

## ORIGINAL ARTICLE

## Mesiotemporal atrophy and hippocampal diffusivity distinguish amnestic from non-amnestic vascular cognitive impairment

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**Background and purpose:** The role of clinical factors, cerebral infarcts and hippocampal damage in vascular cognitive impairment (VCI) subtypes remains unclear.**Methods:** Non-demented patients with carotid stenosis and recent transient ischemic attack/stroke had cognitive assessment and brain magnetic resonance imaging (MRI). Amnestic VCI was defined as memory impairment; non-amnestic VCI was any other subdomain impairment. Associations of MRI metrics [log-transformed total ischemic lesion load (log TILL), mesiotemporal atrophy (MTA) score, hippocampal mean diffusivity (hipMD)] with cognitive performance were assessed.**Results:** A hundred and eight patients, 47 with amnestic VCI and 21 with non-amnestic VCI, were assessed. A higher MTA (odds ratio 12.89,  $P = 0.001$ ) and left hipMD (odds ratio 4.43,  $P = 0.003$ ) contributed to amnestic VCI versus normal. Age-adjusted *fluency* correlated with log TILL ( $P = 0.002$ ). Age-adjusted *memory* was associated with left hipMD ( $P = 0.001$ ), MTA ( $P < 0.001$ ) but not log TILL ( $P = 0.14$ ). Left hipMD, MTA and smoking showed classification potential between amnestic VCI versus normal (area 0.859,  $P < 0.001$ ).**Conclusions:** Neuroimaging assists stratification in amnestic VCI characterized by hippocampal changes and in non-amnestic VCI by higher ischemic burden. MTA and hippocampal diffusivity show diagnostic biomarker potential.**Introduction**

Vascular cognitive impairment (VCI) is the second most common cause of acquired dementia and its prevalence doubles every 5.3 years [1]. VCI is defined as the spectrum of cognitive impairment attributable to cerebrovascular causes, ranging from subtle deficits to dementia [2] and characterized by

various clinical presentations, multiple imaging and heterogeneous pathologies [3]. Four VCI subtypes including amnestic, amnestic plus other domains, non-amnestic single domain, and non-amnestic multiple domains are described [3]. The current criteria are used for all forms of cognitive disorders associated with cerebrovascular disease including cardioembolic, atherosclerotic, ischemic, hemorrhagic or genetic, regardless of the pathogenesis [3]. The criteria feature a range of vascular and cognitive pathologies rather than a validated diagnostic entity [4]. Moreover, they do not categorically define standardized radiological evidence of cerebrovascular disease for VCI or its diverse clinical phenotypes.

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Heterogeneous cerebrovascular pathology including subcortical white matter disease, volume of ischemic lesions, but also global and temporal lobe atrophy are thought to contribute to cognitive impairment after stroke [5]. Cognitive deficits in early subcortical VCI characteristically involve executive dysfunction and reduced processing speed [5] whilst memory loss is considered to develop later [6,7].

Apart from clinical risk factors, especially advancing age and vascular risk [8], the presence of cerebrovascular disease in brain imaging is considered to account for the degree of cognitive impairment [9]. Substantial ischemic burden [10], lacunes and strategic infarcts [11], the location of white matter lesions as well as non-lesional white matter tissue damage have been shown to contribute to VCI [12]. Nevertheless, generalized and hippocampal atrophy on MRI may be as strongly associated with VCI as the extent of vascular pathology [13]. Increased hippocampal mean diffusivity (MD) is frequently found in individuals with amnesic mild cognitive impairment (MCI) and Alzheimer's disease (AD), which implies sensitivity to the neurodegenerative processes associated with AD [14–16]. There is some evidence that ultrastructural hippocampal damage – as measured by hippocampal MD – may also be affected in VCI as MD was shown to be attributed to volumetric and connectivity changes in the hippocampi and memory loss after first stroke or transient ischemic attack [17]. With regard to cognitive subtypes, hippocampal atrophy is known to predict AD in non-amnesic and amnesic MCI but might not be as sensitive in non-amnesic MCI [18], and was found to be less pronounced in non-amnesic versus amnesic MCI [19]. Whether hippocampal volume or MD is differentially altered in amnesic and non-amnesic subtypes in patients with probable VCI is unknown.

This study was aimed to examine the neuroimaging correlates of amnesic versus non-amnesic cognitive impairment in patients with carotid vascular disease and recent cerebrovascular ischemic event who fulfilled the criteria for probable VCI [3]. The associations of clinical and vascular factors such as age, carotid stenosis and index cerebrovascular ischemic events with amnesic versus non-amnesic cognitive impairment in the cohort were assessed in relation to ultrastructural integrity and atrophy of hippocampi and total volume of brain ischemic lesions. It was hypothesized that, in patients with probable VCI, memory impairment would be associated with hippocampal pathology, whereas non-memory cognitive deficits would be related to vascular pathology and risk factors.

