra-high-field strength MRI. We found a measurable disruption

of pulsatility of perforating small vessels in patients with SVD [19]. Since this is currently the most direct way to measure small vessel function, it would be of obvious interest how pulsatility and other novel non-invasive measures of small vessel function relate to microinfarcts.

There is ample evidence that microinfarcts can be caused by microemboli. As briefly mentioned, mouse-model studies whereby microemboli are injected into the blood circulation generate lesions with similar appearance as cortical microinfarcts [18]. Moreover, in humans microinfarcts show a strong association with large cortical infarct (i.e. >5mm) of presumed thromboembolic origin in both neuropathological [4] and in vivo imaging studies (Chapter 2, 5 and 8)[5]. There are several clues that – in line with clinical embolic stroke – microemboli formation can take place at different sites. For example, microinfarcts were correlated with intracranial stenosis in an Asian stroke [20] memory clinic population [5] and population-based cohort [6]. Extracranial stenosis and occlusion of the carotid arteries also appears to be relevant, as microinfarcts were significant more prevalent in the hemisphere ipsilateral to stenosis or occlusion compared with the contralateral hemisphere (Chapter 4) [21,22]. Several studies are also highly suggestive of the involvement of cardiac emboli. Microinfarcts correlated to atrial fibrillation in stroke patients [23] and (sub)clinical cardiac disease in memory clinic patients [24]. In chapter 5 of this thesis, we were the first to explore the occurrence of microinfarcts in patients with heart failure. We found surprisingly high rates of microinfarcts similar to the occurrence rates in stroke and memory clinic population. Our study also suggests that cardiac invasive procedures, such as percutaneous coronary intervention (PCI) can also cause microinfarcts (Chapter 5). Previous studies indeed identified acute microinfarct and large DWI-positive lesions as a common finding after other procedures such as coronary artery bypass graft (CABG) surgery [25].

A number of neuropathological studies noted that microinfarcts appeared to display a predilection for the (cortical) watershed areas [26]. This led to the concept that cerebral hypoperfusion could be an underlying cause of microinfarcts. We considered MRI to be an ideal modality to further investigate this issue, because it allows for simultaneous assessment of cerebral perfusion and whole brain cortical microinfarct detection (this in contrast to discrete sampling of microinfarcts in post-mortem neuropathology studies). We have created 3D representations of the topographical distribution of cortical microinfarcts in memory clinic patients (Chapter 1, 5 and 7) and in patients with internal carotid artery occlusion (Chapter 4). Although microinfarcts are strongly

clustered in the most cranial parts of the fronto-parietal cortex, we found no consistent evidence that microinfarcts preferentially occurred in the watershed areas. One of the complexities, however, is the large interindividual variation in the size and anatomical location of the watershed region. Whether an approach with individually acquired watershed atlases would yield different results remains the question. Other results in this thesis do support the notion that cerebral hypoperfusion is a cause of microinfarcts. Using arterial spin labelling (ASL), a non-invasive MRI perfusion technique whereby blood is magnetically labeled in the cervical arteries, we found that microinfarcts were associated with reduced global cerebral perfusion in memory clinic patients (Chapter 2). Yet, perfusion in the region directly surrounding microinfarcts was not affected. The origin of reduced cerebral perfusion is less straightforward to disentangle in these memory clinic patients with vascular cognitive impairment (VCI), as hypoperfusion could both be a cause as well as the result of their cerebrovascular and neurodegenerative pathologies. This makes it complex to determine the (causal) role of hypoperfusion in the occurrence of microinfarcts in this setting. We therefore also utilized data from two clinical populations where reduced perfusion was more likely to be the primary cause of brain pathology: patients with heart failure and patients with internal carotid artery occlusion (Chapter 4 and 5). Data from these patients was gathered through the Heart-Brain Connection Study, a Dutch research program that investigated the hypothesis that "impaired hemodynamic status of both heart and brain is an important and potentially reversible cause of VCI" [27]. We found that microinfarcts were very common in these two populations with compromised cerebral hemodynamics Moreover, microinfarct presence correlated well with measures of hemodynamic constraint, such as cardiac index in patients with heart failure (Chapter 5) and the number of diseased vessels and blood flow in patients with internal carotid artery occlusion (Chapter 4). Multimodal research also contributes to our understanding of the role of hemodynamics in microinfarct. For example, a CAA mouse model study indicated that when inducing hypoperfusion by bilateral carotid artery stenosis both the severity of CAA-pathology but also the incidence of microinfarcts increases [28]. These findings delineate the complex interplay between hemodynamics, SVD and microinfarcts.

Vascular lesions on brain MRI can have diagnostic value as a marker of underlying pathology, as for example lacunar infarcts and microbleeds are a marker of underlying SVD-pathology. Some of these markers are even suggestive of a particular type of SVD, like for example cortical superficial siderosis in CAA. As microinfarcts can have multiple etiologies, the diagnostic value of microinfarcts is less straightforward. I suggest that

microinfarcts should therefore be interpreted as a sign of broad vascular brain injury. It may, however, be possible that certain characteristics of microinfarcts do reflect etiology. Some authors have suggested that etiology of microinfarcts differs per brain location and that pre(frontal) microinfarcts are more likely to have an embolic cause due to the absence histopathological pathology in these regions [29]. I believe there is currently insufficient evidence to substantiate this claim. One possible way of researching this would be to perform a cluster analysis on microinfarct characteristics, such as topographical brain location, risk factors and (presumed) etiology. To perform this type of analysis, one needs a much larger sample size than in the existing microinfarct cohorts. Due to the time-consuming detection techniques, this is currently not yet feasible.

Perilesional injury associated with microinfarcts

It has been suggested that visible MRI-manifestations of cerebrovascular disease represent only the tip of the iceberg of vascular brain injury. It is evident that the microinfarcts we encounter upon evaluation represent only a fraction of the total brain burden of microinfarcts. This is due to the fact that many smaller microinfarcts (<1mm) escape the detection limit of MRI. Yet also on neuropathological evaluation, the total microinfarct count in individual subjects is severely underestimated, due to selected sampling of tissue representing less than 0.01% of the whole brain [1]. Mathematical estimations indicate that when detecting a single microinfarct on standard neuropathological evaluation, this is indicative of a total brain burden of up to 100s-1000s of chronic microinfarcts [30]. Similarly, finding a single acute microinfarct could indicate an annual incidence of hundreds of new acute microinfarcts, given the limited temporal and spatial resolution of a single DWI scan [31]. This concept was seized in the metaphor of microinfarct as "the cockroaches of the brain", because everybody who has encountered these bugs in their home knows that seeing one of them in your kitchen cupboard means there are hundreds in your wall [30]".

Lesion-related brain injury can also be distant from the lesion, for example when a subcortical infarction causes remote cortical thinning though via degeneration of connecting fiber tracts [32]. Or around the lesion ("perilesional"), for example disruption of the white matter integrity in directly around WMH-lesions [33]. Such remote and perilesional effects might also occur in relation to microinfarcts. Microinfarcts show a relatively consistent association with cortical atrophy in both neuropathology and MRI-studies (Chapter 6). In chapter 6 we explained that the total brain burden of microinfarcts does not explain this difference in cortical volume, due to the tiny lesion

volume of microinfarcts. We hypothesized that atrophy could – at least in part - be due to perilesional effects of microinfarcts and retraced local cortical atrophy to the direct location of the microinfarcts (Chapter 6). We found reduced cortical thickness in a radius of at least 20mm around the microinfarct. In contrast, the maximal radius of the microinfarct itself is 2.5mm. Considering the magnitude of this perilesional effect, it is likely it forms a meaningful contribution to the global cortical atrophy associated with microinfarct. Additional evidence for perilesional atrophy comes from a neuropathology studies in humans and mice. A small neuropathology study in humans identified axonal disorganization in tissue surrounding microinfarcts [34]. In mice neural activity was significantly disturbed around the microinfarct in an area 12-times greater than the microinfarct itself [35]. It is still not entirely clear how injury in the perilesional zone evolves. Some have speculated that mechanisms such as ischemic cortical spreading depression and disrupted cortico-cortical circuits play a role (Summers et al. 2017). In my view, a logical next step would be to carefully examine the perilesional zone on neuropathological samples and in vivo using 7-tesla MRI. Additionally, advanced MRI-techniques, such as fMRI and ASL could also reveal more about the functional problems of the perilesional tissue.

We also performed a study to investigate the perilesional effect of microinfarcts on the white matter connectivity in memory clinic patients (Chapter 7). There are several reports of disruptions in white matter connectivity for other SVD-manifestations [33,36], which in turn correlate well with their impact on cognitive functioning. This is an important finding since it explains to some extent how relatively small lesions, such as lacunar infarcts, can nonetheless have a clinically meaningful impact on brain function. Yet, we found no evidence of disruption of white matter connectivity in memory clinic patients with microinfarcts (Chapter 7). Although we have carefully evaluated the white matter directly underneath the cortical surface using a voxel based approach in brain regions with a high microinfarct density, we cannot exclude the possibility that our methods are not sensitive enough. Neuropathological study in humans, however, also indicates that the subcortical tissue around microinfarcts is relatively spared [34].

