How do different forms of vascular brain injury relate to cognition in a memory clinic population; the TRACE-VCI study.

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Running title: Vascular brain injury patterns and cognition.

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ABSTRACT

Background: Memory clinic patients frequently present with different forms of vascular brain injury due to different etiologies, often co-occurring with Alzheimer pathology.

Objective: We studied how cognition was affected by different forms of vascular brain injury, possibly in interplay with Alzheimer pathology.

Methods: We included 860 memory clinic patients with vascular brain injury on Magnetic Resonance Imaging(MRI), receiving a standardized evaluation including cerebrospinal fluid(CSF) biomarker analyses(n=541). The cognitive profile of patients with different forms of vascular brain injury on MRI(moderate/severe white matter hyperintensities(WMH)(n=398), microbleeds(n=368), lacunar(n=188) and non-lacunar(n=96) infarct(s), macrobleeds(n=16)) was assessed by; 1)comparison of all these different forms of vascular brain injury with a reference group(patients with only mild WMH(n=205) without other forms of vascular brain injury), using linear regression analyses also stratified for CSF biomarker Alzheimer profile and 2)multivariate linear regression analysis.

Results: The cognitive profile was remarkably similar across groups. Compared to the reference group effect sizes on all domains were <0.2 with narrow 95% confidence intervals, except for non-lacunar infarcts on information processing speed(age, sex and education adjusted mean difference from reference group(β)-0.26,p=0.05). Results were similar in the presence(n=300) or absence(n=241) of biomarker co-occurring Alzheimer pathology. In multivariate linear regression analysis higher WMH burden was related to a slightly worse performance on attention and executive functioning(β;-0.08,p=0.02) and working memory(β;-0.08,p=0.04).

Conclusion: Although different forms of vascular brain injury have different etiologies and different patterns of cerebral damage, they show a largely similar cognitive profile in memory clinic patients regardless of co-occurring Alzheimer pathology.

Keywords: cerebrovascular disorders, cerebral small vessel diseases, cognitive disorders, neuropsychological test.

INTRODUCTION

Patients presenting at a memory clinic often show vascular brain injury on Magnetic Resonance Imaging (MRI) of the brain. Of note, vascular brain injury is a heterogeneous construct in this setting. Different forms of vascular brain injury have different etiologies and vascular lesions affect different parts of the brain. This heterogeneity of vascular injury, possible in interplay with co-occurring Alzheimer pathology, might be reflected in distinct cognitive profiles.

The literature on the relationship between vascular brain lesions on MRI and cognition largely concerns studies on non-memory clinic populations, mostly population based cohorts. These studies often evaluate only one type of vascular brain injury in relation to cognition. Comparisons between different forms (for instance lacunar infarcts versus microbleeds) are generally not reported [1]. Other studies address different forms of vascular brain injury as one combined entity, generally as a construct for "small vessel disease" [2-4]. Conventionally, small vessel disease, in particular with subcortical lesions, is considered to be related to impairment of the cognitive domains executive functioning and information processing speed, with relative preservation of memory [1, 3, 5]. If memory impairment is present, it typically involves retrieval problems, which may respond to cuing with preserved recognition [6]. Alzheimer's disease (AD), in contrast, mainly involves episodic memory impairment affecting the encoding and storage of information [6]. These concepts on etiology-function relationships are commonly used in clinical practice, when assigning potential clinical significance to vascular brain injury on MRI in the diagnostic work up of patients with cognitive complaints. However, thus far, there are only few studies in a memory clinic population that have actually assessed how different forms of vascular brain injury and possible co-occurring Alzheimer pathology relate to cognition, independent of the assigned clinical diagnosis or assumed etiology. This often unrecognized knowledge gap can lead to circular reasoning. Pre-existing concepts on functional impact of vascular brain lesions may bias the clinical diagnosis and the relevance that is assigned to these lesions. For example, in a patient with prominent episodic

memory deficits we would consider a likely diagnosis of AD, and might consider moderate to severe white matter hyperintensities (WMH) on MRI as co-morbidity. However, if we would encounter exactly the same MRI in another patient with a deficit in executive functioning, we might label the WMH as the primary etiology.

This study compared the cognitive profile between patients with different forms of vascular brain injury (WHM, lacunar infarct(s), non-lacunar infarct(s), microbleed(s) or macrobleed(s)) in a memory clinic cohort. We also assessed whether this relation changed according to the presence or absence of cerebrospinal fluid (CSF) biomarker evidence of Alzheimer pathology. Patients were derived from the "Utrecht-Amsterdam clinical features and prognosis in vascular cognitive impairment" (TRACE-VCI) study population [7].

MATERIALS AND METHODS

Study population

Patients were included from the TRACE-VCI study population, using cross sectional data. The rationale and design of the TRACE-VCI study has been published previously [7]. In short, the aim of the TRACE-VCI study was to determine the influence of different forms of vascular brain injury on the cognitive profile and prognosis of patients with cognitive complaints in a memory clinic setting. We included patients regardless of cognitive severity, including patients with no objective cognitive impairment (NOCI), mild cognitive impairment (MCI) and dementia, attending the memory clinics between September 2009 and December 2013. All patients showed evidence of vascular brain injury (i.e. possible vascular cognitive impairment (VCI)) on MRI, which was operationalized as the presence of at least one of the following neuro-imaging markers: (1) mild WMH also known as Fazekas scale grade 1 [8] and an increased vascular risk defined as the presence of ≥ 2 vascular risk factors (hypertension, hypercholesterolemia, diabetes mellitus, obesity, current smoking or a reported history of a vascular event other than stroke; full definitions in design paper [7]) (2) moderate to severe WMH, also known as Fazekas scale grade ≥ 2 or (3) ≥ 1 lacunar infarct(s) (4) ≥ 1 non-lacunar (large vessel) infarct(s) (5) ≥ 1 cerebral microbleed(s) (6) ≥ 1 intracerebral hemorrhage(s) (ICH) /macrobleed(s).

Subjects were included from the outpatient clinic of the VU University Medical Center (VUMC), registered in the Amsterdam Dementia Cohort (N = 664) and from two outpatient memory clinics of the University Medical Center Utrecht (UMCU) (N = 196) [7]. One patient from the original reported cohort [7] was erroneously included (glioma with bi-hemispheric involvement) and therefore the current study included 860 patients. Patients with a presumed primary etiology other than vascular brain injury or neurodegeneration (e.g. brain tumors) were excluded. The presence of co-occurring etiologies, in addition to vascular injury, such as neurodegenerative pathology or

depression was accepted, because many patients with VCI have neurodegenerative diseases as a co-morbid etiology and depression can be a manifestation of cerebrovascular disease [9, 10].

Each patient underwent a standardized extensive one-day memory clinic evaluation including an interview, physical and (cognitive) neurological examination, laboratory testing, extensive neuropsychological testing and a MRI-scan of the brain. Lumbar puncture was performed in a subset of the study population [7]. In the VUMC, collection of CSF was a standard procedure for research purposes. Only patients with contraindications, such as the use of anticoagulants, an unsuccessful procedure or refusal of the puncture did not undergo the procedure. In the UMCU, collection of CSF biomarkers was not a standard procedure and only performed at the discretion of the doctor and the patient, also in the context of research. All patients provided informed consent prior to research related procedures.

Cognitive assessment

We used the Dutch version of the Mini-Mental State Examination (MMSE; maximum score of 30) as a cognitive screening test [11]. The severity of cognitive symptoms was assessed using the Clinical Dementia Rating (CDR; 0-3) global score [12]. All participants underwent an extensive neuropsychological examination, with some variation between centers and over time. Harmonization of the test battery had been established through a Dutch multicenter university memory clinic research program on diagnosis and prognosis of cognitive impairment and dementia [13]. Tasks that were available for the majority of patients (>70%) were included. The tasks were summarized in five major widely used cognitive domains to reduce the amount of neuropsychological variables for statistical analysis and clinical interpretation: (1) memory, (2) attention and executive functioning, (3) information processing speed, (4) perception and construction and (5) working memory.

The domain memory was assessed by the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT) [14] and the Visual Association Test (VAT) part A [15].

The domain attention and executive functioning was assessed using the ratio of the Trail Making Test part B and A (TMT-B and TMT-A) [16], the Stroop Color Word Test [17, 18] and the category naming tasks (animal naming, one minute) and lexical fluency tasks (one minute per letter) [19].