## Methods

### Study population

The study population was identical to that recently reported [12] and was co-recruited from the Imaging in Carotid Artery Disease (ICAD) study [20] for which patients with a recent non-disabling cerebrovascular event and an ipsilateral carotid artery stenosis of >30% were included. Participants were identified from stroke services clinics or inpatients at Nottingham University Hospitals NHS Trust between November 2010 and January 2014. Patients who were incompetent to consent or those with MRI contraindications were excluded. As part of the original protocol for the ICAD study, clinically indicated planned carotid intervention led to exclusion. Consenting participants were interviewed and clinically assessed for eligibility for this cognitive cross-sectional sub-study including MRI safety, evaluation of cerebrovascular disease, vascular risk factors, literacy, dexterity and ability to perform cognitive tools tasks. Non-English speaking, aphasia, complete blindness and dense dominant-hand paresis excluded a few patients from cognitive tests. Addenbrooke's Cognitive Examination – revised version (ACE-R) was used due to excellent sensitivities and specificities for the diagnosis of cognitive impairment [21,22]. ACE-R reliably tests episodic memory, which is a known function of the left hippocampus [23]. Assessments were conducted on the day of MRI by trained researchers (AAH, RJS). The ACE-R subscales were used to define amnesic subtypes [22,24] based on age-specific cut-offs for memory loss. Non-amnesic VCI was defined as normal memory function but impairment in at least one other domain (*fluency*  $\leq 9$  out of 14, *attention and orientation*  $\leq 17/18$ , *memory*  $\leq 18/26$  and *visuospatial*  $\leq 15/16$  in those aged 50–59 years; *fluency*  $\leq 8/14$ , *attention and orientation*  $\leq 17/18$ , *memory*  $\leq 19/26$  and *visuospatial*  $\leq 14/16$  in 60–69 years; *fluency*  $\leq 9/14$ , *attention and orientation*  $\leq 16/18$ , *memory*  $\leq 17/26$  and *visuospatial*  $\leq 14/16$  in those over 70 years [24]). Since aphasia is likely to bias results due to the verbal testing procedures of ACE-R [25], our eligibility criteria excluded aphasic and non-English participants, and language subdomain correlations were not assessed in this cohort. It should be noted that the subdomain-specific cognitive definitions are more inclusive than the global cognitive impairment definition and patients with single domain MCI may not qualify for global cognitive impairment according to the ACE-R < 82 definition.

All participants received secondary preventative treatment for stroke according to the current

guidelines and no treatment was withheld or modified for the purpose of the study.

All participants provided written informed consent in accordance with the study protocol approved by the local ethics committee.

### Imaging protocol

The imaging protocol has been previously reported [12]. In brief, all participants underwent 3 T MRI (Philips, Eindhoven, Netherlands; Achieva version 3.1.2 software) including whole brain axial diffusion tensor imaging (DTI) (spin echo single-shot diffusion weighted echo planar imaging,  $b = 1000 \text{ s/mm}^2$ , 15 directions, TR 4.2 s, TE 57 ms, FOV  $224 \times 135 \times 224$ , matrix  $112 \times 112$ , 45 slices of 3 mm thickness) and axial fluid attenuated inversion recovery (FLAIR) (TR/TE/TI 10.5/0.14/2.75 s, FOV  $224 \times 135 \times 192$ , matrix  $224 \times 181$ , 45 slices of 3 mm thickness).

Diffusion tensor imaging pre-processing, including motion correction, eddy currents, skull stripping and fitting of the DTI data to the diffusion tensor model to compute fractional anisotropy and mean diffusivity maps was performed using the diffusion toolbox of FSL [26]. Standard image reconstruction and further analysis was performed using FSL.

### Total brain ischemic lesion load (TILL)

The NeuRoi image processing tool [27] was used to manually (by DM, supervised by DPA) outline all infarcted and ischemic appearing lesions including white matter hyperintense lesions, cortical and subcortical chronic infarctions as determined on FLAIR images and acute lesions defined on diffusion weighted images as previously described [12] (Fig. 1a, b). Lesion volume was calculated as the sum of individual lesion areas  $\times$  (slice thickness + slice gap) to give the TILL which was normalized to brain volume and log transformed (log TILL). Brain volume was measured with SIENA, part of the FMRIB software library [28].

### Mesiotemporal atrophy (MTA) scores

For each individual, MTA was assessed on reconstructed coronal FLAIR images using Scheltens' scale [29]. As previously described [12], the presence of MTA was defined using averaged age-ranged specific cut-offs for the mean MTA scores, i.e. MTA  $>1.5$  in participants  $<75$  years of age, MTA  $>2$  in patients  $\geq 75$  years or MTA  $>2.5$  in those  $\geq 85$  years of age [30].

### Hippocampal mean diffusivity assessment

In order to avoid partial volume effects from cerebrospinal fluid (CSF), MD maps were thresholded using a visually optimized cut-off retaining only voxels with MD  $\leq 1.5 \times 10^{-3} \text{ mm}^2/\text{s}$  [31]. Left and right hippocampal MD were determined by manually drawing regions of interest (ROIs) on three consecutive images (size 27 voxels,  $216 \text{ mm}^3$ ) (Fig. 1c). The grand average over all left and right regional hippocampal MD was calculated to reflect hippocampal MD (hipMD) and separately reported for the left hippocampal MD (left hipMD). In addition, hippocampal formation was visually inspected on FLAIR, MD and DWI to assess the presence or absence of acute/chronic lesions.