Functional impact of microinfarcts

Cognitive dysfunction associated with and presumed to be caused by vascular brain damage has been termed vascular cognitive impairment (VCI) [37]. Since VCI entails a broad spectrum of vascular brain pathologies, the functional impacts of microinfarcts could certainly be classed under this concept.

There has been quite some evidence that points towards a role of microinfarcts in cognitive decline and dementia. A systematic review of microinfarcts in neuropathology studies reported the highest incidences in patients with vascular dementia, followed by patients with Alzheimer's disease [4]. A number of autopsy studies involving community dwelling elderly found that microinfarcts were associated with lower average global cognition and increased odds of dementia, independent of macroscopic infarcts and Alzheimer's pathology [38]. The advent of in vivo microinfarct detection greatly facilitates research into the cognitive impact of microinfarct and several studies have investigated this using a cross-sectional design. In our cohort of patients from our memory clinics with VCI, we also found an association with the diagnosis vascular dementia (Chapter 2 and 8). Moreover, we observed worse cognitive functioning in the domains perception & construction, attention & executive functioning and possibly processing speed (Chapter 2). Interestingly, there appears to be some overlap in the affected domains visuocontruction and executive function across studies [5,23]. However, it is also important to acknowledge that the association between microinfarcts and cognition is not consistent across populations. In this thesis, we found no association between microinfarcts and cognition in patients with heart failure (Chapter 5). The reason for this is not entirely clear. Yet, it is important to realize that microinfarcts rarely exist as solitary brain lesion, but usually occur in the context other vascular and neurodegenerative damage. This context may determine the impact on cognition, for example by affecting the cognitive reserve. On the whole the cognitive profile of microinfarcts in memory clinic and stroke patients appears to be characterized by deficits in "cortical" domains, such as visuocontruction and language, but also in "subcortical" domains, such as attention and processing speed. While these subcortical features are the hallmark of advanced SVD [8], the cortical features may well be related to the cortical localization of microinfarcts in these studies.

Less research has been carried out to establish the impact of microinfarcts on cognitive decline over time. In Asian memory clinic patients, cortical microinfarcts at baseline were associated with accelerated decline in memory and language domains [39]. Another studies in stroke patients indicated decline in visuospatial performance after a follow up of 28 months [23]. These studies clearly allude to the causative role of microinfarcts in cognitive deterioration. Yet, the mechanisms by which microinfarcts affect cognition are still not fully understood. Considering the small size of tissue damage it is unlikely that microinfarcts themselves directly cause cognitive impairment. Within this thesis, we observed that cortical atrophy was a significant mediator in the relationship between

microinfarct-presence and cognitive performance (Chapter 6), suggesting a role of the perilesional injury on the functional impact of microinfarct. Another possibility is that microinfarcts are above all a proxy of disease severity of underlying etiology. Although the concomitant vascular brain injury is certainly important when considering the impact on cognition, the effects of microinfarcts remained unaltered when corrected for MRI markers of cerebrovascular disease [39]. More research into the longitudinal effect of microinfarcts on cognition is needed before their role as biomarker of cognitive decline can be confirmed. On note, other areas of functional impact have remained large unexplored. Considering the high incidence of gait and psychiatric problems in patients with SVD, these topics also warrant further investigation.

Within this thesis, we have shown that acute microinfarcts on DWI are associated with poor clinic outcome (including risk of stroke and institutionalization) in memory clinic patients (chapter 8). Similarly, (acute) microinfarcts were also associated with poor clinic outcome, including (vascular) death and recurring stroke, in patients with acute hemorrhagic stroke [40] and ischemic stroke [20]. These studies highlight the potential prognostic value of acute microinfarcts. We have argued that these lesions could be viewed as a marker of "active" cerebrovascular disease. More longitudinal studies are needed before microinfarcts can be used to reliably identify populations at risk for cognitive decline or future stroke.

Outstanding challenge in microinfarct research: need for automated detection

This thesis provided a starting point for an epidemiological approach of the causes and consequences of microinfarct in vivo. Although we used reasonable subject numbers in each research project, statistical power for some of the questions addressed was nonetheless limited, due to fact that microinfarcts only occurred in the minority of subjects. Looking into the future, microinfarct research would greatly benefit from studies on a truly epidemiological scale with several thousands of subjects. On the one hand this seems increasingly feasible, as research groups all over the world are starting research collaborations and exchanging (MRI)data, such as the METACOHORTS initiative [41] . On the other hand, in vivo microinfarct is still hampered by its time-consuming detection. Detection of cortical microinfarct on 3 tesla MRI is performed completely manually and takes on average 20-30 minutes per scan even more than an hour on 7 tesla. Acute microinfarcts are somewhat faster to detect on DWI, taking up on average 10 minutes per scan. Moreover, the sensitivity of manual ratings is also

not optimal (when comparing to a ground truth established by multiple visual raters). Automatic detection of microinfarcts could solve this problem. This option seems within reach: Our group has published an automated detection tool for microbleeds [42] and recently organized a challenge in which developers could evaluate their automated WMH method on a standardized dataset [43]. Results from our WMH challenge suggest that advanced machine learning techniques may be a potentially strategy for automated microinfarct detection. Currently, efforts to develop an automated microinfarct detection tool are still ongoing in our group.

Clinical implications

From my perspective as neurology resident I evidently asked myself the question how microinfarcts should guide the clinical management of my patients. How should I advise my colleagues when they encounter microinfarcts? A decade ago, microinfarcts were only observed at autopsy and rarely, if ever, incorporated in clinical consultation. Nowadays microinfarcts are increasingly recognized as MRI-manifestation of cerebrovascular disease in the research setting and sporadically entering the clinical stage.

When addressing clinical implications, it is important to consider that microinfarcts most commonly occur in the context of other cerebrovascular pathologies. In my view these co-occurring cerebrovascular manifestations, on which there clearly is a much more elaborate clinical knowledge base already, should primarily determine the approach to diagnosis, prognosis and treatment. In other words, microinfarcts currently do generally not direct our clinical management. Nonetheless, I think it is important to address what to do when one encounters a microinfarct and accentuate some of the differences between chronic cortical microinfarcts and acute microinfarcts on DWI in this respect.

Detection of chronic cortical microinfarcts is relatively hard and time consuming, also making it less likely that they are spontaneously detected in the clinical setting. However, when microinfarcts are encountered, a clinical should make an effort to place them in the clinical context of the patient. Are there vascular risk factors? Is there evidence for concurrent SVD, atherosclerosis of cardiac disease? With regards to treatment, I recommend handling microinfarcts like other forms of "silent" cerebrovascular disease, such as asymptomatic lacunar infarction and WMHs. That includes identifying and optimizing vascular risk factors according to the primary prevention guidelines [44].

It is my personal experience that acute cerebral microinfarcts are increasingly encountered in the clinic. This may be due to the fact that DWI-sequences are more often performed, not only in a stroke setting but for example also in memory clinics. Moreover, they are quite easily detectable due to their contrasting hyperintense aspect. When encountering an acute microinfarct, the first step for the clinician is to carefully determine whether there has been a symptomatic event (i.e. TIA or "transient neurological attack") which could be attributed to the lesion. If so, a diagnostic work-up and treatment for a TIA is indicated. If an acute microinfarct is not evidently symptomatic, deciding on the appropriate management is less straightforward, as one could argue that they are "silent", but still a sign of "active" cerebrovascular disease. Nonetheless, I would recommend treating asymptomatic acute microinfarcts – like chronic microinfarcts - as "silent" cerebrovascular disease according to primary prevention guidelines, albeit with even higher vigilance for other –covert- manifestations of cardiovascular disease.

These considerations highlight the knowledge gaps that hamper full incorporation of microinfarcts into the clinical management of patients today. It is clearly of interest to clinicians whether stringent vascular risk management, including blood pressure management, or anti-platelet medications, such as aspirin, can influence prognosis and clinical outcomes in patients with microinfarcts. However, before one can consider a clinical trial, some issues need to be further clarified. For example, we first need to identify which populations are at risk for microinfarcts and would be a suitable target population for a trial. We also need to determine the intervention that is suitable for that setting, in light of the different etiologies (and thus treatment targets) of microinfarcts. To further investigate these issues, large datasets are needed. As previously stated, automated microinfarct detection is a crucial next step in order to advance these developments and benefit from large datasets that are already available from established observational cohorts and previous intervention studies.

Conclusion

This thesis explored possible causes and consequences of microinfarcts in vivo on 3 tesla MRI. We established an etiological role for cerebral hypoperfusion and confirmed the notion that microinfarcts – depending on disease context – can have multiple causes, including SVD and microemboli. The potential cognitive impact of microinfarcts has been well established in cross-sectional and recently also longitudinal studies. It is, however, not yet clear by which mechanisms microinfarcts and their co-occurring pathologies cause cognitive symptom, although perilesional injury may play a role.

Development of automated detection of microinfarcts will greatly facilitate their future study that will provide better insight in implications for clinical diagnosis and treatment.