The domain information processing speed was assessed by the TMT-A, the Stroop Color Word Test I and II and the Digit Symbol-Coding Test (DSCT) of the WAIS-III or the Letter Digit Substitution Test (LDST) [20, 21]. The cognitive domain perception and construction was assessed using the Fragmented Letters and Dot Counting subtests of the Visual Object and Space Perception Battery (VOSP) [22].

The domain working memory was assessed by the Digit Span of the Wechsler Adult Intelligence Scale – 3rd edition (WAIS-III) [20]. After publication of the design article [7], more data regarding the Digit Span were collected. The availability and raw test scores are shown in the supplementary, table 1. More information about the tests is provided in the design article of the TRACE-VCI study [7].

Z-scores based on the total study population were created for each individual test (inversed Z-scores for the TMT and Stroop Color Word Test). The test Z-scores were averaged to create Z-scores per domain. If patients were unable to perform a test for various reasons, the test was defined as a missing variable. If individual test scores were missing, the domain Z-score was based only on the available tests.

Laboratory testing

Plasma glucose level, HbA1c levels and CSF were collected in a subset of the study population. CSF concentrations of amyloid-β_{42} ($A\beta_{42}$), tau and/or total tau phosphorylated at threonine 181 (p-tau) were measured at a central laboratory for clinics at the Department of Clinical Chemistry of the VUMC [23]. CSF samples were stored at -20˚C until biomarker analysis (within 2 months). $A\beta_{42}$, total tau, and p-tau were measured with commercially available ELISAs (Innotest β-

amyloid($_{1-42}$), Innotest hTAU-Ag and Innotest Phosphotau($_{181p}$), respectively; Innogenetics, Ghent, Belgium) on a routine basis [23]. Patients with a ratio of total tau to amyloid-β_{42} of more than 0.52 were classified as having a positive CSF biomarker Alzheimer profile [24].

MRI assessment

Brain MRI scans were performed on 3.0 Tesla (809 out of 860 patients (94%)) or 1.5 Tesla MRI scanners (51 patients (6%)). Most scans were performed on a General Electric (GE) (619 patients (72%)) or Philips (239 patients (28%)) MRI scanner. The MRI scan protocol included the following sequences: 3D T1-weighted, T2-weighted, T2*-weighted/susceptibility-weighted imaging (SWI) and fluid-attenuated inversion recovery (FLAIR) sequences. A total of 849 (99%) patients were scanned using all of these sequences. In 11 patients (1%) a 2D T1-weighted sequence was acquired instead of a 3D T1-weighted sequence and/or no FLAIR sequence was available. There were 12 patients with no microbleed rating because of a missing or unreadable T2*-weighted scan, therefore in 848 out of 860 patients (99%) microbleeds were scored. Further details of the MRI sequence parameters were described in the design article of the TRACE-VCI-study [7].

WMH were rated using the Fazekas scale (WMH grade 0-3: none or a single punctate lesion, multiple punctate lesions (mild WMH), beginning confluency of lesions (moderate WMH), large confluent lesions (severe WMH)) on FLAIR images [8]. Non-lacunar and lacunar infarct(s), microbleed(s) and ICH/macrobleed(s) were all rated in line with the STRIVE (standards for reporting vascular changes on neuroimaging) criteria [25]. Ratings were performed by or under the supervision of a neuroradiologist (in training).

Statistical analysis

Statistical analyses were performed using SPSS (version 21; SPSS, Chicago, IL, USA). The computed Z-scores of the five cognitive domains were used to evaluate the cognitive profile in relation to the vascular brain injury type in two ways.

Firstly, the mean cognitive domain Z-scores for each form of vascular brain injury were compared to a reference group, which was defined as patients with *only* mild WMH (Fazekas score 1), ≥ 2 vascular risk factors and no other forms of vascular brain injury. A linear regression model was used, adjusting for sex, age and educational level.

Secondly, multivariate linear regression analysis was performed including the lesion burden for all forms of vascular brain injury (i.e. WMH entered as Fazekas scale 0, 1, 2 or 3, microbleed(s) as 0, 1-4 or ≥ 5, lacunar infarct(s) as 0, 1 or ≥ 2 and non-lacunar infarct(s) as 0 or ≥ 1) with sex, age and educational level, number of vascular risk factors (0-6) and CSF biomarker Alzheimer profile as covariates.

Presence or absence of Alzheimer pathology, as reflected in positive or negative CSF biomarker profile can influence the cognitive profile. Moreover, the relation of vascular lesion burden to cognition may vary according to disease stage (NOCI, MCI and dementia). Therefore, we performed pre-specified post hoc multivariate linear regression analyses, stratified according to CSF biomarker status and in separate analyses also to disease stage, relating lesion burden for all forms of vascular brain injury to cognition.

A p-value of less than 0.05 was considered significant.

RESULTS

Characteristics of the study population

Mean age of the cohort was 67.7 years (SD ± 8.5) and 46% were female. The median MMSE score was 25 (IQR 22-28) points out of 30 and the median CDR was 0.5 (IQR 0.5-1) out of 3. Dementia was diagnosed in approximately half of patients (n=449 (52%)), of whom 304 (68%) were clinically diagnosed with AD. CSF was collected in 541 (63%) patients. In total, 300 (56%) of these patients had a positive CSF biomarker Alzheimer profile (ratio of total tau to amyloid-β_{42} > 0.52). Table 1 is providing details on demographic characteristics, vascular risk factors, severity of cognitive impairment and the results of CSF biomarkers in the study population.

Please insert table 1 here

Distribution of different forms of vascular brain injury

A total of 500 (58%) patients presented with *only* one single form of vascular brain injury (table 2). Hence, the other 360 (42%) patients showed mixed forms of vascular brain injury. WMH burden was Fazekas 0 in 71 (8%) patients, 1 in 391 (46%), 2 in 289 (34%) and 3 in 109 (13%) patients. Microbleeds were absent in 480 (56%) patients, whereas 276 (32%) had 1-4 and 91 (11%) patients had 5 or more microbleed(s). Lacunar infarcts were absent in 672 (78%) patients, whereas 81 (9%) had 1 and 106 (12%) patients had ≥ 2 lacunar infarct(s). Non-lacunar infarct were absent in 764 (89%) patients and 96 (11%) patients had ≥ 1 infarct(s). Macrobleeds were infrequent (< 2%) in this cohort. We therefore did not consider macrobleeds as a separate form of vascular brain injury in our analyses on cognitive profiles.

In the 541 patients with CSF biomarker Alzheimer analysis, lacunar infarcts were significantly more present in patients with a negative versus a positive CSF biomarker Alzheimer profile (24% and 14%), whereas the other forms of vascular brain injury showed similar numbers (table 2).

Please insert table 2 here

Lesion type and cognitive profile

For all cognitive domains, data were available from at least 97% of participants, except for perception and construction (82%). The evaluated neuropsychological tests and the mean raw test scores in the whole study population are shown in the supplementary, table 1, including the number of each available test per cognitive domain. The overall cognitive profile of our study population, showing the mean unadjusted domain Z-scores for the different forms of vascular brain injury, is demonstrated in figure 1. Because Z-scores were calculated at test level across the whole population, mean domain z-scores across the whole population can deviate from 0. For all different forms of vascular brain injury, the difference in cognition compared to the reference group was remarkably small on all domains, with effect sizes of less than 0.2 with narrow 95% confidence intervals. Only patients with non-lacunar infarcts showed a slightly significant worse performance on information processing speed (age, sex and education adjusted mean difference from reference group (β) -0.26, (CI; -0.52 -0.01), p = 0.05; figure 1). Additional adjustments for the 15-item Geriatric Depression Scale (GDS) [26] (available in 814 out of 860 patients (95%)) did not materially change the results (data not shown).

Please insert figure 1 here

A post-hoc analysis, that excluded patient with mixed pathologies, showed similar results; the cognitive profile for the different single forms of vascular brain injury was largely similar to the reference group (supplementary figure 1), but the group of patients with non-lacunar infarcts was too small to evaluate (only 4 patients). We also evaluated the location of microbleeds (lobar, deeply located or mixed) showing no significant effect.