### Statistical analysis

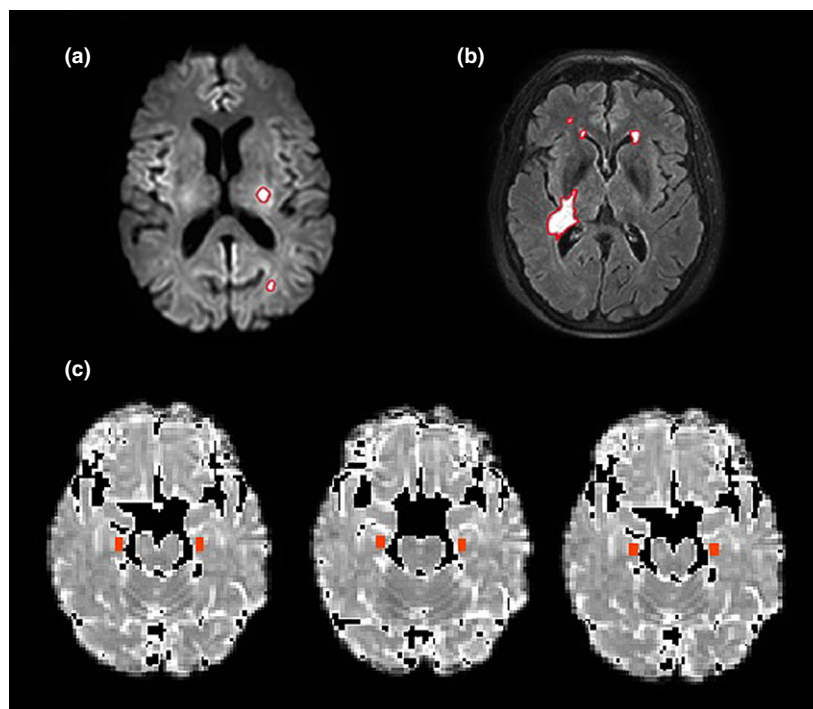
The study sample size was predetermined in the parent study (ICAD) [20] and a power calculation using SamplePower 2.0 (SPSS, version 19.0, Chicago, IL, USA) was performed for amnesic and non-amnesic cognitive impairment subgroups.

The Student  $t$  test, Mann–Whitney  $U$  test and chi-squared test were used to compare the data. One-way ANOVA, Tukey's test and Kruskal–Wallis analyses were performed in order to examine the associations of clinical factors as well as neuroimaging markers between the three groups: vasculopathy with normal cognition, amnesic VCI and non-amnesic VCI. The normality of the data was assessed before the analysis and mention is made whenever the data were not normally distributed.

Binary univariate analysis was applied to determine the associations between the factors.

Multivariate logistic forward stepwise regression analysis was used to study associations of age, smoking, presenting ischemic event, log TILL, MTA scores and left hipMD between the amnesic and non-amnesic VCI subgroups. To quantify the associations of neuroimaging markers and clinical risk contributors to amnesic VCI, estimated probabilities calculated from multivariate logistic regression models for the receiver operating characteristic (ROC) curve were used. The ROC curve model, which is a measure of discrimination and directly applicable to a classification setting [32], was used in order to define important clinical risk factors and imaging markers in the classification of amnesic VCI.

To probe the independence of MTA scores from CSF thresholded hipMD, the correlations between both imaging markers were assessed and the associations of clinical and imaging factors were independently tested in the subgroup with normal MTA scores.



**Figure 1** Manual outlines for volumetry of (a) acute ischemic lesions on standard diffusion images (DWI) and (b) chronic ischemia on FLAIR images. (c) Outlines of hippocampi by drawing ROIs on CSF-thresholded mean diffusivity (MD) map to derive average hippocampal MD. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

Partial correlation analyses, controlled for age, were used to assess the contributions of log TILL or left hipMD to each cognitive subdomain including *fluency*, *visuospatial*, *attention and orientation*, and *memory*. Applying the Bonferroni correction because four subdomain and four MRI metrics were used, the results with a  $P$  value less than 0.0031 were considered significant.

Statistical analysis was performed using SPSS software (version 19.0, Chicago, IL, USA).  $P < 0.05$  was considered statistically significant. Data are reported as mean  $\pm$  standard deviation unless stated otherwise.

## Results

The study has 89% power to detect a correlation of 0.3 for the sample size. There is 80% power to detect a standardized mean difference of 0.61 for amnesic versus normal, 0.77 for non-amnesic versus normal and 0.75 for amnesic versus non-amnesic subgroups.

### General findings and cognitive profile

Of 147 recruited participants eligible for the cognitive sub-study, 108 [66 men (age  $74.3 \pm 9.5$ ), 42 women (age  $75.2 \pm 8.8$ )] had good image quality for analysis (Table 1). Of these, 53 presented with stroke and had larger volumes of total ischemic lesion compared with 55 patients presenting with transient ischemic attack ( $P = 0.03$ ).

Mean total ACE-R score was  $81.5 \pm 10.5$ . Sixty-eight participants (63%) showed MCI in at least one domain (including 15 not classed as overall cognitive impairment based on ACE-R  $>82$ ). In total, 47 (43.5%) subjects showed memory impairment (using established cut-off scores [24]), of whom 12 had pure amnesic and 35 memory plus other cognitive domain impairment. Twenty-one participants (19.4%) were classed as non-amnesic cognitive impairment affecting one domain in 11/21 and multiple domains in 10/21.

### Clinical risk factors and neuroimaging findings in cognitive subgroups

Compared with never-smokers, a history of smoking was associated with amnesic VCI versus normal cognition ( $P = 0.014$ , Table 1). Peripheral vascular disease and presenting symptoms differed in the three-group analysis ( $P = 0.048$ ,  $P = 0.045$ , respectively), but there was no pairwise significance. No other clinical factor differed between the amnesic, non-amnesic and normal cognition groups (Table 1).