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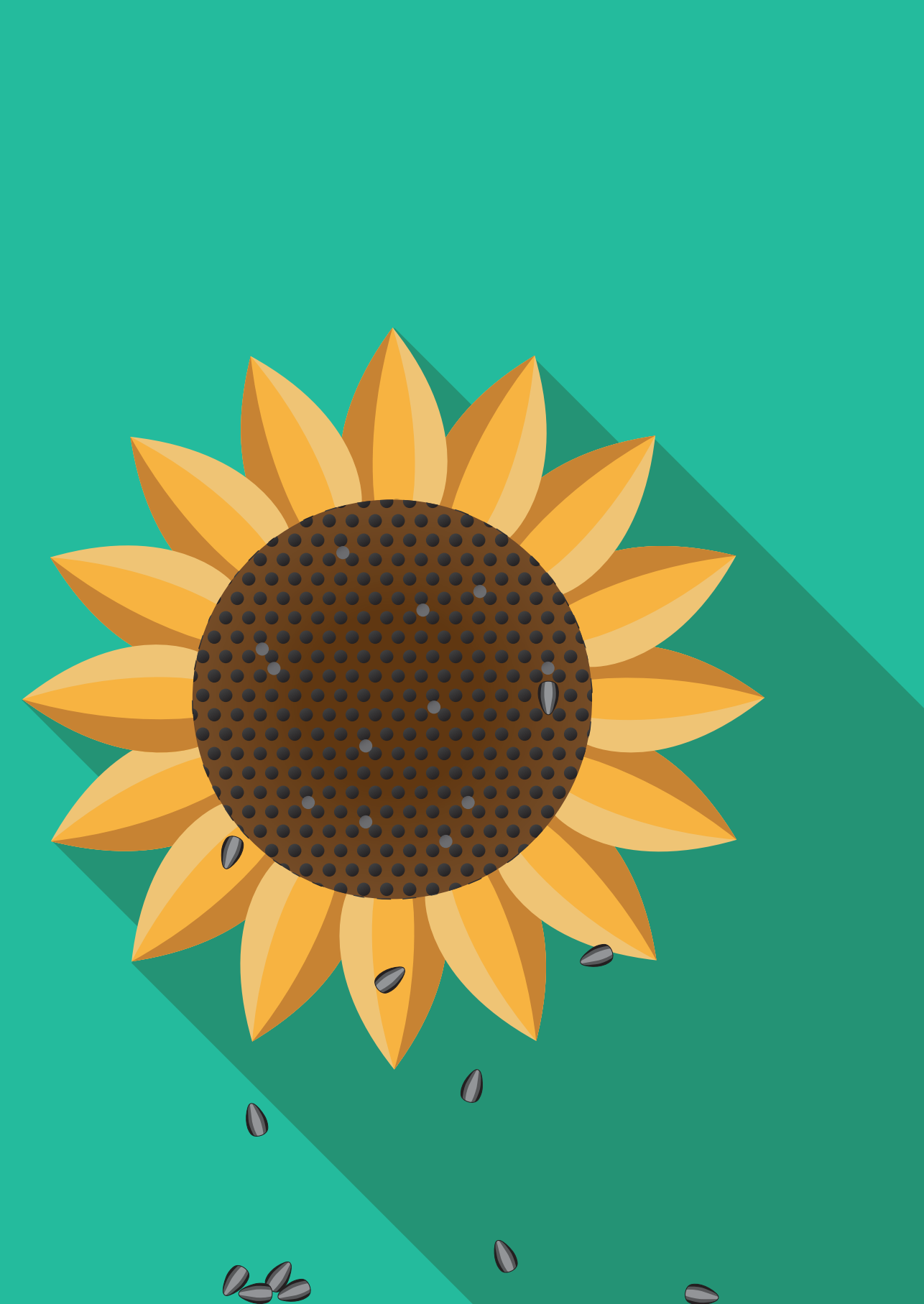
APPENDICES



SUMMARY

Cerebral microinfarcts are small brain lesions presumed to be caused by ischemia. They are a common finding upon post-mortem histopathological examination, especially in patients with vascular dementia and Alzheimer's disease [1] and an independent predictor of ante-mortem cognitive decline [2]. The size of typical microinfarcts on neuropathological evaluation ranges between 50 μm and a few mm [1]. Since this is too small to detect with the naked eye and the lesions could only be observed post-mortem, microinfarcts have been dubbed "the invisible lesion" [3]. This changed with the advent of high field strength MRI, which allowed the visualization of in vivo microinfarcts on 7 tesla MRI [4]. Once identified on 7 tesla, microinfarcts also appeared to be visible on widely available 3 tesla MRI. This opened up the opportunity to study microinfarcts in vivo on a larger scale.

- *The overall aim of this thesis was to identify causes and consequences of in vivo cerebral microinfarct om 3 tesla MRI*

Risk factors and mechanisms of cerebral microinfarcts

Previous neuropathology and in vivo MRI studies have suggested that microinfarcts relate to common vascular risk factors and probably have multiple cerebrovascular causes, including small vessel disease, microemboli and possibly cerebral hypoperfusion [1,5]. We studied occurrence of microinfarcts, risk factors and associated clinical features, and potential underlying causes in three clinical cohorts. These cohorts reflect differing primary (cerebro)vascular pathologies, which provided a complementary view on the different etiological aspects of microinfarcts. In our cohort of memory clinic patients with vascular cognitive impairment, small vessel disease was presumed to be the primary underlying pathology. This population was, however, less suited to investigate the (causal) relationship between cortical microinfarcts and cerebral hypoperfusion, since hypoperfusion could both be a cause well as the result of underlying cerebrovascular and neurodegenerative pathologies in these patients. We therefore also sought after patients in which reduced cerebral perfusion was likely to be the primary cause of brain pathology. Through the Heart-brain Connection study we were able to utilize data from patients with heart failure and patients with internal carotid artery occlusion. Besides, these populations are known to have a high risk of embolic stroke, which is also a risk factor for cortical microinfarcts.

In **chapter 2**, we aimed to determine the occurrence of cortical microinfarcts and associated clinical features in memory clinic patients with possible vascular cognitive

impairment. We found that occurrence of cortical microinfarcts was higher (20%) in patients than controls (10%). Presence of cortical microinfarcts was associated with white matter hyperintensities and large cerebral infarcts of presumed embolic origin, but found not with classical vascular risk factors. These results reflect the heterogeneous etiology of microinfarcts in patients with vascular cognitive impairment.

In **chapter 3**, we investigated the hypothesis that cortical microinfarcts are related to cerebral hypoperfusion. We examined both global and local (i.e. in the direct region around microinfarcts) cerebral perfusion in memory clinic patients. Using arterial spin labeling, a non-invasive MRI perfusion technique, we found that patients with cortical microinfarcts had a 12% lower *cerebral blood flow* and 22% increased *spatial coefficient of variation* (measure reflecting arterial transit time). Perfusion in the region directly surrounding cortical microinfarcts was not different from a reference region in the contralateral hemisphere. These findings indicate that microinfarcts are primarily related to global reductions in cerebral perfusion.

In **chapter 4**, we studied cortical microinfarcts in patients with internal carotid artery (ICA) occlusion, as a model for cerebral hemodynamic compromise. We assessed at the spatial distribution of cortical microinfarcts in relation to the side of ICA occlusion and examined the relation between cortical microinfarcts and the condition of the remaining arterial supply. Microinfarcts were far more common in the patients (54%) than in the reference group (6%). In patients with an ICA occlusion, cortical microinfarcts were localized more often in the hemisphere ipsilateral to the occlusion than in the contralateral hemisphere. Lastly, microinfarcts were associated with a higher number of occluded or stenosed cervical arteries and cerebral blood flow through these arteries tended to be lower. These results support an etiological role of hemodynamics in the development of cortical microinfarcts.

In **chapter 5**, we studied the relation between cortical microinfarcts and hemodynamic status in patients with chronic heart failure. Patient with heart failure are at risk for vascular brain injury, but also suffer compromised cerebral hemodynamic and the latter is a suspected risk factor for cortical microinfarcts. Cortical microinfarct occurrence was higher in patients (17%) than reference participants (7%). In patients with heart failure, cortical microinfarcts were related to office hypertension and cerebral infarcts of presumed embolic origin. Most notably, we found that cortical microinfarcts related to lower cardiac index (cardiac output corrected for body surface area), but not with

cause or duration of heart failure. These results show that vulnerability for vascular brain injury in patients with heart failure extends to cortical microinfarcts and suggests a hemodynamic origin of cortical microinfarcts.

Together these studies show that cortical microinfarcts generally relate to vascular risk factors, although this differs according to context of the specific population. These studies also support a probable role for cerebral hypoperfusion, although – again depending on the disease context - other causes, including small vessel disease and microemboli are also likely to be involved.

Identifying perilesional injury associated with cerebral microinfarcts

It is increasingly recognized that the microinfarcts we encounter upon evaluation represent only a fraction of the total brain burden of microinfarcts. This is due to the fact that many smaller microinfarcts (<1mm) escape the detection limit of MRI or remain undetected due to selective sampling in neuropathological evaluation [5]. Additionally, like other cerebrovascular MRI-markers, microinfarcts may present with lesion-related brain injury distance or directly surrounding the lesion ("perilesional"). These effects may help to explain why these tiny lesions can make a substantial contribution to, for example cognition. In two studies we investigated whether cortical microinfarcts were associated with perilesional injury in cortical tissue and subcortical white matter tracts.