Multivariate analysis, including lesion burden

In multivariate analysis, WMH burden was related to worse performance on attention and executive functioning (-0.08 per Fazekas point, (CI; -0.14 -0.01), p=0.02; table 3) and working memory (-0.08 per Fazekas point, (CI; -0.15 -0.00), p=0.04; table 3), with similar result after adjusting for other forms of vascular brain injury, vascular risk factors and CSF biomarker Alzheimer profile. This implies that in patients with severe WMH (Fazekas 3) versus no WMH (Fazekas 0), the effect size of the deficits on these domains is around 0.25, which equals around 10 percentile points of the Gaussian distribution curve. Patients with lacunar and non-lacunar infarcts had worse performance on information processing speed than patients without infarcts, but these effects were attenuated after additional adjustment for other forms of vascular brain injury (table 3). Higher numbers of microbleeds (as a categorical variable) tended to be related to worse cognitive performance, but the results were not statistically significant (table 3).

Please insert table 3 here

Influence of disease stage

We performed stratified analyses based on the severity of cognitive impairment (NOCI, MCI and dementia). Comparing the different groups of vascular brain injury with the reference group showed similar cognitive profiles to the overall study sample in the subgroup of patients with NOCI albeit with some variation in effect sizes and bigger confidence intervals due to smaller samples compared to the total study population (supplementary figure 2). The same was also true for patients with dementia (supplementary figure 4). In patients with MCI, however, those with moderate/severe WMH or lacunar infarcts showed a slightly significant better performance on memory (age, sex and education adjusted mean difference from reference group (β) 0.22, (CI; -0.00 0.43), p = 0.05 and (β) 0.28, (CI; 0.06 0.49), p = 0.01; supplementary figure 3). Patients with MCI and non-lacunar infarcts showed a slightly significant worse performance on information processing

speed (age, sex and education adjusted mean difference from reference group (β) -0.35, (CI; -0.62 - 0.09), p = 0.01; supplementary figure 3).

Influence of lesion type considering co-occurring Alzheimer pathology

Patients with a positive CSF biomarker Alzheimer profile performed significantly worse on all cognitive domains, especially memory (-0.78, (CI; -.92 -.63), p<0.0005; table 3; also figure 3), compared to patients with a negative profile (table 3). Effect sizes substantially exceeded those of any form of vascular brain injury (table 3; model D). .

We repeated the analysis on the relation between vascular lesion type and cognition after stratification for a positive or negative CSF biomarker Alzheimer profile. This showed very similar results as for the total study population, although the sample sizes resulted in somewhat more variation in effects sizes and wider 95% confidence intervals. Again, compared to the reference group, the largest effect sizes were seen for patients with non-lacunar infarcts, regardless of the CSF biomarker Alzheimer profile being negative or positive (figure 2a and 2b).

Please insert figure 2a and 2b here

Please insert figure 3 here

DISCUSSION

This study demonstrates that memory clinic patients with different forms of vascular brain injury on MRI showed largely similar cognitive profiles, regardless of CSF biomarker Alzheimer status or severity of cognitive impairment. Patients with non-lacunar infarcts performed worse than the reference group on the cognitive domain information processing speed. Moreover, in the multivariate models WMH burden was associated with worse attention and executive functioning and working memory, but effect sizes were relatively small. On the other hand, a positive CSF biomarker Alzheimer profile by itself did markedly affect cognitive performance on all domains. These observations may certainly impact our appreciation of vascular brain lesions on MRI in clinical practice. At memory clinics neuropsychological tests in combination with patterns of vascular and non-vascular brain injury on MRI scans guide clinical diagnoses on the etiology of cognitive dysfunction. For each individual patient, the clinician assigns relevance to potential vascular brain injury in the context of cognitive dysfunction. However, the current results showed that, in this setting, patterns and severity of vascular brain injury actually explain little of the interindividual variation in cognition.

Our study is different from most of the existing literature in that we evaluated patients referred for suspected cognitive problems in a memory clinic setting. The majority of previous studies are population based, stroke clinic based or involve hospital populations selected on the presence of certain forms of vascular brain injury, rather than cognitive complaints. Moreover, previous studies mainly address one particular form of vascular brain injury, without making comparisons between the cognitive profile of different forms of vascular brain injury. WMH are the form of vascular brain injury that have been most widely investigated. Studies in large population based cohorts or cognitively healthy elderly generally do report a relation between WMH burden and cognition [27, 28]. Yet, a meta-analysis showed that effect sizes were generally small: general intelligence (Fisher z-score -0.10 (CI; -0.19 to -0.04)), memory (-0.08 (CI; -0.13 to -0.06)), processing

speed (-0.11 (CI; -0.17 to -0.07)), attention and executive functions (-0.11 (CI; -0.16 to -0.07)), and perception/construction (-0.15 (CI; -0.21 to -0.07)) [29]. In a hospital based cohort, including patients with minor cognitive complaints without formal cognitive impairment or patients with incidental vascular brain injury on brain imaging, higher burden of WMH was also associated with worse performance on these domains [30]. Although effect sizes were slightly larger than reported in the meta-analysis [29], they were also small to modest [30]. The small effect sizes that we observe for WMH burden are in line with these observations.

Regarding microbleeds, population based studies generally do report an association with worse cognitive functioning [31-33]. Of note, effect sizes and explained variance generally also were small. The Rotterdam Scan Study, for example, showed that only subjects with at least 5 microbleeds (i.e. less than 2% of the study population) had significantly worse performance than subjects without microbleeds, again with modest effects (i.e. 0.5 MMSE points) [32]. Similarly, a meta-analysis including both population and hospital based cohorts, observed small effects for the presence versus absence of microbleeds (0.3 MMSE points) [34]. Another meta-analysis evaluating the effect of microbleeds on the cognitive profile in patients diagnosed with AD showed nonsignificant small effects (effect size (hedges g) =-0.155 (CI; -0.465 0.155)) [35].

Previous studies on the impact of non-lacunar and lacunar infarcts and ICH on cognition largely focused on acute post-stroke deficits [36-40]. Fewer studies reported on so called “silent infarcts” that appear on CT or MRI without a patient having experienced acute stroke symptoms connected to this lesion. The latter are clearly most relevant for the present study, but mainly focused on cognitive decline over time [41-43] or just used the MMSE as outcome measurement. A population based cohort, showed that patients with silent brain infarcts scored 1.6 MMSE points less than patients without silent brain infarcts [44]. The results of the ISSYS study, investigating silent strokes on brain imaging in hypertensive patients have not been published yet [45]. We show that also on top of co-existing Alzheimer pathology, presence of non-lacunar infarcts is associated with worse performance on information processing speed.

Previous cross-sectional studies evaluating the relation between vascular brain injury and cognition in a memory clinic setting mostly included patients according to specific diagnoses, such as probable AD or vascular dementia. In general, the presence of WMH was associated with a small decrease in cognitive functioning, observed across different cognitive domains, mostly with effect sizes around 0.1 in patients with AD and 0.25 in patients with MCI [46]. Regarding microbleeds in memory clinic populations, only very high burden (e.g. 8 or more microbleeds, which occurred in a subset of only 5% of patients with AD [47]) has been associated with worse cognitive performance [47, 48]. The other forms of vascular brain injury are less studied in memory clinic populations, only showing a slightly worse cognitive performance for lacunar infarcts, not statistically significant [49].

Taken together, the results of our study converge with those of previous studies reviewed above. Although there are significant associations between different forms of vascular brain injury and cognition, effect sizes were generally small. There may be exceptions, of course, particularly in individual patients with exceptionally high lesion burden. Such rare cases will have little impact on the overall results of a large cohort study such as ours, but could still be clinically relevant. Hence, overall, the variance in cognitive performance attributable to the presence or absence of different forms of vascular brain injury is relatively small, which raises questions to their diagnostic value in understanding the particular cognitive profile of patients presenting at a memory clinic. Yet, this clearly does not negate the value of MRI in a memory clinic population. Apart from understanding why a patient has a particular cognitive profile, the pattern of vascular brain injury on MRI can provide important leads on the underlying etiology, for example cerebral amyloid angiopathy. Moreover, the pattern and burden of vascular brain injury should guide decisions on vascular risk management [50], which may be particularly important in vulnerable individuals such as memory clinic patients. In light of the current results, the latter may currently represent the main clinical value of detecting vascular brain injury on MRI in memory clinic patients.