There were no ischemic lesions noted in the hippocampi of the study cohort. Left hipMD was significantly higher in amnesic VCI, compared with cognitively intact subjects [mean difference 0.359, 95% confidence interval (CI) 0.112–0.606, SE 0.10398,  $P = 0.002$ ] (Fig. 2). Conversely, left hipMD did not differ between non-amnesic VCI and normal

**Table 1** Demographic characteristics and risk factors in participants with normal cognition, amnestic and non-amnestic cognitive impairment at the time of recruitment into the study

Characteristic	Normal cognition ( <i>n</i> = 40)	Amnestic VCI ( <i>n</i> = 47)	Non-amnestic VCI ( <i>n</i> = 21)	<i>P</i>
Age, mean years ± SD	72.4 ± 8.8	75.5 ± 9.8	77.1 ± 7.6	0.131
Female, <i>n</i> (%)	18 (45%)	13 (27.7%)	11 (52.4%)	0.094
AF	7 (17.5%)	12 (25.5%)	5 (23.8%)	0.65
PVD	3 (7.5%)	12 (25.5%)	2 (9.5%)	0.048 <sup>b</sup>
IHD	12 (30%)	17 (36.2%)	5 (23.8%)	0.57
Hypertension	33 (82.5%)	38 (80%)	17 (81%)	0.98
Non-smoker (versus ex- or current)	10 (25%)	9 (19.1%)	9 (42.9%)	0.014 <sup>c</sup>
Diabetes mellitus	8 (20%)	12 (25.5%)	4 (19%)	0.76
DWI lesions positive	14 (35%)	24 (51%)	8 (38.1%)	0.28
Laterality (left side)	17 (42.5%)	23 (49%)	12 (57.1%)	0.54
Presenting symptom <sup>a</sup> (stroke)	14 (35.5%)	29 (61.7%)	10 (47.6%)	0.045 <sup>b</sup>
Higher education (university/college)	5 (12.5%)	2 (4%)	1 (4.7%)	0.3
Degree of stenosis (ipsilateral to ischemic event), <i>n</i>				
30%–49%	21 (52.5%)	20 (42.6%)	9 (42.9%)	0.56
50%–69%	14 (35.5%)	23 (48.9%)	8 (38.1%)	
>70%	5 (12.5%)	4 (8.5%)	4 (19%)	
Degree of stenosis (contralateral to ischemic event), <i>n</i>				
0%–29%	29 (72.5%)	31 (66%)	11 (52.4%)	0.35
30%–49%	5 (12.5%)	3 (6.4%)	5 (23.8%)	
50%–69%	3 (7.5%)	9 (19.1%)	3 (14.3%)	
>70%	3 (7.5%)	4 (8.5%)	2 (9.5%)	
Time between presenting symptom and MRI, mean days ± SD	35.8 ± 27	33.5 ± 32.8	37 ± 28.2	0.51 <sup>d</sup>
Hippocampal MD × 10 <sup>-3</sup> mm <sup>2</sup> /s	0.752 ± 0.047	0.781 ± 0.046	0.754 ± 0.029	0.006 <sup>c</sup>
Left hippocampal MD × 10 <sup>-3</sup> mm <sup>2</sup> /s	0.746 ± 0.055	0.782 ± 0.045	0.756 ± 0.04	0.003 <sup>c</sup>
TILL mm <sup>3</sup> ± SD (log TILL)	13083 ± 18160 (-5.6 ± 1.48)	20188 ± 20622 (-4.94 ± 1.136)	19965 ± 21749 (-4.89 ± 1.271)	0.022 <sup>c,d</sup>
Presence of MTA	2 (5%)	19 (40%)	2 (9.5%)	<0.001 <sup>c</sup>

AF, atrial fibrillation; DWI, diffusion weighted imaging; PVD, peripheral vascular disease; IHD, ischemic heart disease; log TILL, logarithmic normalized total ischemic lesion load; MD, mean diffusivity; MRI, magnetic resonance imaging; MTA, mesiotemporal atrophy; VCI, vascular cognitive impairment. <sup>a</sup>Indexed cerebrovascular ischemic symptoms at presentation; <sup>b</sup>no pairwise significance; <sup>c</sup>*P* < 0.05 and, where *P* value is significant, it reflects normal cognition versus amnestic VCI; all associations were analyzed using ANOVA except when the data were not normally distributed; <sup>d</sup>Kruskal–Wallis analysis (data not normally distributed).

cognition groups (*P* = 0.75) with a trend reduction versus amnestic VCI (mean difference 0.265, *P* = 0.097).

Log TILL showed a trend to be higher in the amnestic VCI group versus normal cognition (mean difference 0.709, 95% CI -0.003–1.421, SE 0.2995, *P* = 0.051). However, log TILL did not differ between the non-amnestic versus normal (*P* = 0.11) or amnestic (*P* = 0.99) subgroups.

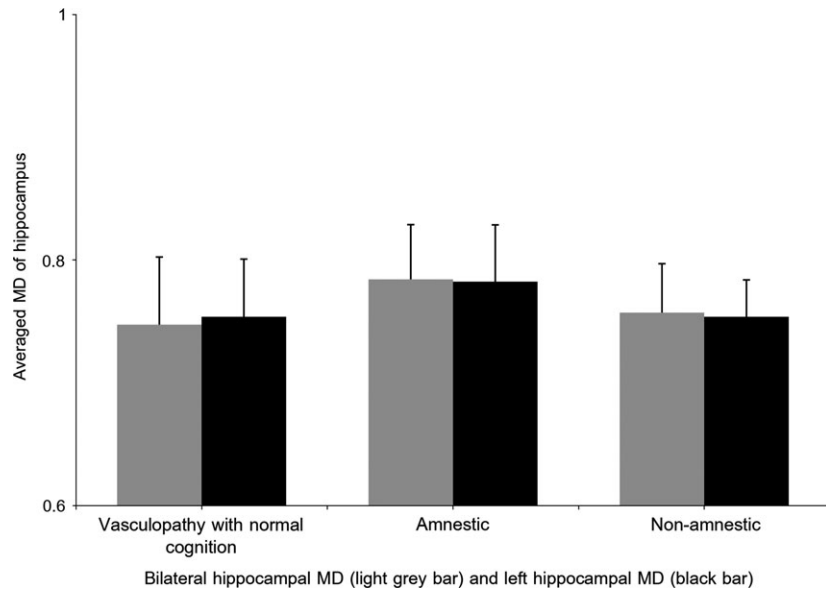
#### Amnestic VCI versus vasculopathy with normal cognition