In **chapter 6** we sought evidence that cortical microinfarcts were associated with perilesional cortical atrophy in memory clinic patients. We found that cortical microinfarcts were associated with a lower mean global cortical thickness and lower mean total grey matter volume, also after correction for demographics and intracranial volume. Moreover, we found that brain regions with microinfarcts had a lower cortical thickness and cortical thickness was significantly reduced within the local surrounding area of microinfarcts. These results suggest a perilesional area around cortical microinfarcts many times larger than the microinfarct core, which helps us understand the impact of cortical microinfarcts on overall brain structure and function.

In **chapter 7** we investigated whether cortical microinfarcts are clustered in specific brain regions and if presence of cortical microinfarcts was associated with reduced white matter connectivity in tracts projecting to these regions. We found that cortical microinfarcts displayed strong local clustering in highly interconnected frontal, pre- and postcentral brain regions in our memory clinic patients. Using diffusion imaging-based

tractography we concluded that white matter connections projecting to these regions were not disproportionally impaired in patients with compared to patients without cortical microinfarcts. These results indicate that the major white matter networks are probably not involved in cortical microinfarct-associated cognitive impairment.

Functional impact of microinfarcts

The functional impact of microinfarcts on cognition has been classed under the concept of vascular cognitive impairment. We investigated whether presence of cortical microinfarcts was associated with cognitive impairment in a cross-sectional design across two populations.

In **chapter 2**, we found that presence of cortical microinfarcts in memory clinic patients was associated with a diagnosis of vascular dementia and reduced performance in multiple cognitive domains, including attention & executive functioning and perception & construction. These associations were independent of other markers of vascular injury.

In **chapter 4**, we examined the relation between cortical microinfarcts and cognitive performance in patients with heart failure, but found no association between these two.

In summary, the cognitive complaints associated with cortical microinfarcts in memory clinic patients are characterized by deficits in both cortical and subcortical domains. The impact of cortical microinfarcts on cognitive performance differs per population. This could be due to the amount of co-occurring vascular and neurodegenerative pathology, which may determine the cognitive reserve.

In **chapter 8**, we examined occurrence of acute cerebral microinfarcts on diffusion-weighted imaging (DWI) in memory clinic patients and related their presence to vascular risk and cognitive profile, CSF and neuroimaging markers, and clinical outcome. We found that acute microinfarcts occurred in 2% of the patients and were most common in patients with vascular dementia. Acute microinfarcts were associated with a high burden of both small and large vessel disease on MRI. Finally, patients with acute microinfarcts at baseline had 3-fold increased risk of poor clinical outcome after 2 year. These findings suggest that acute microinfarcts in a memory clinic setting could be regarded as a marker of active vascular disease.

Conclusion

In this thesis, we have provided further support for a likely role for cerebral hypoperfusion as underlying cause of microinfarcts. Moreover, we confirmed the notion that - depending on the disease context- other causes, including small vessel disease and microemboli, are likely to be involved. Within our memory clinic cohort, cortical microinfarcts were associated with cognitive impairment in both cortical and subcortical domains. It is not yet clear by which mechanisms microinfarcts cause cognitive symptoms, although the "perilesional" injury in immediately surrounding cortical tissue may play a role. Finally, we established that acute microinfarcts on DWI are predictor of poor clinical outcome, suggesting these lesions may be viewed as a marker of active vascular disease.

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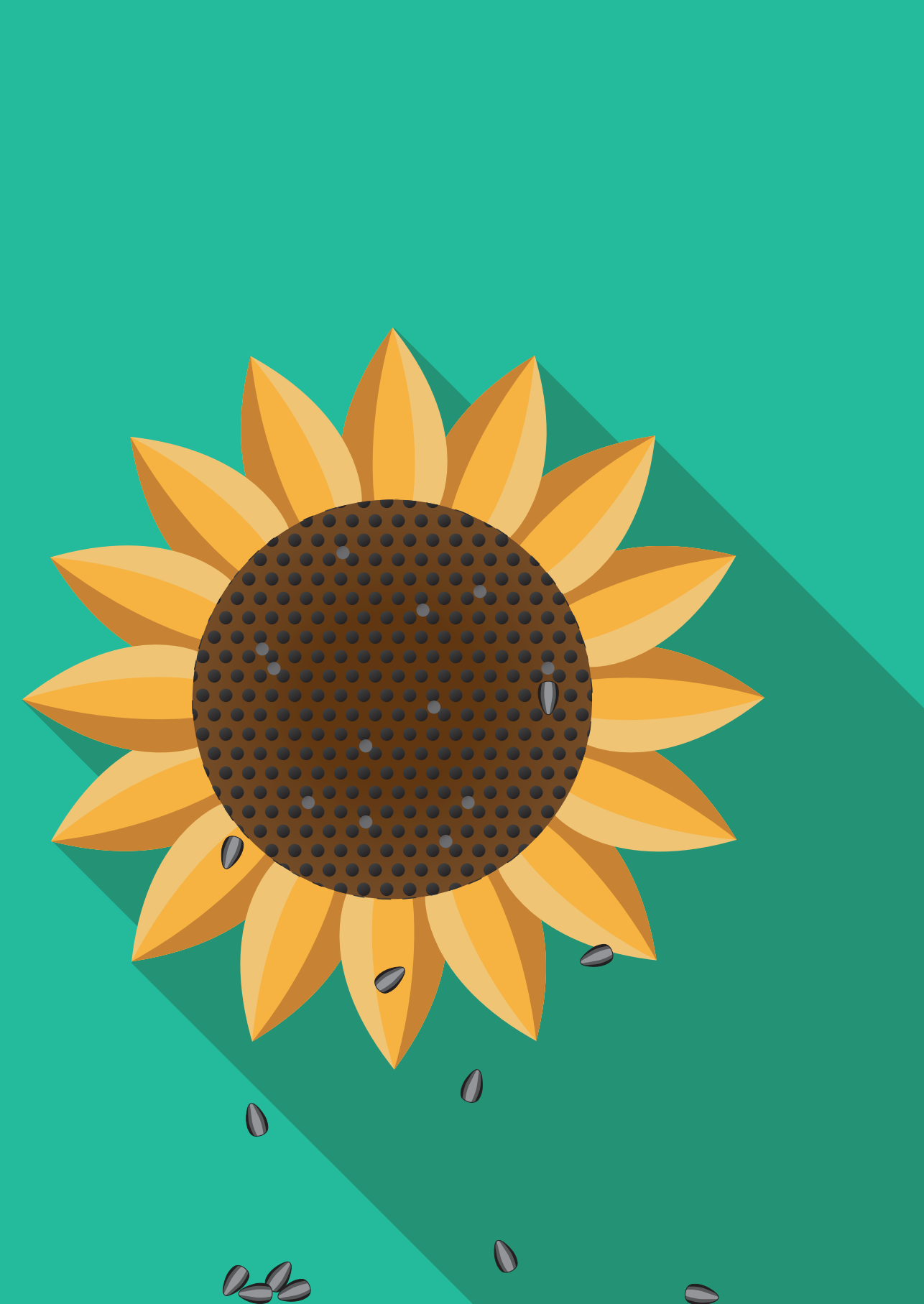


NEDERLANDSE SAMENVATTING

Casus

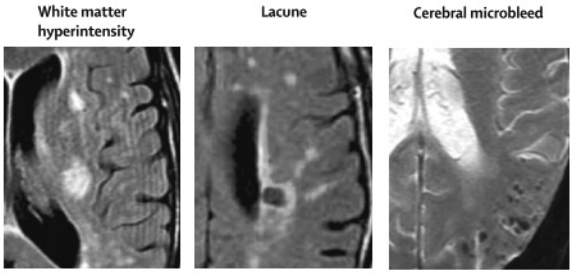
Mevrouw Jansen is 75 jaar oud en komt samen met haar dochter bij de huisarts. Haar dochter maakt zich zorgen, ze vindt haar moeder in het afgelopen half jaar een stuk trager en warriger geworden. Mevrouw Jansen vertelt de huisarts dat ze gestopt is met haar vrijwilligerswerk in het filmhuis. Ze vond het te stressvol om tegelijk achter de bar te staan én kaartjes te verkopen en raakte hierdoor overstuur. Ook lukt het haar niet meer de wekelijkse kruiswoordpuzzel in de krant op te lossen zoals ze dat vele jaren deed. De huisarts verwijst haar door naar de neuroloog, die constateert dat er lichte stoornissen zijn van de planningsfunctie en concentratie. Daarnaast heeft zij een verhoogde bloeddruk en is er op de MRI-scan hersenschade door "small vessel disease" zichtbaar. De neuroloog stelt de diagnose "mild cognitive impairment" en adviseert de huisarts om de bloeddruk te behandelen.

Inmiddels is het 3 jaar later. De klachten zijn de afgelopen jaren redelijk stabiel gebleven tot mevrouw Jansen op 78-jarige leeftijd met een herseninfarct wordt opgenomen in het ziekenhuis op de afdeling neurologie. Gelukkig verbeteren de verlammingsverschijnselen van de linker arm binnen enkele dagen en kan ze weer met ontslag naar huis. Een paar maanden later blijkt dat het toch niet goed gaat. Ze heeft al dagen niet voor zichzelf gekookt en vergeet rekeningen te betalen. Ook voelt ze zich somber en komt ze amper de deur nog uit. Ze bezoekt met haar dochter opnieuw de neuroloog, die constateert dat er nu sprake is van "vasculaire dementie".