The strengths of our TRACE-VCI study include its large number of included memory clinic patients, with different levels of cognitive dysfunction, vascular brain injury and clinical diagnosis, with an extensive clinical and MRI evaluation. This inclusion procedure supports generalizability of our findings to patients who attend a memory clinic with any form of vascular brain injury and allow an unbiased assessment of the potential impact of vascular brain injury in this clinical setting. Of note, however, our findings might not be generalizable to VCI in other settings; such as for example post-stroke cognitive impairment. Moreover, the VUMC and UMCU are tertiary referral clinics (e.g. for a second opinion), with some overrepresentation of relative young patients.

CSF was available in a substantial subset of patients, allowing us to explore the impact of vascular brain injury on cognition both in the absence and presence of biomarker evidence of concomitant Alzheimer pathology. CSF biomarkers amyloid-β_{42} (reduced) and tau (increased) levels are sensitive and specific to identify Alzheimer pathology, also in de presence of vascular brain injury [51]. A limitation of the study is that there were some differences in neuropsychological tests that were applied in different patients. We decided not to impute or recode missing variables, since the available raw data was still relatively complete. Furthermore, in 31% of the patients a T2*-weighted sequence was performed without SWI, whereas SWI was performed in the others. Differences in sensitivity between these protocols may have affected microbleed detection. Moreover, the use of a categorical measure of microbleed burden (none, 1-4, ≥ 5) for analytical purposes may have underestimated the impact of microbleeds in a (small) subgroup of patients with very high microbleed counts.

Another limitation might be that post hoc analyses created small subgroups. Nonetheless, despite a lower power, these analyses showed the same pattern of cognitive profile in patients with a negative or positive CSF biomarker Alzheimer profile and also in patients with dementia, MCI and NOCI. Finally, we did not have a reference group without any vascular injury, which is inherent to the design of TRACE-VCI study. However, it should be noted that our primary aim was to compare different forms of vascular brain injury rather than comparing the cognitive profile of patients with

vascular brain injury to patients without any form of vascular brain injury. Yet, due to this design, the effect of vascular burden might be underestimated.

In conclusion, different forms of vascular brain injury on MRI explain relatively little of the variance in cognition in memory clinic patients, regardless of CSF biomarker Alzheimer status and severity of cognitive dysfunction. The diagnostic value of MRI in understanding the cognitive profile of individual patients according to type and severity of vascular brain injury in this setting may therefore often be limited. Nevertheless, MRI can help to identify underlying etiologies and direct patient management.

ACKNOWLEDGEMENTS

The authors would like to express their thanks to all the members of the TRACE-VCI study group (in alphabetical order, per department);

VU University Medical Center, Amsterdam, The Netherlands:

Alzheimer Center and Department of neurology: M.R. Benedictus, J. Bremer, W.M. van der Flier, A.E. Leeuwis, J. Leijenaar, N.D. Prins, P. Scheltens, B.M. Tijms.

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Department of Clinical Chemistry: C.E. Teunissen.

Department of Medical Psychology: T. Koene.

University Medical Center Utrecht, Utrecht, The Netherlands:

Department of Neurology: E. van den Berg, G.J. Biessels, J.M.F. Boomsma, L.G. Exalto, D.A. Ferro, C.J.M. Frijns, O.N. Groeneveld, R. Heinen, N.M van Kalsbeek, J.H. Verwer.

Department of Radiology/Image Sciences Institute: J. de Bresser, H.J. Kuijf.

Department of Geriatrics: H.L. Koek.

Onze Lieve Vrouwe Gasthuis (OLVG) West, Amsterdam, The Netherlands:

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Leiden University Medical Center, Leiden, The Netherlands:

Department of Radiology: J. de Bresser.

National Institute for Health Research (NIHR) and University College London Hospitals NHS Foundation Trust (UCLH) biomedical research center, London, United Kingdom:

Department of Radiology: F. Barkhof.

Funding

The TRACE-VCI study is supported by Vidi grant 91711384 from ZonMw, The Netherlands, Organisation for Health Research and Development and grant 2010T073 from the Dutch Heart Association to Geert Jan Biessels.

Research of the VUMC Alzheimer center is part of the neurodegeneration research program of the Amsterdam Neuroscience. The VUMC Alzheimer Center is supported by Stichting Alzheimer Nederland and Stichting VUMC fonds. The clinical database structure was developed with funding from Stichting Dioraphte.

Conflict of Interest

The authors have no conflict of interest to report.

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TABLES

Table 1: Demographics, vascular risk factors, severity of cognitive impairment and CSF biomarker analysis in the study population.

	Number of patients (N=860)		
Demographic characteristics			
Female, n (%)	398 (46)		
Mean age (y) (SD)	67.7 (±8.5)		
Level of education (Verhage scale range 1-7), median (IQR) (n = 856)[A]	5 (4-6)		
Vascular risk factors (n (%))			
Hypertension	729 (85)		
Hypercholesterolemia	386 (45)		
Diabetes mellitus	159 (18)		
Current smoker (n=852)	173 (20)		
Obesity (BMI ≥ 30) (n=848)	176 (21)		
History of reported vascular events			
History of reported stroke	78 (9)		
History of reported vascular events other than stroke[B]	86 (10)		
Severity of cognitive impairment and clinical diagnosis (n (%))			
NOCI	198 (23)		
MCI	213 (25)		
Dementia	449 (52)		
Vascular		37	
Neurodegenerative		385	
Alzheimer's disease			304
Frontotemporal dementia			25
Lewy body dementia			20
Others[C]			36
Unknown etiology		27	
CSF biomarker analysis (N = 541)			
$A\beta_{42}$, median (IQR) (pg/mL)	617 [463 – 927.5]		
Tau, median [IQR] (pg/mL)	371 [254 – 651.5]		
p- tau (N=539), median (IQR) (pg/mL)	56 [39 – 81]		
Tau / $A\beta_{42}$ ratio, median (IQR) (pg/mL)	0.64 [0.28 – 1.29]		
Positive CSF biomarker Alzheimer profile (ratio tau / $A\beta_{42}$, > 0.52), n (%)	300 (56)		

Abbreviations: $A\beta_{42}$, amyloid-β_4; CSF, cerebrospinal fluid; IQR, interquartile range; MCI, mild cognitive impairment; NOCI, no objective cognitive impairment; p-tau, tau phosphorylated at threonine 181; SD, standard deviation; if there were missing data the number (n) is specifically mentioned.

[A] Verhage scale [52].

[B] Myocardial infarction, surgery or endovascular treatment for coronary artery disease, any arterial occlusion or surgical intervention of a peripheral artery (such as an abdominal or leg artery) or carotid artery intervention (stenting or endarterectomy).

[c] Such as Primary Progressive Aphasia [53], Cortical Basal Syndrome [54] and Progressive Supranuclear Palsy [55].

[D] Dementia of unknown origin, further examination needed to state diagnosis.

Table 2: Occurrence of different forms of vascular brain injury .

Vascular brain injury	Mixed and single vascular brain injury				Single vascular brain injury			
	Total N=860	**AD CSF +** N=300	**AD CSF -** N=241	**p-value**	**Total** N=500	**AD CSF +** N=195	**AD CSF -** N=149	**p-value**
Mild WMH and ≥ 2 VRF	307 (36)	100 (33)	95 (39)	.14	205 (41)*	73 (37)*	66 (44)*	.42
Moderate/severe WMH	398 (46)	141 (47)	97 (40)	.12	157 (31)	68 (35)	39 (26)	.06
≥1 Lacunar infarct(s)	188 (22)	41 (14)	57 (24)	.00	13 (3)	5 (3)	4 (3)	1.0
≥1 Non-lacunar infarct(s)	96 (11)	15 (5)	18 (8)	.23	4 (1)	1 (1)	1 (1)	1.0
≥1 Microbleed(s)[1]	368 (43)	136 (45)	101 (42)	.39	119 (24)	48 (25)	39 (26)	.98
≥1 Macrobleed(s)	16 (2)	0 (0)	2 (1)	.20	2 (0)	0 (0)	0 (0)	-

Abbreviations: AD, Alzheimer's disease; AD CSF +, positive cerebrospinal fluid biomarker Alzheimer profile; AD CSF -, negative cerebrospinal fluid biomarker Alzheimer profile; CSF, cerebrospinal fluid; VRF, vascular risk factors; WMH, white matter hyperintensities. Data are presented as n (%). CSF available from 541 patients. First the data for the overall cohort (both mixed and single forms of vascular brain injury) are shown, the column % is larger than 100% since a patient can have multiple types of vascular brain injury. P-values were calculated using chi-square tests, the Fisher's exact test was used if a low number of patients was evaluated.