Univariate regression analysis showed that high hipMD [odds ratio (OR) 3.75, 95% CI 1.4–10.1; *P* = 0.009], high left hipMD (OR 4.43, 95% CI 1.66–11.83, *P* = 0.003), abnormal MTA (OR 12.89, 95% CI 2.77–59.9, *P* = 0.001) and high log TILL (OR

1.43, 95% CI 1.04–1.95; *P* = 0.027) were significant factors associated with amnestic VCI but not right hipMD (*P* = 0.1). In addition, the only clinical factors with significant associations included presenting with stroke (OR 2.99, 95% CI 1.25–7.19, *P* = 0.014) and smoking (OR 3.82, 95% CI 1.47–9.93, *P* = 0.006). In multivariate forward stepwise regression analysis of clinical and imaging factors, four factors independently contributed to amnestic VCI, compared with normal cognition: abnormal MTA scores (OR 18.48, 95% CI 3.3–103, *P* = 0.001), left hipMD (OR 4.88, 95% CI 1.57–15.16, *P* = 0.006), presenting with stroke (OR 4.44, 95% CI 1.42–13.86, *P* = 0.01) and smoking (OR 3.9, 95% CI 1.14–13.4, *P* = 0.031). The corresponding area under the ROC curve was 0.859 (95% CI 0.784–0.934; *P* < 0.001) (Fig. 3).

Given the strong association with MTA and the potential for including patients with comorbid AD

**Figure 2** Bars to display the group mean and standard deviations of left (light grey bars) and bilateral (black bars) hippocampal mean diffusivity (MD) between vasculopathy with normal cognition, amnesic and non-amnesic VCI. (MD units,  $10^{-3} \text{ mm}^2/\text{s}$ ).



pathology, the analysis was repeated in the subgroup of patients with normal MTA scores (MTA-ve), i.e. no apparent hippocampal atrophy and hence a low likelihood of subclinical AD ( $n = 28/85$  amnesic VCI,  $n = 38/85$  normal cognition). The associations of bilateral hippocampal ultrastructural damage (OR 3.04, 95% CI 1.03–8.98,  $P = 0.044$ ) and left hipMD (OR 3.75, 95% CI 1.32–10.66,  $P = 0.013$ ) in MTA-ve amnesic VCI were confirmed.

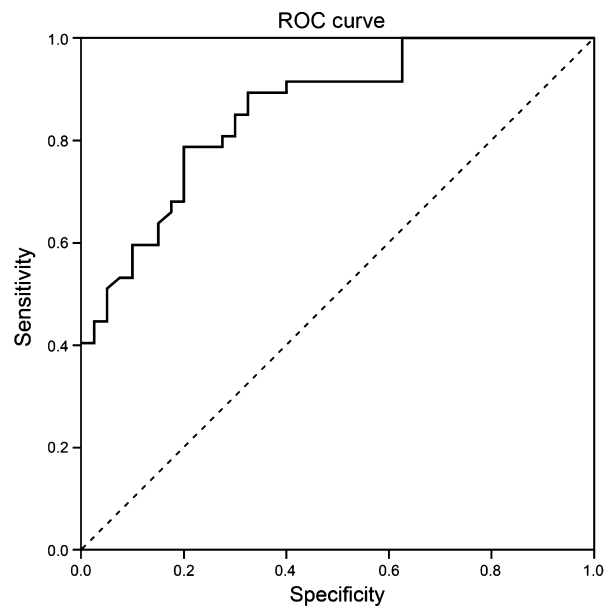
To further corroborate the independence of hipMD from macroscopic atrophy scores, the association of all MTA scores with total and left hipMD was assessed, which proved non-significant ( $P = 0.08$ ,  $P = 0.31$ , respectively). Furthermore, there was no relationship in a general linear model between total or left hipMD and all MTA scores in the normal, amnesic and non-amnesic groups ( $P = 0.67$  and  $0.17$  respectively).

#### Non-amnesic probable VCI versus vasculopathy with normal cognition

Using a univariate regression model, log TILL (OR 1.4, 95% CI 0.99–2.22,  $P = 0.056$ ) and age (OR 1.08, 95% CI 1.00–1.16,  $P = 0.051$ ) showed trends for associations with non-amnesic VCI compared with the normal cognition subgroup. There were no significant variables in the multivariate logistic regression analysis.

#### Amnesic versus non-amnesic probable VCI

In separate binary logistic regression models, entering a single explanatory variable in each model, amnesic VCI was only associated with left hipMD (OR 4.31,



**Figure 3** ROC curve with diagonal reference (dotted line) for associations of amnesic vascular cognitive impairment with hippocampal mean diffusivity (hipMD), MTA status (normal/abnormal), stroke at presentation and smoking (continuous line) compared with vasculopathy with normal cognition.