Vascular cognitive impairment

Deze casus illustreert een typisch beloop van een patiënt met de diagnose *vasculaire dementie*. Vasculaire dementie wordt veroorzaakt door vaatschade in de hersenen en is na de ziekte van Alzheimer de belangrijkste oorzaak voor dementie. Bij dementie in het algemeen is er sprake van cognitieve achteruitgang, waardoor iemand niet meer onafhankelijk kan functioneren in het dagelijks leven. Vaak ontstaan de cognitieve klachten geleidelijk in de tijd, het stadium met deze subtielere klachten wordt *mild cognitive impairment* genoemd. Alle cognitieve klachten – van subtiele concentratiestoornissen tot gevorderde dementie – waarbij gedacht wordt dat vaatschade een rol speelt als oorzaak worden geschaard onder de term *vascular cognitive impairment* [1]. Vascular cognitive impairment vormt het belangrijkste onderzoeksdomein van dit proefschrift.

Verschillende vormen van vaatschade kunnen bijdragen aan vascular cognitive impairment. Zo kan er zoals bij mevrouw Jansen sprake zijn van een beroerte, dat wil zeggen schade in de hersenen door een herseninfarct (waarbij een bloedvat is afgesloten) of een hersenbloeding (waarbij een bloedvat is geknapt). Een beroerte gaat gepaard met plotselinge verschijnselen, zoals een verlamming van een arm of problemen met spreken. Echter, bij een groot deel van de patiënten met vascular cognitive impairment is er nooit sprake geweest van plotselinge uitvalsverschijnselen, maar is er wel degelijk vaatschade in de hersenen aanwezig. Een belangrijke oorzaak van deze "stille" vaatschade is *small vessel disease*, een ziekte waarbij de wand van de allerkleinste bloedvaatjes is verdikt door een langdurig verhoogde bloeddruk of door ophoping van het amyloïd-eiwit in de vaatwand. Small vessel disease gaat gepaard met een aantal kenmerkende vormen van hersenschade, die kunnen worden opgespoord met behulp van een MRI-scan van de hersenen. In figuur 1 een aantal van deze typische afwijkingen te zien, zoals schade aan de verbindingsbanen van de hersenen (white matter hyperintensities), kleine herseninfarcten diep in de hersenen (*lacunar infarcts*) en kleine puntbloedingen (*microbleeds*).

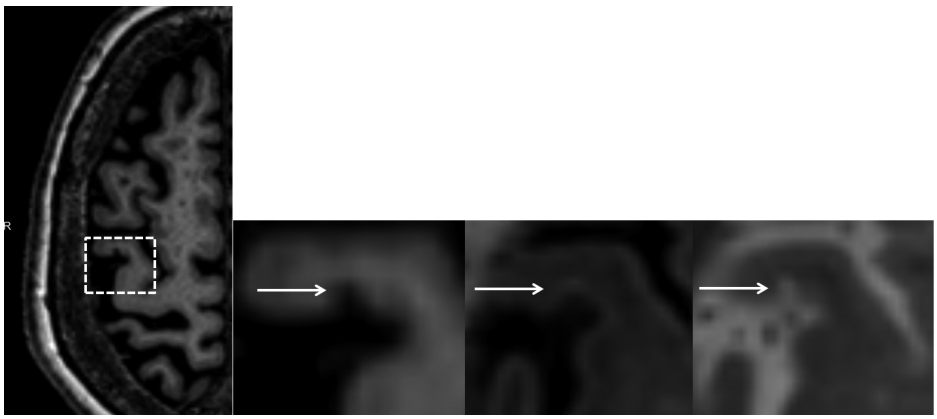


Figuur 1: Voorbeelden van hersenschade door small vessel disease, zoals voorgesteld in de STRIVE-criteria [2].

Een MRI-scan van de hersenen speelt dus een belangrijke rol bij het opsporen van vaatschade in de hersenen bij patiënten met cognitieve klachten. Toch is het niet altijd zo rechttoe rechtaan en hebben sommige patiënten veel cognitieve klachten, maar is de vaatschade die we zien op de MRI-scan beperkt. De afgelopen jaren komen er steeds meer aanwijzingen dat een standaard MRI-scan niet alle aanwezige hersenschade bij patiënten met small vessel disease goed laat zien. Geavanceerde beeldvormingstechnieken, zoals de krachtige 7 tesla MRI-scanner met een bijzonder hoge resolutie, ondersteunen het idee dat we nu slechts tegen het topje van de ijsberg van vaatschade in de hersenen aankijken. Dit proefschrift gaat over een vorm van hersenschade die voorheen niet goed zichtbaar was op de MRI-scan: de microinfarcten.

Microinfarcten: de "onzichtbare" afwijking

Microinfarcten zijn hele kleine herseninfarcten, die zijn ontstaan door een bloed en/of zuurstoftekort van hersenweefsel. Microinfarcten worden al meer dan 20 jaar gerapporteerd in autopsiestudies waarbij hersenweefsel van overleden personen onder de microscoop wordt onderzocht. Deze studies laten zien dat microinfarcten vooral voorkomen bij personen met (vasculaire) dementie of uitgebreide vaatschade in de hersenen [3]. Omdat de gemiddelde doorsnede van microinfarcten in deze microscopische studies varieert tussen 50 micrometer en een paar millimeter zijn ze niet goed zichtbaar met het blote oog en werd verondersteld dat ze ook niet op MRI te zien zouden zijn. Hierdoor hebben microinfarcten de bijnaam de "onzichtbare" afwijking gekregen [4]. Toch is het in 2013 in de VCI-onderzoeksgroep van het UMC Utrecht gelukt om de nét iets grotere microinfarcten met behulp van de 7 tesla MRI-scanner aan te tonen in levende personen [5]. Het gedeelte microinfarcten met de grootste diameter bleek zelfs zichtbaar op een 3 tesla MRI-scanner [6]. Dit laatste is een groot voordeel, omdat de 3 tesla MRI-scanner in meeste Nederlandse ziekenhuizen gebruikt wordt voor patiëntenzorg én in diverse cohortonderzoeken van mensen met cognitieve stoornissen. Deze ontwikkeling vormde een schakelstuk voor het oplossen van belangrijke vraagstukken rondom microinfarcten, zoals hoe microinfarcten ontstaan en wat hun rol is bij cognitieve achteruitgang.



Figuur 2: Een voorbeeld van een microinfarct op een 3 tesla MRI-scanner [7].

Oorzaken en mechanismen van cerebrale microinfarcten

In het eerste deel van dit proefschrift heb ik me gericht op de risicofactoren en onderliggende mechanismes die betrokken zijn bij het ontstaan van microinfarcten. Uit de eerdere (onder andere) autopsiestudies weten we dat hierbij waarschijnlijk

drie mechanismes betrokken zijn [4,8]. Allereerst afwijkingen aan de lokale kleine bloedvaatjes, (1) small vessel disease. Daarnaast kunnen microinfarcten waarschijnlijk worden veroorzaakt door bloedpropjes van elders in het lichaam, die noemen we (2) *trombo-embolieën*. Tot slot is gesuggereerd dat te weinig bloed toevoer – bijvoorbeeld door een tijdelijk lage bloeddruk – microinfarcten kan veroorzaken, dit wordt (3) *cerebrale hypoperfusie* genoemd. We hebben de relatie tussen microinfarcten en deze mechanismes in verschillende onderzoekssettings bestudeerd.

In **hoofdstuk 2** onderzochten we hoe vaak microinfarcten voorkwamen in een groep patiënten met vascular cognitive impairment. Het bleek dat microinfarcten twee keer zo vaak voorkwamen in patiënten (20% van de gevallen) ten opzichte van gezonde leeftijdsgenoten (10% van de gevallen). Daarnaast vonden we een relatie tussen microinfarcten en andere vormen van hersenschade op de MRI-scan door zowel small vessel disease als trombo-embolieën. De bevindingen uit hoofdstuk 2 zijn een belangrijke bevestiging dat de microinfarcten die we detecteren op MRI meerdere oorzaken kunnen hebben.

In **hoofdstuk 3** hebben we de relatie tussen microinfarcten en hersendoorbloeding onderzocht met behulp van *arterial spin labeling*. Dit is een innovatieve MRI-techniek waarbij bloeddoorstroming van de hersenen kan worden gemeten door bloed magnetisch te labelen in de halsslagaders en dit signaal weer op te pikken in het hersenweefsel, zodat je een overzichtskaart krijgt van de hersendoorbloeding. Patiënten van de geheugenpolikliniek mét microinfarcten bleken een 12% lagere hersendoorbloeding te hebben dan patiënten zonder microinfarcten. De verminderde doorbloeding bleek niet specifiek voor de hersenregio rondom het microinfarct, maar een globaal verschijnsel over het gehele hersenoppervlak. Deze resultaten zijn niet eenvoudig te interpreteren. Ze vertellen ons namelijk niet of microinfarcten werkelijk het gevolg zijn van de verminderde hersendoorbloeding. De relatie tussen microinfarcten en doorbloeding kan ook verklaard worden door het feit microinfarcten vaker voorkomen bij patiënten met verder gevorderde hersenschade en dat deze hersenschade de verminderde doorbloeding veroorzaakt. We hebben daarom gezocht naar een klinisch model waarin we kunnen veronderstellen dat hersendoorbloeding het primaire probleem is en daarbij gebruik gemaakt van de onderzoeksgegevens van de *Hart-Breinconnectie*. Dit is een Nederlands onderzoekscollectief van verschillende medische disciplines uit meerdere universitaire centra dat de relatie tussen hersendoorbloeding en cognitieve problemen onderzoekt.