[1] Microbleed(s) ratings were available for 848 (99%) patients.

* these groups were used as reference groups in statistical analysis.

Table 3: Multivariate linear regression analysis in all forms of vascular brain injury including lesion burden.

Total study population (N=860)					
	Memory	**Attention and executive functioning**	**Information processing speed**	**Perception and construction**	**Working memory**
Model A: adjusted for age, sex and education					
WMH	-.05 (-.12 .03)	**-.08 (-.14 -.01)***	-.02 (-.10 .07)	-.04 (-.13 .05)	**-.08 (-.15 -.00)***
Microbleed(s)	-.03 (-.11 .06)	-.07 (-.14 .01)	-.09 (-.19 .01)	-.09 (-.19 .02)	-.03 (-.11 .06)
Lacunar infarct(s)	.04 (-.04 .13)	-.02 (-.10 .06)	**-.10 (-.20 -.00)***	.02 (-.09 .12)	-.03 (-.11 .05)
Non-lacunar infarct(s)	-.08 (-.26 .11)	.01 (-.16 .17)	**-.25 (-.46 -.04)***	.08 (-.15 .30)	-.15 (-.33 .03)
Model B: model A, each lesion type additionally adjusted for all other forms of vascular brain injury					
Patients included	**N = 835**	**N = 829**	**N = 819**	**N = 693**	**N = 816**
WMH	-.07 (-.14 .01)	**-.09 (-.16 -.02)***	.01 (-.09 .10)	-.05 (-.14 .05)	**-.08 (-.16 -.00)***
Microbleed(s)	-.04 (-.12 .05)	-.06 (-.14 .02)	-.09 (-.19 .02)	-.09 (-.19 .02)	-.03 (-.11 .06)
Lacunar infarct(s)	.08(-.02 .17)	.02 (-.06 .10)	-.07 (-.18 .04)	.04 (-.08 .15)	.02 (-.08 .11)
Non-lacunar infarct(s)	-.11 (-.30 .08)	-.01 (-.18 .16)	-.21 (-.43 .02)	.04 (-.20 .28)	-.14 (-.32 .05)
Model C: model B, with the number of vascular risk factors (VRF) (0-6)					
Patients included	**N = 835**	**N = 829**	**N = 819**	**N = 693**	**N = 816**
WMH	-.06 (-.14 .02)	**-.08 (-.15 -.01)***	.01 (-.09 .10)	-.05 (-.14 .05)	**-.08 (-.16 -.01)***
Microbleed(s)	-.03 (-.11 .06)	-.06 (-.13 .03)	-.09 (-.19 .02)	-.09 (-.19 .02)	-.04 (-.13 .05)
Lacunar infarct(s)	.07 (-.03 .16)	.02 (-.07 .10)	-.07 (-.19 .04)	.04 (-.08 .15)	.02 (-.07 .12)
Non-lacunar infarct(s)	-.12 (-.31 .07)	-.02 (-.19 .16)	-.21 (-.43 .01)	.04 (-.19 .28)	-.14 (-.33 .05)
VRF	.03 (-.02 .09)	.02 (-.03 .07)	.00 (-.06 .07)	-.00 (-.07 .06)	-.02 (-.07 .04)
Model D: Model B, with CSF biomarker Alzheimer profile (N=541)					
Patients included	**N = 527**	**N = 524**	**N = 519**	**N = 445**	**N = 518**
WMH	**-.11 (-.20 -.02)***	**-.10 (-.19 -.01)***	-.05 (-.17 .07)	-.06 (-.17 .06)	**-.10 (-.20 -.00)***
Microbleed(s)	.04 (-.06 .14)	-.04 (-.14 .06)	-.03 (-.16 .11)	.03 (-.09 .16)	.02 (-.08 .13)
Lacunar infarct(s)	-.03 (-.15 .09)	-.02 (-.14 .09)	**-.19 (-.35 -.04)***	-.04 (-.19 .11)	-.00 (-.13 .12)
Non-lacunar infarct(s)	-.28 (-.57 .01)	-.27 (-.54 .01)	**-.40 (-.78 -.03)***	-.19 (-.58 .20)	-.17 (-.48 .14)
CSF Alzheimer profile	**-.78 (-.92 -.63)***	**-.39 (-.53 -.25)***	**-.50 (-.69 -.32)***	**-.45 (-.62 -.27)***	**-.19 (-.34 -.03)***

Abbreviations: CSF, cerebrospinal fluid; VRF, vascular risk factors; WMH, white matter hyperintensities.
WMH burden entered as; Fazekas scale 0-3. Lacunar infarct(s); 0 = no lacune, 1 = one lacune, 2 = 2 lacunes or more. Non-lacunar infarct(s); 0 = no infarct and 1 = 1 or more infarct(s). Microbleed(s); 0 = no microbleed, 1 = 1-4 microbleed(s) and 2 = 5 or more microbleeds. CSF biomarker Alzheimer profile; 0 = negative CSF biomarker Alzheimer profile and 1 = positive CSF biomarker Alzheimer profile. Vascular risk factor(s) as amount of factors 0-6.
* $p < 0.05$

FIGURES

Figure 1: Cognitive performance in relation to type of vascular brain injury.