95% CI 1.15–16.1,  $P = 0.031$ ), abnormal MTA scores (OR 6.45, 95% CI 1.34–30.9,  $P = 0.02$ ) and smoking (OR 3.17, 95% CI 1.02–9.79,  $P = 0.045$ ). Conversely, neither log TILL ( $P = 0.88$ ), age ( $P = 0.44$ ) nor presenting ischemic symptom ( $P = 0.28$ ) were associated with amnesic versus non-amnesic VCI. The results of multivariate forward stepwise regression analysis showed that only abnormal MTA was a significant

contributing factor with odds ratio the same as above. In the subgroup with normal MTA scores ( $n = 28$  amnestic versus  $n = 19$  non-amnestic), left hipMD was significantly associated with amnestic VCI (OR 5.42, 95% CI 1.21–24.24,  $P = 0.027$ ), compared with the non-amnestic subgroup.

### Neuroimaging findings and subdomain cognitive performance

*Fluency* significantly correlated with higher log TILL after adjusting for age ( $r = -0.3$ ,  $P = 0.002$ ). Performance for *visuospatial* ( $r = -0.18$ ,  $P = 0.058$ , age corrected) or *attention and orientation* ( $r = -0.19$ ,  $P = 0.051$ , age corrected), however, was not significantly correlated with log TILL.

*Memory* decline was associated with higher left hipMD ( $r = -0.324$ ,  $P = 0.001$ , age corrected) and higher MTA scores ( $r = -0.35$ ,  $P < 0.001$ , age corrected), which were considered significant after Bonferroni correction. However, *memory* was not significantly associated with log TILL ( $P = 0.14$ , age corrected), presenting with stroke ( $P = 0.15$ , age corrected) or smoking ( $P = 0.009$ , age corrected). MTA scores were not significantly associated with *fluency* ( $P = 0.018$ , age corrected) or *attention and orientation* ( $P = 0.005$ , age corrected).

## Discussion

In this study of patients with recently symptomatic carotid artery disease, distinct association profiles for clinical risk factors and neuroimaging markers of amnestic and non-amnestic probable VCI have been described. Ultrastructural left hippocampal damage, indexed as increased mean diffusivity (hipMD), MTA scores, presence of stroke and smoking habit were significant predictors of amnestic VCI. Conversely, older people were more likely to have non-amnestic VCI, and higher total ischemic lesion burden showed a tendency to predict non-amnestic VCI. This dissociation was further confirmed by divergent patterns of correlation with performance in cognitive subdomains: total ischemic lesion burden was significantly correlated with performance in verbal *fluency*, whilst MTA scores and hipMD were associated with *memory* performance.

A predominant effect from hippocampal ultrastructural damage, particularly the left hippocampus, in patients with amnestic VCI was shown in our study. Notably, no evidence was found to suggest that extra-hippocampal cerebral ischemic lesion load, age or vascular risk factors contributed to *memory* loss accounting for hippocampal damage. Memory function, in

particular long recall and delayed recognition, are both typically considered as hippocampal-dependent functions [33]. Whilst the role of the right hippocampus is on allocentric processing of space, the left hippocampus is more involved in context-dependent episodic memory [23]. Hippocampal damage on diffusion MRI and hippocampal atrophy were reported to predict cognitive impairment after stroke [17]. However, the relationship between hippocampal atrophy and especially hippocampal ultrastructural tissue damage and cognitive impairment in VCI has remained unclear [4]. Although in one study, hipMD after cerebrovascular ischemic events was thought to precede hippocampal atrophy and disconnection [17], that study did not correct for partial volume effects making it impossible to exclude atrophy effects. Using careful ROI selection and CSF thresholding, we have now shown that the associations of atrophy indexed as MTA scores and hippocampal tissue damage (atrophy corrected MD) with memory loss in VCI are independent. Although elevated hipMD can be predictive of memory decline in healthy elderly individuals [34] and prodromal AD [35], there is insufficient evidence to prove that hipMD changes cause hippocampal atrophy. Elevated mean diffusivity of the anterior-dorsal hippocampus was observed in spontaneous hypertensive rats, a recognized model of VCI even before behavioural deficits were noted [36]. In our study, significant ischemic lesions in the hippocampi and parahippocampal regions were excluded by careful review of imaging. It is plausible that high MD, which indicates a local increase of free water diffusivity in the parenchyma, is linked to reduction in myelin content, axonal decline or depletion of extracellular matrix [37].

Mesiotemporal atrophy scores were significant predictors of the amnestic VCI cohort, whereas MTA scores were not significantly impaired in our non-amnestic subgroup. The MTA score strongly supports the clinical diagnosis of AD at early stages of the disease, and it is related to memory dysfunction and delayed recall [29]. Although our study population represents VCI, we have shown a subgroup with predominant memory loss, amnestic VCI, with radiological evidence of MTA. This amnestic VCI conceivably might represent a mixed type pre-manifest dementia with additional pathology of AD. Whilst this remains speculative without evidence for amyloid pathology using CSF or positron emission tomography (PET)-imaging, our findings are well in line with a previous report of less severe MTA scores in non-amnestic post-stroke VCI compared with amnestic subtypes [38].

Neuropsychological studies have shown VCI attentional-executive deficits [39,40] with frequently

observed subcortical vascular pathology and interruption in the frontostriatal circuits [41–43]. Similarly, a trend for associations with *fluency* impairment and total ischemic lesion volume as a measure of vascular pathology was found in our cohort. Further studies are required to clearly demonstrate the pathways and mechanisms in non-amnesic VCI; elaboration and refining lesion and tract injury have been shown to have diagnostic potential [12]. In our study population, ultrastructural hippocampal damage was not associated with non-amnesic VCI. This profile is further supported by the current evidence that cortical and subcortical infarcts primarily affect non-memory domains of cognition, mostly executive function [7,44]. The extent of infarcts tended to play roles in non-amnesic VCI; likewise, non-amnesic MCI has been reported to be suggestive of vascular disease and more infarcts in a small cohort [19]. The associations between *fluency* – a non-amnesic subdomain – and total ischemic burden additionally support primary vascular pathology in non-amnesic VCI.