In **hoofdstuk 4** hebben we patiënten met een verstopping van één van de halsslagaders onderzocht als model voor verminderde hersendoorbloeding. Het vóórkomen van microinfarcten bij deze patiënten bleek veel hoger (54% van de gevallen) dan in een referentiepopulatie (6% van de gevallen). De belangrijkste bevinding van deze studie was echter dat microinfarcten vaker lijken voor te komen in de hersenhelft aan de kant van de verstopte halsslagader. Ook was de aanwezigheid van microinfarcten geassocieerd met een ernstigere mate van vernauwing en verminderde bloeddoorstroming in de overige halsslagaders. In **hoofdstuk 5** hebben we de relatie onderzocht tussen microinfarcten en hersendoorbloeding in patiënten met hartfalen met een verminderde pompkracht van het hart. Ook nu weer als model voor verminderde hersendoorbloeding, maar nu met een oorzaak nog verder van de hersenen gelegen; aan de basis van de bloeddoorstroming. Patiënten met hartfalen hadden een verhoogd risico op microinfarcten (17% van de gevallen) ten opzicht van de referentiepersonen (7% van de gevallen). Daarnaast vonden we dat de aanwezigheid van microinfarcten gepaard ging met een 11% lagere *cardiac index*, een maat voor de pompkracht van het hart. De bevindingen van zowel hoofdstuk 4 als 5 lijken een directere aanwijzing te geven dat microinfarcten kunnen worden veroorzaakt door een verminderde hersendoorbloeding.

Hersenschade rondom microinfarcten

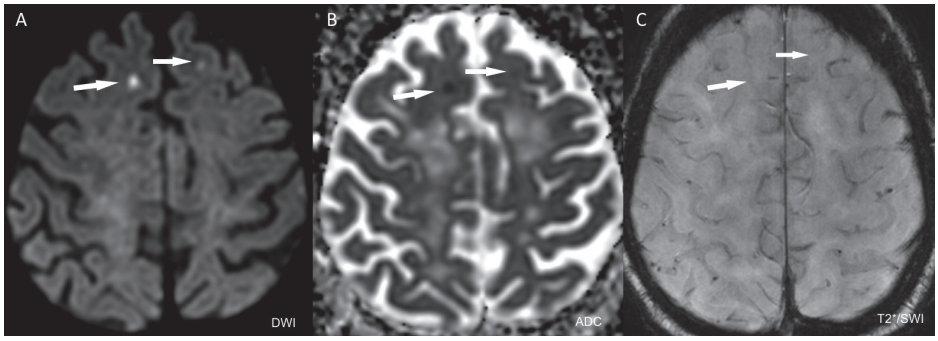
Microinfarcten zijn erg kleine hersenafwijkingen. Toch suggereren (autopsie)studies dat ze weldegelijk een grote impact kunnen hebben op het functioneren van de hersenen. Hoe kunnen één of twee microinfarcten nou zo'n verschil maken? Ten eerste is het goed te realiseren dat de 3 tesla MRI-scan slechts een fractie van de totale hoeveelheid microinfarcten in het brein zichtbaar maakt. We kunnen namelijk alleen het deel van de microinfarcten met grootste diameter (vanaf ongeveer 1-2mm) zien, terwijl we uit autopsiestudies weten dat er veel meer kleinere microinfarcten in de hersenen aanwezig zijn. Sommige onderzoekers beweren dat er in de hersenen van aangedane personen misschien wel een paar duizend microinfarcten kunnen zitten [9]. Hieraan hebben microinfarcten overigens de bijnaam "de kakkerlakken" van het brein te danken en dit is ook de reden dat er een kakkerlak te zien op de achterkant van dit proefschrift Toch blijft het totale volume van deze microinfarcten samen zeer gering, zelfs minder dan 0.1% van het hersenvolume. Één van mogelijke oorzaken is dat microinfarcten gepaard gaan met bijkomende hersenschade rondom het microinfarct, dit wordt ook wel *perilesional* schade genoemd. In het tweede deel van het proefschrift onderzochten we de aanwezigheid van perilesional schade in zowel de aangrenzende hersenschors (cortex) als de onderliggende verbindingsbanen (witte stofbanen).

In **hoofdstuk 6** hebben we gekeken naar de dikte van de hersenschors in patiënten van de geheugenpolikliniek mét en zonder microinfarcten. We vonden dat patiënten met microinfarcten een dunnere hersenschors hebben ten opzichte van patiënten zonder microinfarcten. Een opvallende bevinding daarbij is dat het hersenschors dunner is in een vrij grote breinregio rondom het microinfarct. Het lijkt er dus op dat de perilesional schade vele malen groter is dan het microinfarct zelf. Mogelijk wordt dit veroorzaakt door verstoorde verbindingen tussen de hersencellen in de hersenschors. In **hoofdstuk 7** hebben we onderzocht of microinfarcten effect hebben op het onderliggende hersennetwerk met *diffusion-tensor imaging*, een MRI-techniek waarbij de verbindingsbanen van de hersenen in kaart kunnen worden gebracht. We vonden dat microinfarcten een sterke voorkeur hebben voor bepaalde hersengebieden (met name in het topje van het brein), maar dat de hersenenconnecties in deze regio's niet slechter waren in patiënten mét microinfarcten in vergelijking met de patiënten zonder microinfarcten. Het lijkt er dus op dat microinfarcten met name gepaard gaan met een dunnere hersenschors rondom de afwijking, maar niet zozeer effect hebben op de onderliggende verbindingsbanen van de hersenen.

De impact van microinfarcten op hersenfunctie

Zoals besproken in de introductie van dit hoofdstuk gaat hersenschade vaak gepaard met een verslechtering van cognitie, ook wel vascular cognitive impairment genoemd. In het derde deel van dit proefschrift hebben we onderzocht of de aanwezigheid van microinfarcten gepaard gaat met een slechtere hersenfunctie. In **hoofdstuk 2** vonden we dat patiënten van de geheugenpolikliniek mét microinfarcten vaker de diagnose vasculaire dementie hadden en daarnaast slechter presteerden op de cognitieve taken van het executief functioneren en visuoperceptie dan patiënten zonder microinfarcten. Deze relatie met slechter cognitief functioneren bleef bestaan wanneer het statistisch model werd gecorrigeerd voor leeftijd, geslacht en andere aanwezige vaatschade. In **hoofdstuk 4** hebben we het cognitief functioneren van patiënten met hartfalen mét microinfarcten vergeleken met patiënten zonder microinfarcten, maar constateerden uiteindelijk geen verschil. De vraag is hoe het komt dat microinfarcten wel een verschil maken in cognitief functioneren bij patiënten van de geheugenpolikliniek, maar niet bij patiënten met hartfalen. De oorzaak hiervan zou kunnen liggen in de aanwezigheid van andere aanwezige hersenschade, waardoor de cognitieve reserve is afgenomen. Microinfarcten fungeren dan als het ware als de druppel die de emmer doet overlopen. Of dit daadwerkelijk zo is, moet nog verder worden onderzocht.

Tot slot hebben we in **hoofdstuk 8** een ander type microinfarct onderzocht in patiënten met vascular cognitive impairment. Acute microinfarcten kunnen worden gedetecteerd op een diffusie gewogen MRI-scan tot ongeveer twee weken nadat ze ontstaan zijn. Ze zijn – in tegenstelling tot de chronische microinfarcten – zowel in de hersenschors als de verbindingsbanen van de hersenen te zien. Een voorbeeld van een acuut microinfarct is te zien in figuur 3. Acute microinfarcten waren in ongeveer 2% van de patiënten te zien en bleken sterk geassocieerd met een slechte klinische uitkomst na een periode van twee jaar. Ze gaven een hoger risico op een beroerte en patiënten met acute microinfarcten moesten vaker in een verpleeghuis worden opgenomen vanwege cognitieve achteruitgang. Mogelijk kunnen we acute microinfarcten in de toekomst gaan gebruiken als aanwijzing voor de neuroloog dat een patiënt zeer actief vaatschade in de hersenen aan het ontwikkelen is. Wel moet worden opgemerkt dat het nog niet duidelijk is hoe we deze patiënten het beste kunnen behandelen.



Figuur 3: Een voorbeeld van een acuut microinfarct op een DWI-scan [10].

Conclusie

Dit proefschrift gaat over de oorzaken en gevolgen van microinfarcten die zichtbaar zijn op een 3 tesla MRI-scan. We hebben bevestigd dat er meerdere mechanismes betrokken zijn bij het ontstaan van microinfarcten, waaronder small vessel disease en trombo-embolieën. Daarnaast hebben we in dit proefschrift sterke aanwijzingen gevonden dat microinfarcten veroorzaakt kunnen worden door een slechtere doorbloeding van de hersenen. De aanwezigheid van microinfarcten gaat gepaard met verminderd cognitief functioneren en de diagnose vasculaire dementie. Het is nog niet duidelijk hoe microinfarcten precies leiden tot een verslechtering van het (cognitief) functioneren, mogelijk speelt de perilesional schade een rol. Dit proefschrift laat zien dat het gebied met hersenschade in de hersenschors rondom een microinfarct vele malen groter is dan het microinfarct zelf. Tot slot toonden we aan dat de aanwezigheid van een acuut

microinfarct een sterke voorspeller is van een slechte klinische uitkomst. Toekomstige studies zullen moeten uitwijzen hoe we patiënten met (acute) microinfarcten het beste kunnen behandelen om cognitieve verslechtering te voorkomen.