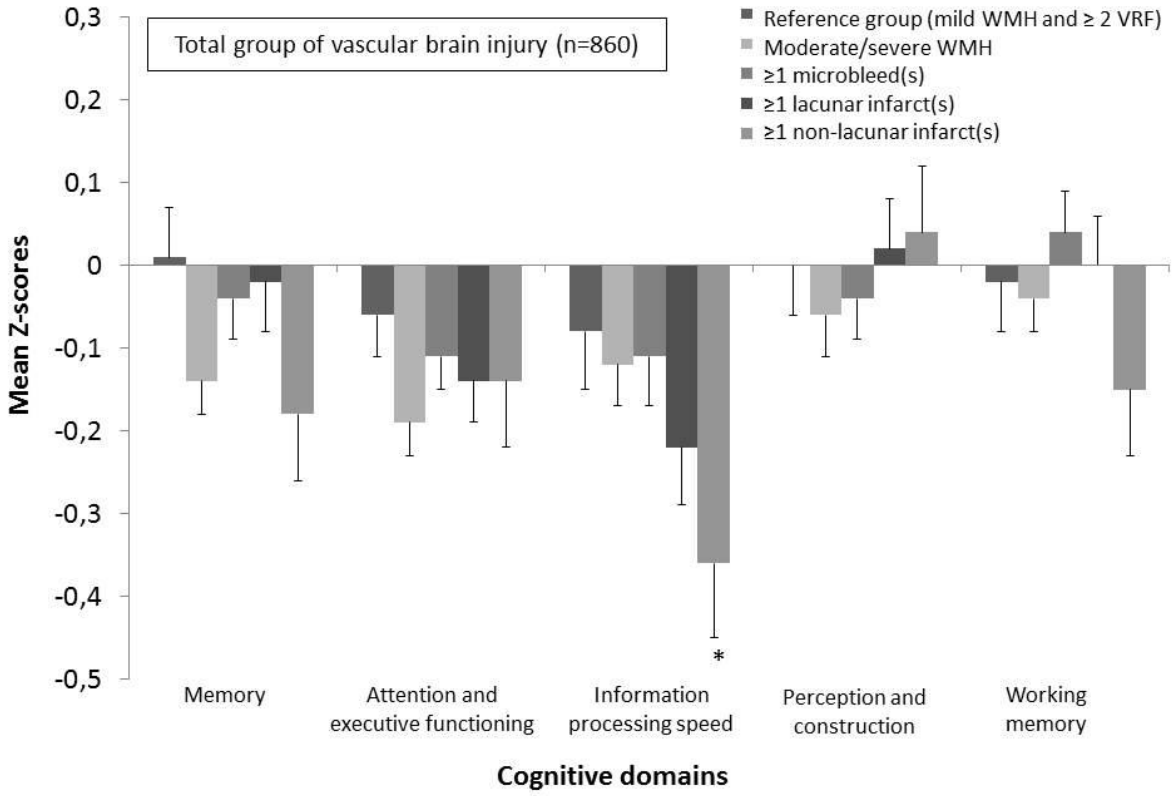
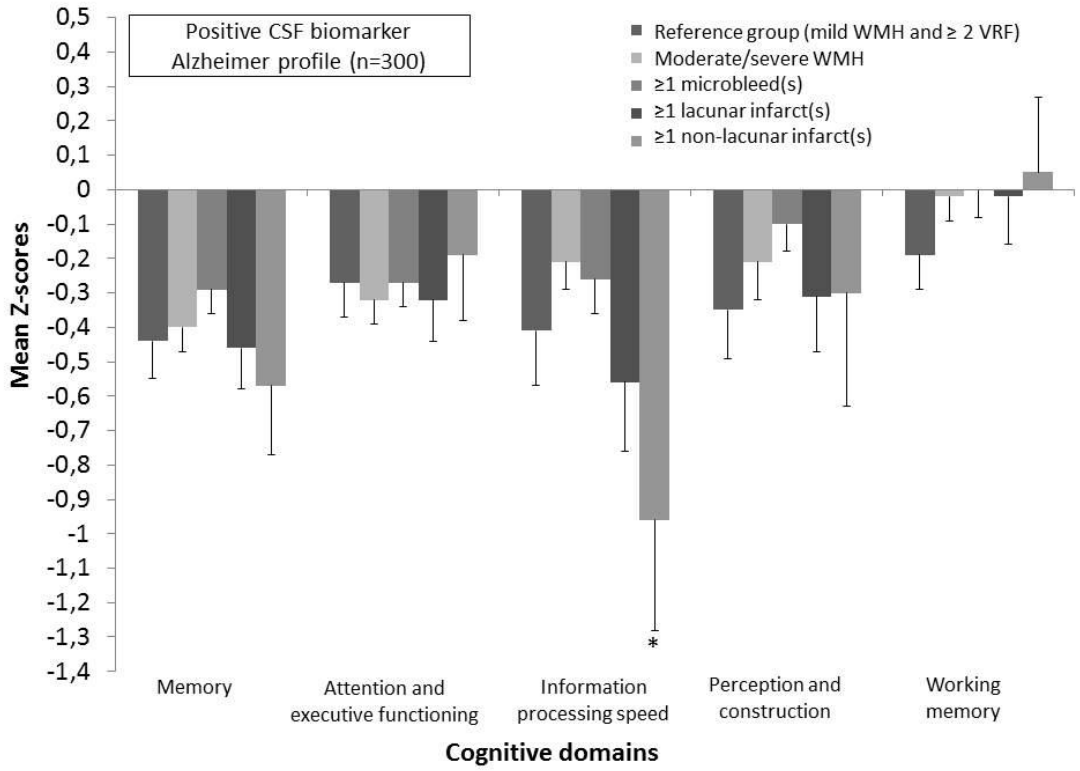


Figure 1: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in the total study population (n=860). Reference group (only mild WHM and ≥ 2 VRF (n=205)), moderate/severe WMH (n=398), ≥1 microbleed(s) (n=368), ≥1 lacunar infarct(s) (n=188), ≥1 non-lacunar infarct(s) (n=96). Note that a single patient can be represented in multiple groups (i.e. both in moderate/severe WMH and ≥1 microbleed(s)), but that the reference group by definition does not overlap with any of the other groups. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
* p value = 0.05; estimated mean difference from reference group (β) -0.26 (CI; -0.52 -0.01).
Abbreviations: VRF, vascular risk factors; WMH, white matter hyperintensities.

Figure 2: Cognitive performance in relation to type of vascular brain injury, stratified according to CSF biomarker Alzheimer profile.

2a



2b

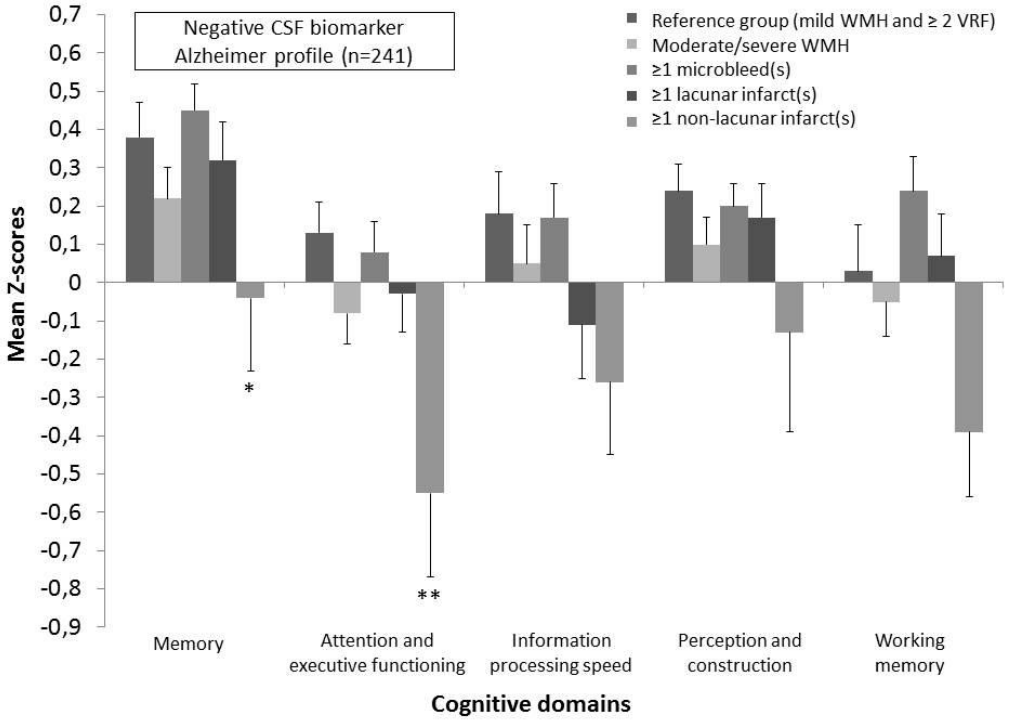


Figure 2: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in patients stratified by CSF biomarker Alzheimer profile (n=241). Note that a single patient can be represented in multiple groups (i.e. both in moderate/severe WMH and microbleeds), but that the reference group by definition does not overlap with any of the other groups. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
Abbreviations: CSF, cerebrospinal fluid; VRF, vascular risk factors; WMH, white matter hyperintensities.

2a: Reference group (only mild WHM and ≥ 2 VRF (n=73)), moderate/severe WMH (n=141), ≥1 microbleed(s) (n=136), ≥1 lacunar infarct(s) (n=41), ≥1 non-lacunar infarct(s) (n=15).
* p value = 0.04; estimated mean difference from reference group (β) -0.79 (CI; -1.56 -0.22).

2b: Reference group (only mild WHM and ≥ 2 VRF (n=66)), moderate/severe WMH (n=97), ≥1 microbleed(s) (n=101), ≥1 lacunar infarct(s) (n=57), ≥1 non-lacunar infarct(s) (n=18).
* p = 0.04; estimated mean difference from reference group (β) -0.39 (-0.75 0.02).
** p = 0.002; estimated mean difference from reference group (β) -0.61 (-0.99 -0.24).

Figure 3: Influence of CSF biomarker Alzheimer profile on the cognitive profile.