Our study does not allow pathological confirmation, but provides radiological evidence for selective left hippocampal damage in amnesic VCI compared to the non-amnesic group who showed more cerebrovascular disease burden. Coexisting preclinical AD is expected in 11%–20% of people aged 65 and older [45,46], confirmation of which would have required availability of amyloid and tau CSF markers or amyloid-PET, which our study is lacking. ACE-R cut-off scores for detection of subdomain impairment after stroke have good validity to detect executive, visuospatial and attention impairment but not memory [24]. Nevertheless, age-adjusted overall memory scores showed similar results in our study. The third limitation is the cross-sectional design that does not allow comment on the predictive value of our neuroimaging findings to predict the clinically most relevant conversion to vascular dementia. Longitudinal neuroimaging studies are required to better clarify the natural progression of VCI subtypes, and to assess the value of neuroimaging predictors of conversion to different types of dementia.

We describe two distinctive VCI subtypes to replace the current criteria with a heterogeneous descriptive entity to allow clinical and imaging stratification for clinical trials and future treatment strategies. It is plausible that amnesic VCI may represent the subgroup of VCI closer to Alzheimer's pathology and may be selected for pre-manifest Alzheimer's/mixed type dementia prevention trials. Moreover, in clinical trials of VCI, focusing on non-amnesic VCI subtypes especially with absent MTA may offer a pure VCI cohort for intervention trials in which subsiding

cerebrovascular pathology is targeted. Conversely, for treatment trials aiming to prevent further hippocampal pathology, we anticipate potential gain from stratification in amnesic and non-amnesic VCI with or without MTA abnormality and characterization using hippocampal mean diffusivity.

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### References

1. Ganguli M. Principles and practice of geriatric psychiatry. Chapter 38, In: Abou-Saleh MT, Katona C, Kumar A, eds. *Epidemiology of Dementia*, 3rd edn. Hoboken, NJ: Wiley, 2011.
2. Iadecola C. The pathobiology of vascular dementia. *Neuron* 2013; **80**: 844–866.
3. Gorelick PB, Scuteri A, Black SE, *et al.* Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011; **42**: 2672–2713.
4. O'Brien JT, Thomas A. Vascular dementia. *Lancet* 2015; **386**: 1698–1706.
5. Pohjasvaara T, Mantyla R, Salonen O, *et al.* How complex interactions of ischemic brain infarcts, white matter lesions, and atrophy relate to poststroke dementia. *Arch Neurol* 2000; **57**: 1295–1300.
6. O'Sullivan M, Morris RG, Huckstep B, Jones DK, Williams SC, Markus HS. Diffusion tensor MRI correlates with executive dysfunction in patients with ischemic