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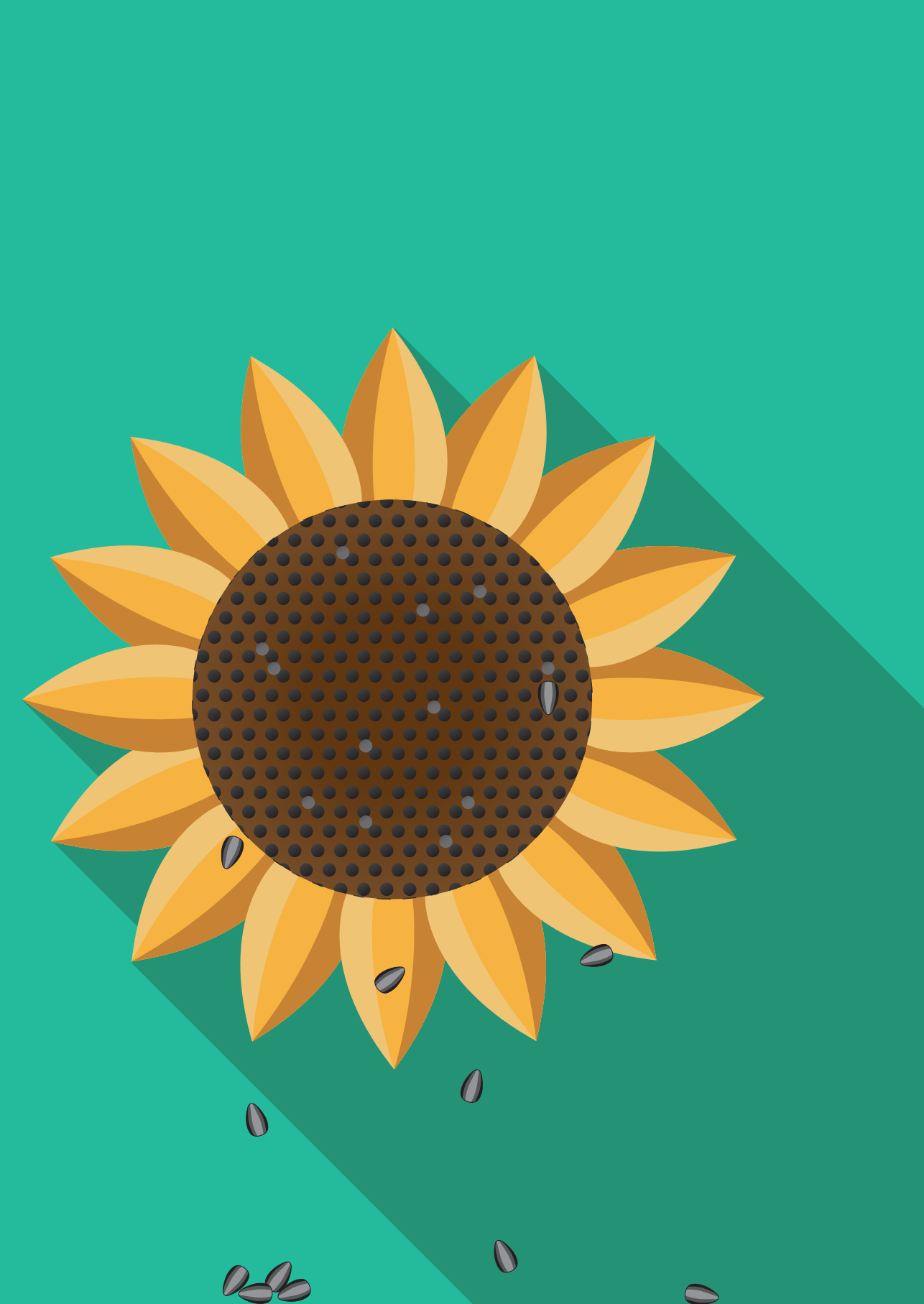

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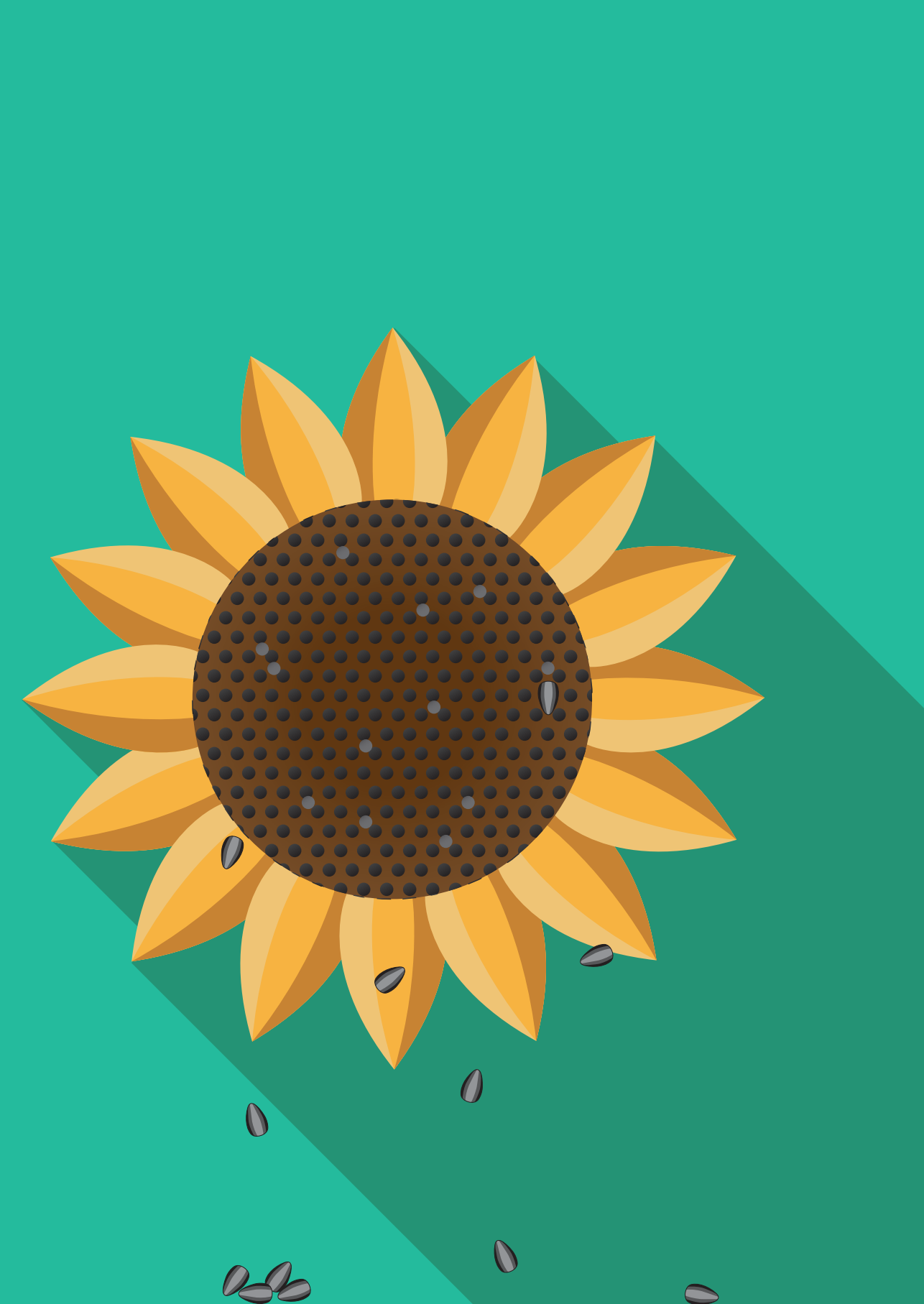
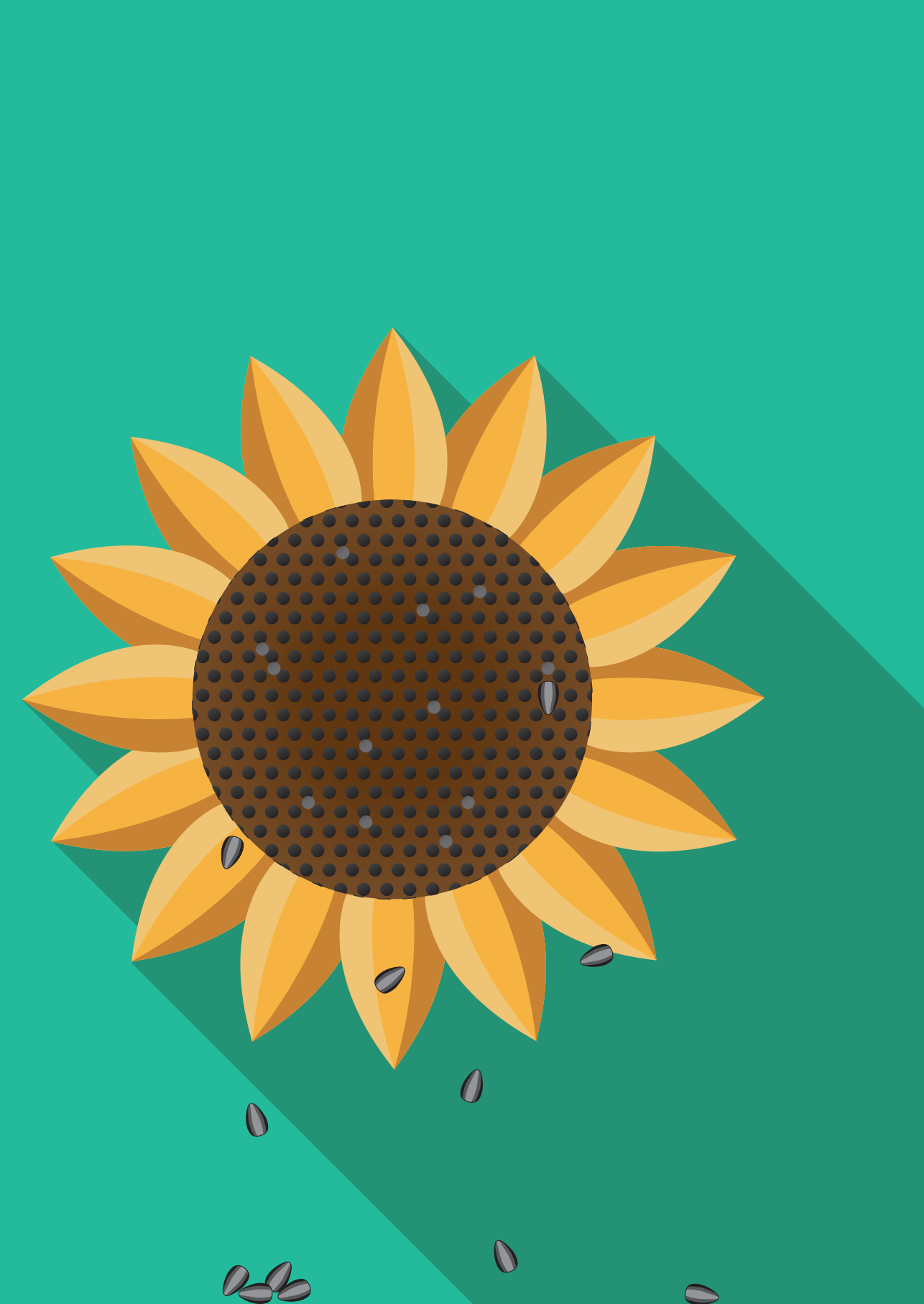