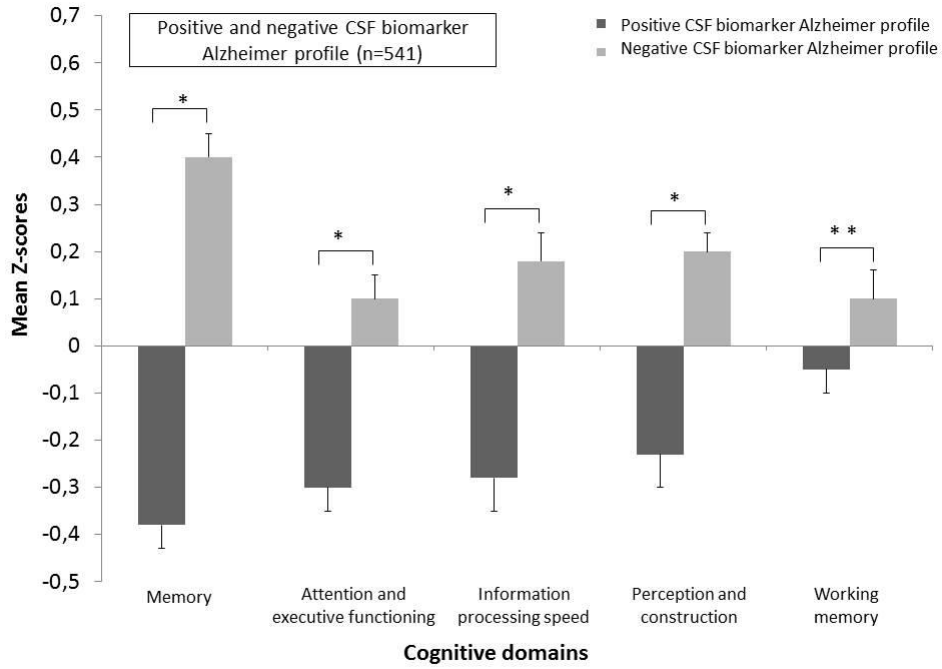


Figure 3: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in patients with a positive or negative CSF biomarker Alzheimer profile (n=541). Univariate analyses of variance were performed with CSF biomarker Alzheimer profile as fixed factor and age, sex and education as covariates.
* p value < 0.0005 ** p value = 0.04.
Abbreviations: CSF, cerebrospinal fluid.

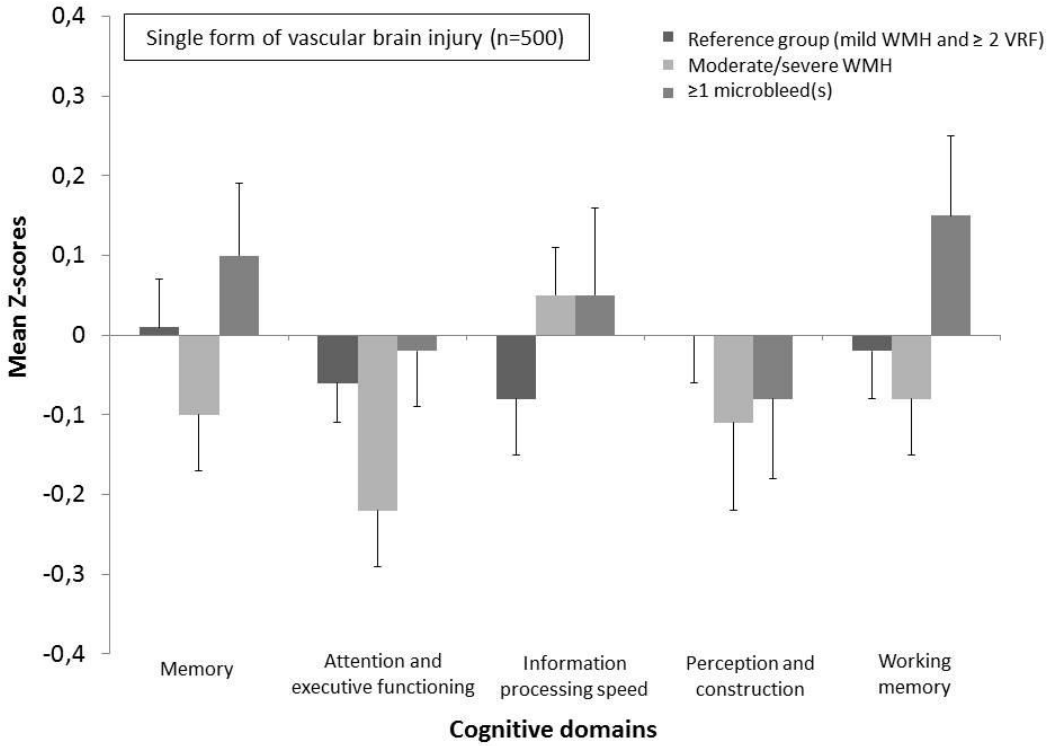
SUPPLEMENTARY AREA

Supplementary table 1: Neuropsychological test scores and cognitive domains in the study population.

Neuropsychological tests and cognitive domains	number of patients N total=860 (%)	Raw test scores (mean ± SD)
Working memory	**834 (97)**	
WAIS-III Digit Span forward	834 (97)	5.4 (±1.1)
WAIS -III Digit Span backward	828 (96)	4.0 (±1.1)
Memory	**853 (99)**	
RAVLT trials 1-5	814 (95)	28.9 (±11.5)
VAT part A	782 (91)	8.9 (±3.8)
RAVLT delayed recall	809 (94)	4.3 (±3.7)
RAVLT recognition	805 (94)	25.0 (±4.1)
Attention and executive functioning	**847 (98)**	
ratio TMT part B/TMT part A	635 (74)	3.1 (±1.4)
Stroop Color Word Test III/(I and II)	731 (85)	1.3 (±0.8)
Category fluency (animals)	833 (97)	15.0 (±6.6)
Letter fluency		
N+A *or*	66 (8)	17.5 (±7.8)
D+A+T	625 (73)	26.9 (±13.2)
Information processing speed	**836 (97)**	
TMT part A	791 (92)	72.0 (±55.1)
Stroop Color Word Test I	795 (92)	60.3 (±25.5)
Stroop Color Word Test II	787 (92)	86.1 (±40.6)
WAIS-III		
DSCT *or*	65 (8)	42.0 (±15.2)
LDST	695 (81)	33.1 (±12.7)
Perception and construction	**705 (82)**	
VOSP Fragmented Letters	696 (81)	17.4 (±4.0)
VOSP Dot Counting	682 (79)	9.3 (±1.3)

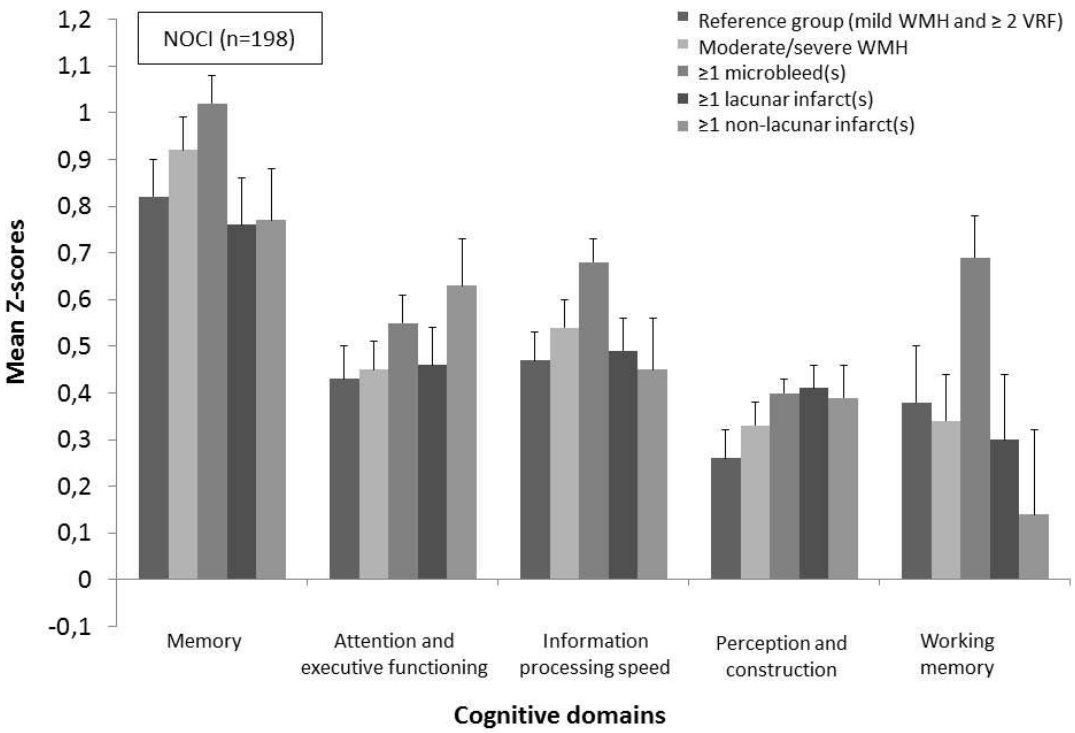
Abbreviations: RAVLT, Rey Auditory Verbal Learning Test; WAIS-III, Wechsler Adult Intelligence Scale – 3rd edition; VAT, Visual Association Test; TMT, Trail Making Test; DSCT, Digit Symbol-Coding Test; LDST, Letter Digit Substitution Test; VOSP, Visual Object and Space Perception Battery; SD, standard deviation.