- leukoaraiosis. *J Neurol Neurosurg Psychiatry* 2004; **75**: 441–447.
7. Traykov L, Baudic S, Thibaudet MC, Rigaud AS, Smaghe A, Boller F. Neuropsychological deficit in early subcortical vascular dementia: comparison to Alzheimer's disease. *Dement Geriatr Cogn Disord* 2002; **14**: 26–32.
  8. Wiesmann M, Kiliaan AJ, Claassen JA. Vascular aspects of cognitive impairment and dementia. *J Cereb Blood Flow Metab* 2013; **33**: 1696–1706.
  9. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993; **43**: 250–260.
  10. Ylikoski R, Jokinen H, Andersen P, et al. Comparison of the Alzheimer's Disease Assessment Scale Cognitive Subscale and the Vascular Dementia Assessment Scale in differentiating elderly individuals with different degrees of white matter changes. The LADIS Study. *Dement Geriatr Cogn Disord* 2007; **24**: 73–81.
  11. Benjamin P, Lawrence AJ, Lambert C, et al. Strategic lacunes and their relationship to cognitive impairment in cerebral small vessel disease. *Neuroimage Clin* 2014; **4**: 828–837.
  12. Meng D, Hosseini AA, Simpson RJ, Tench CR, Dineen RA, Auer DP. Lesion topography and microscopic white matter tract damage contribute to cognitive impairment in symptomatic carotid artery disease. *Radiology* 2017; **282**: 502–515.
  13. Fein G, Di Sclafani V, Tanabe J, et al. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease. *Neurology* 2000; **55**: 1626–1635.
  14. Bosch B, Arenaza-Urquijo EM, Rami L, et al. Multiple DTI index analysis in normal aging, amnesic MCI and AD. Relationship with neuropsychological performance. *Neurobiol Aging* 2012; **33**: 61–74.
  15. Fellgiebel A, Wille P, Muller MJ, et al. Ultrastructural hippocampal and white matter alterations in mild cognitive impairment: a diffusion tensor imaging study. *Dement Geriatr Cogn Disord* 2004; **18**: 101–108.
  16. Li YD, Dong HB, Xie GM, Zhang LJ. Discriminative analysis of mild Alzheimer's disease and normal aging using volume of hippocampal subfields and hippocampal mean diffusivity: an *in vivo* magnetic resonance imaging study. *Am J Alzheimers Dis Other Demen* 2013; **28**: 627–633.
  17. Kliper E, Ben Assayag E, Korczyn AD, et al. Cognitive state following mild stroke: a matter of hippocampal mean diffusivity. *Hippocampus* 2016; **26**: 161–169.
  18. Vos SJ, van Rossum IA, Verhey F, et al. Prediction of Alzheimer disease in subjects with amnesic and non-amnesic MCI. *Neurology* 2013; **80**: 1124–1132.
  19. He J, Farias S, Martinez O, Reed B, Mungas D, Decarli C. Differences in brain volume, hippocampal volume, cerebrovascular risk factors, and apolipoprotein E4 among mild cognitive impairment subtypes. *Arch Neurol* 2009; **66**: 1393–1399.
  20. Hosseini AA, Simpson RJ, Altaf N, Bath PM, MacSweeney ST, Auer DP. MRI plaque hemorrhage for risk stratification in carotid artery disease with moderate risk under current medical therapy. *Stroke* 2017; **48**: 678–685.
  21. Lischka AR, Mendelsohn M, Overend T, Forbes D. A systematic review of screening tools for predicting the development of dementia. *Can J Aging* 2012; **31**: 295–311.
  22. Crawford S, Whitnall L, Robertson J, Evans JJ. A systematic review of the accuracy and clinical utility of the Addenbrooke's Cognitive Examination and the Addenbrooke's Cognitive Examination-Revised in the diagnosis of dementia. *Int J Geriatr Psychiatry* 2012; **27**: 659–669.
  23. Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. *Neuron* 2002; **35**: 625–641.
  24. Morris K, Hacker V, Lincoln NB. The validity of the Addenbrooke's Cognitive Examination-Revised (ACE-R) in acute stroke. *Disabil Rehabil* 2012; **34**: 189–195.
  25. Bickerton WL, Demeyere N, Francis D, et al. The BCOS cognitive profile screen: utility and predictive value for stroke. *Neuropsychology* 2015; **29**: 638–648.
  26. Behrens TEJ, Johansen-Berg H, Woolrich MW, et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci* 2003; **6**: 750–757.
  27. University of Nottingham. <http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx> (accessed 01/05/2017)
  28. Smith SM, De Stefano N, Jenkinson M, Matthews PM. Normalized accurate measurement of longitudinal brain change. *J Comput Assist Tomogr* 2001; **25**: 466–475.
  29. Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in 'probable' Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry* 1992; **55**: 967–972.
  30. Ferreira D, Cavallin L, Larsson EM, et al. Practical cut-offs for visual rating scales of medial temporal, frontal and posterior atrophy in Alzheimer's disease and mild cognitive impairment. *J Intern Med* 2015; **278**: 277–290.
  31. Dichgans M, Putz B, Boos D, Auer DP. Role of subvoxel free fluid on diffusion parameters in brain tissue with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and its correlation with physical disability: histogram analysis of standard and fluid-attenuated MR diffusion. *AJNR Am J Neuroradiol* 2003; **24**: 1083–1089.
  32. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation* 2007; **115**: 928–935.
  33. Manns JR, Eichenbaum H. Evolution of declarative memory. *Hippocampus* 2006; **16**: 795–808.
  34. Carlesimo GA, Cherubini A, Caltagirone C, Spalletta G. Hippocampal mean diffusivity and memory in healthy elderly individuals: a cross-sectional study. *Neurology* 2010; **74**: 194–200.
  35. Zhang B, Xu Y, Zhu B, Kantarci K. The role of diffusion tensor imaging in detecting microstructural changes in prodromal Alzheimer's disease. *CNS Neurosci Ther* 2014; **20**: 3–9.
  36. Lopez-Gil X, Amat-Roldan I, Tudela R, et al. DWI and complex brain network analysis predicts vascular cognitive impairment in spontaneous hypertensive rats undergoing executive function tests. *Front Aging Neurosci* 2014; **6**: 167.
  37. Gouw AA, Seewann A, Vrenken H, et al. Heterogeneity of white matter hyperintensities in Alzheimer's disease: post-mortem quantitative MRI and neuropathology. *Brain* 2008; **12**: 3286–3298.
  38. Kim BJ, Oh MY, Jang MS, et al. Medial temporal atrophy and memory dysfunction in poststroke cognitive impairment – no dementia. *J Clin Neurol* 2012; **8**: 43–50.

39. Nyenhuis DL, Gorelick PB. Diagnosis and management of vascular cognitive impairment. *Curr Atheroscler Rep* 2007; **9**: 326–332.
40. Hachinski V, Iadecola C, Petersen RC, *et al.* National Institute of Neurological Disorders and Stroke–Canadian Stroke Network vascular cognitive impairment harmonization standards. *Stroke* 2006; **37**: 2220–2241.
41. O'Brien JT, Erkinjuntti T, Reisberg B, *et al.* Vascular cognitive impairment. *Lancet Neurol* 2003; **2**: 89–98.
42. Stebbins GT, Nyenhuis DL, Wang C, *et al.* Gray matter atrophy in patients with ischemic stroke with cognitive impairment. *Stroke* 2008; **39**: 785–793.
43. Delano-Wood L, Abeles N, Sacco JM, Wierenga CE, Horne NR, Bozoki A. Regional white matter pathology in mild cognitive impairment: differential influence of lesion type on neuropsychological functioning. *Stroke* 2008; **39**: 794–799.
44. Carey CL, Kramer JH, Josephson SA, *et al.* Subcortical lacunes are associated with executive dysfunction in cognitively normal elderly. *Stroke* 2008; **39**: 397–402.
45. Wang R, Fratiglioni L, Laukka EJ, *et al.* Effects of vascular risk factors and APOE epsilon4 on white matter integrity and cognitive decline. *Neurology* 2015; **84**: 1128–1135.
46. de Jong D, Jansen RW, Kremer BP, Verbeek MM. Cerebrospinal fluid amyloid beta42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia. *J Gerontol A Biol Sci Med Sci* 2006; **61**: 755–758.