ABOUT THE AUTHOR

Doeschka Aniek Ferro was born on July 31 1984 in Zwolle, the Netherlands. In 2002 she obtained her VWO diploma at the Etty Hillesum Lyceum in Deventer. She decided life should take her somewhere new and studied English literature for a year at the London Metropolitan University. In 2003 she obtained a place at University College London to study psychology and received her BSc (Hons) in 2006. During her studies she had worked as an administrative assistant at the National Hospital for Neurology at Queens Square - around the corner from her university - where she gained enthusiasm for the field of medicine, particularly neurology. She moved back to the Netherlands and enrolled at the Vrije Universiteit (VU) in Amsterdam to study medicine and an MSc in clinical neuropsychology. She did a research internship at the Sophia Children's Hospital in Rotterdam studying executive functioning in very preterm children at schooling age (Supervision: Dr. Moens & Prof. Oosterlaan, VU) and a research internship at the VU medical center studying the effect of adolescent fitness on cognition in adulthood as part of the Amsterdam Growth and Health Longitudinal Study (Supervision: Dr. Deijen & Prof. Dent, VUmc). In 2010 she obtained her BSc in medicine and her MSc in clinical neuropsychology and continued the clinical part of her medical training. In her final year of medical school in 2013 she successfully applied for an elective clinical internship at the memory clinic of Columbia University in New York (USA), where she developed a strong interest in dementia research. After graduating in the summer of 2013 she started working as a neurology resident at the UMC Utrecht (Supervision: Prof. Wokke, Dr. Seute & Prof. Biessels). In 2015 she started as a PhD researcher on the Heart-Brain Connection Study under supervision of Prof. Biessels, Prof. Hendrikse and Dr. Zwanenburg. After defending her thesis on cerebral microinfarcts in March 2020, she will continue her residency training which she plans to complete in 2022. Doeschka lives together with her partner Malte in the (op)Vrolikstraat in Amsterdam.



PUBLICATIONS

This thesis:

Ferro DA, van Veluw SJ, Koek HL, Exalto LG, Biessels GJ (2017) Cortical Cerebral Microinfarcts on 3 Tesla MRI in Patients with Vascular Cognitive Impairment. *J. Alzheimer's Dis.* **60**, 1443–1450.

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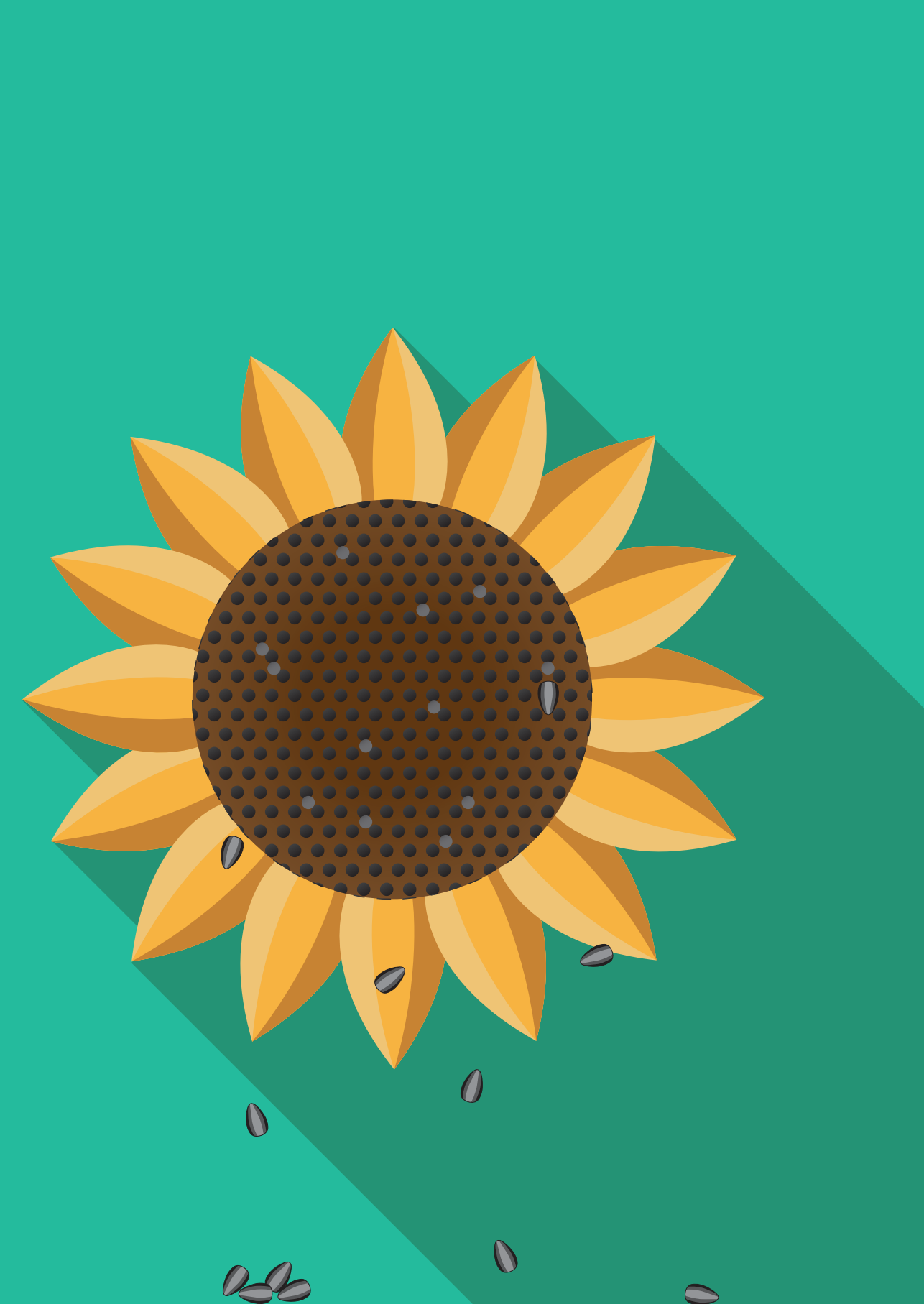

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Zon
Ik sta een tijdje met mijn vriendje op de dijk.

Kijk, zegt hij: de zon zakt in de zee
en het licht zakt langzaam mee

en de schaduwen verdwijnen
en de kleuren van de dag

en als het donker wordt
dan is het avond

en is het avond
wordt het nacht.

Mooi hè, zegt hij.
En we zwijgen.

Het is mooi en
goed bedacht.

Ester Naomi Perquin

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