Supplementary figure 1: Cognitive performance in relation to form of vascular brain injury, only in patients with one single form of vascular brain injury.



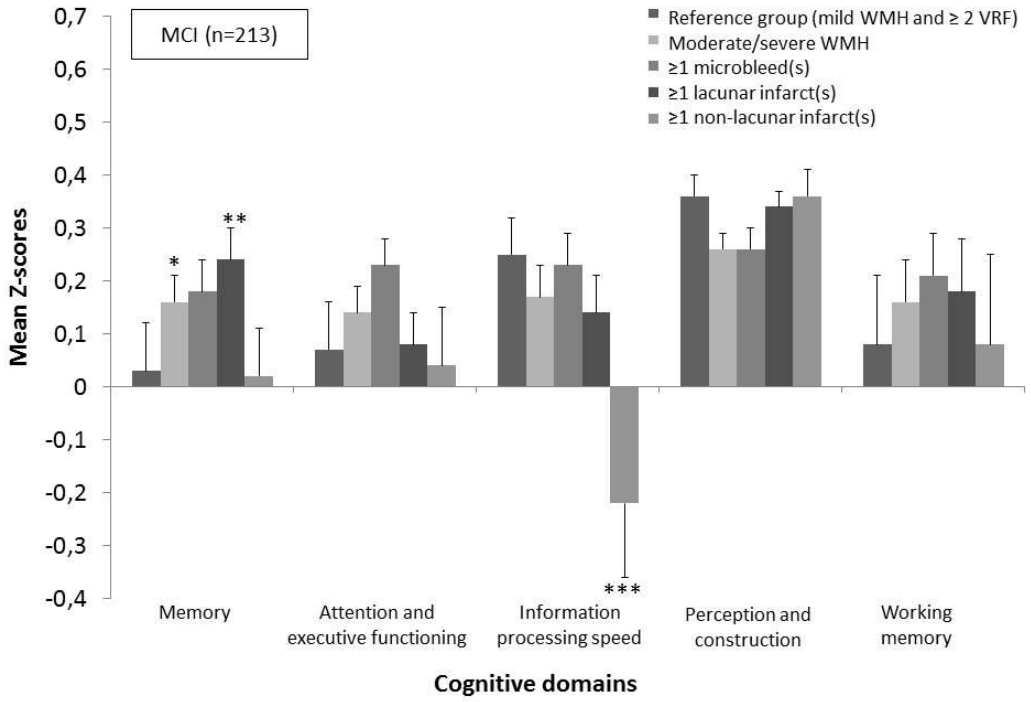
Supplementary figure 1: Mean unadjusted domain Z-scores for the single forms of vascular brain injury (n=500). Reference group (only mild WHM and ≥ 2 VRF n=205), moderate/severe WMH (n=157) and ≥1 microbleed(s) (n=119). Patients with ≥1 lacunar infarct(s) (n=13), ≥1 non-lacunar infarct(s) (n=4) and ≥1 macrobleed(s) (n=2) are not shown, because of small numbers. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
Abbreviations: VRF, vascular risk factors; WMH, white matter hyperintensities.

Supplementary figure 2: Cognitive performance in relation to form of vascular brain injury, only in patients with NOCI.



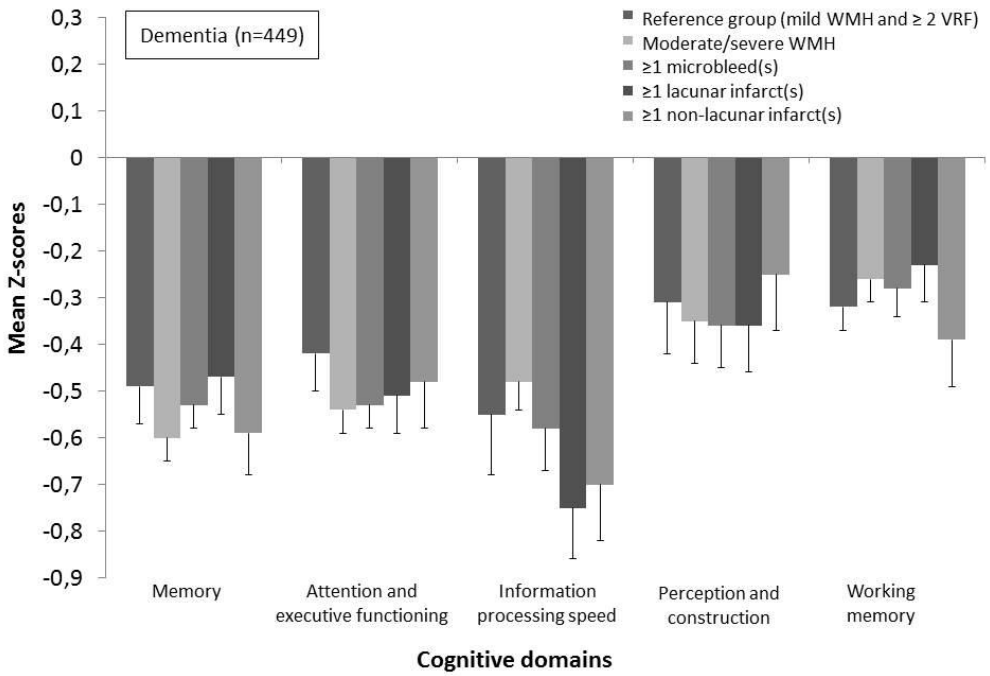
Supplementary figure 2: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in patients with NOCI in the total study population (n=198). Reference group (mild WHM and ≥ 2 VRF (n=62)), moderate/severe WMH (n=68), ≥1 microbleed(s) (73), ≥1 lacunar infarct(s) (n=32), ≥1 non-lacunar infarct(s) (n=16). Note that a single patient can be represented in multiple groups (i.e. both in moderate/severe WMH and ≥1 microbleed(s)), but that the reference group by definition does not overlap with any of the other groups. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
Abbreviations: CSF, cerebrospinal fluid; NOCI, no objective cognitive impairment; VRF, vascular risk factors; WMH, white matter hyperintensities.

Supplementary figure 3: Cognitive performance in relation to form of vascular brain injury, only in patients with MCI.



Supplementary figure 3: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in patients with MCI in the total study population (n=213). Reference group (mild WHM and ≥ 2 VRF (n=40)), moderate/severe WMH (n=104), ≥1 microbleed(s) (94), ≥1 lacunar infarct(s) (n=63), ≥1 non-lacunar infarct(s) (n=28). Note that a single patient can be represented in multiple groups (i.e. both in moderate/severe WMH and ≥1 microbleed(s)), but that the reference group by definition does not overlap with any of the other groups. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
* p value = 0.05; estimated mean difference from reference group (β) 0.22 (CI; -0.00 0.43).
** p value = 0.01; estimated mean difference from reference group (β) 0.28 (CI; 0.06 0.49).
*** p value = 0.01; estimated mean difference from reference group (β) -0.35 (CI; -0.62 -0.09).
Abbreviations: CSF, cerebrospinal fluid; MCI, mild cognitive impairment; VRF, vascular risk factors; WMH, white matter hyperintensities;

Supplementary figure 4: Cognitive performance in relation to form of vascular brain injury, only in patients with dementia.



Supplementary figure 4: Mean unadjusted domain Z-scores for the different forms of vascular brain injury in patients with dementia in the total study population (n=449). Reference group (only mild WHM and ≥ 2 VRF (n=103)), moderate/severe WMH (n=226), ≥1 microbleed(s) (201), ≥1 lacunar infarct(s) (n=93), ≥1 non-lacunar infarct(s) (n=52). Note that a single patient can be represented in multiple groups (i.e. both in moderate/severe WMH and ≥1 microbleed(s)), but that the reference group by definition does not overlap with any of the other groups. Each form of vascular brain injury was compared to the reference group with regression analysis adjusted for age, sex and education.
Abbreviations: CSF, cerebrospinal fluid; VRF, vascular risk factors; WMH, white matter hyperintensities.