

# Relationship between Cerebral Perfusion on Arterial Spin Labeling (ASL) MRI with Brain Volumetry and Cognitive Performance in Mild Cognitive Impairment and Dementia due to Alzheimer's Disease

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## Abstract

**Context:** Cerebral blood flow (CBF) measurement using arterial spin labelling (ASL) MRI sequences has recently emerged as a prominent tool in dementia research. **Aims:** To establish association between quantified regional cerebral perfusion and gray matter (GM) volumes with cognitive measures in mild cognitive impairment (MCI) and early Alzheimer's Dementia (AD), using three dimensional fast spin echo pseudo-continuous ASL MRI sequences. **Settings and Design:** Hospital-based cross-sectional study. **Methods and Material:** Three age-matched groups, i.e., 21 cognitively normal healthy controls (HC), 20 MCI and 19 early AD patients diagnosed using neuropsychological tests and who consented for multimodality 3T MRI were recruited for the study. **Statistical Analysis Used:** Statistical parametric mapping and regions of interest (ROI) multivariate analysis of variance was used to ascertain differences between patients and controls on MRI-volumetry and ASL. Linear regression was used to assess relationship between CBF with GM atrophy and neuropsychological test measures. **Results:** Compared to HC, patients with MCI and AD had significantly lower quantified perfusion in posterior cingulate and lingual gyri, over hippocampus in MCI, with no differences noted between MCI and AD. Atrophy over the middle temporal gyrus and hippocampus differentiated AD from MCI. No significant positive correlations were noted between perfusion and GM volumes in ROI with the exception of temporal neocortex. Significantly positive coefficient b-value ( $p < 0.01$ ) were apparent between global cognition with CBF in precuneus, temporal neocortex and precuneus volume, with negative b-values noted between medial temporal CBF for global cognition and recall scores. **Conclusions:** ROI-based CBF measurements differentiated MCI and AD from HC; volumetry of medial and neocortical temporal GM separates AD from MCI. Correlations between CBF and neuropsychology are variable and require further longitudinal studies to gauge its predictive utility on cognitive trajectory in MCI.

**Keywords:** Alzheimer's disease, Cerebral blood flow (CBF), mild cognitive impairment, perfusion MRI, Pseudo-continuous arterial spin labeling (pCASL)

## INTRODUCTION

Research in mild cognitive impairment (MCI) has been focused on identifying biomarkers for individuals who are at risk for developing AD. Promising among magnetic resonance imaging (MRI) based biomarkers is the detection of abnormalities in cerebral blood flow (CBF) in patients with AD and MCI that potentially predict progressive neuronal loss.<sup>[1]</sup> Several studies have reported positive correlation between CBF and metabolism, suggesting the former's utility as an alternative biomarker for cerebro-vascular brain function and brain aging, although these cannot be used interchangeably.<sup>[2,3]</sup> Arterial Spin Labeling Magnetic Resonance Imaging (ASL-MRI), a truly non-invasive technique, employs arterial blood as an endogenous contrast for detecting regional CBF perfusion in patients with AD and MCI.<sup>[4-6]</sup> Besides, the several advantages of ASL-MRI such as its non-invasiveness, freedom from exposure to ionizing radiation, intravenous contrast agents or radioactive isotopes and short acquisition time at higher

magnetic field strengths (3T) make ASL a potential alternative to FDG PET.<sup>[7,8]</sup>

To date, few studies have addressed correlation between quantified CBF variations with neuropsychological parameters in patients with AD and MCI and have largely relied on

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qualitative visual assessments.<sup>[9]</sup> Using ASL, many studies have consistently quantified significant hypoperfusion involving the posterior cingulate gyrus and precuneus, among many regions studied in MCI due to AD.<sup>[3,10,11]</sup> Positive correlations have been noted between global cognitive scores and CBF values in posterior cingulate cortex and temporo-parietal association cortices.<sup>[9]</sup> No consistent correlation has been established between CBF and gray matter volume changes in the corresponding regions in patients with AD and MCI in the existing literature.<sup>[11]</sup> Specifically in one study, concordance with CBF reductions and GM atrophy over the entorhinal cortex and cingulate cortex has been noted in MCI in comparison to healthy controls.<sup>[9]</sup> Thus, the aim of the present study was to detect and quantify perfusion distribution abnormalities in the whole brain for early AD and MCI patients at an initial stage using three dimensional fast spin echo pseudo-continuous ASL (3D FSE pCASL) MRI sequence. We aimed to study the correlation between topography of perfusion abnormalities with underlying regions of GM atrophy in MCI and AD. We also studied correlation between quantified perfusion measures and neuropsychological test scores in MCI and early AD.

## MATERIALS AND METHODS

### Subjects

In this study, 65 subjects were prospectively recruited from a Memory and Neurobehavioral Disorders Clinic of a hospital in the South Indian state of Kerala. Five subjects were excluded from further analysis; four due to strong head motion during ASL-MRI, and one participant due to structural abnormalities that could produce artefacts in CBF maps which interfere with ASL interpretation (dolichoectasia of intracranial vessels). The final sample consisted thus of 21 cognitively normal healthy controls (HC), 20 MCI patients and 19 AD patients. The participants were cognitively screened by the vernacular (south Indian language Malayalam) adaptation of Addenbrooke's Cognitive Examination battery (ACE-M).<sup>[12,13]</sup> All subjects provided written consent according to procedures approved by the Institutional Ethics Committee of our institute.

Participants with a global cognitive assessment score, as determined on ACE-M, between 88 and 100, clinical dementia rating (CDR) score of 0, with formal education of >10 years, no subjective memory complaints, and with no serious neurological/psychiatric issues were selected as HC. Participants with CDR of <2 and ACE-M score between 60 and 78 were confirmed as AD according to standard NINCDS-ADRDA diagnostic criteria.<sup>[14]</sup> Amnesic MCI patients were diagnosed as per modified Petersen's criteria with CDR of ≤0.5 and ACE-M score between 78 and 88 with evidence of impairment in the memory domain, defined as test performance on at least 2 tests (which also included the ACE-M sub-component scores) for a given domain falling below mean-1.5SD of the normative scores for the corresponding age and education status.<sup>[15,16]</sup> Neuropsychological tests used to diagnose amnesic MCI and to exclude non-amnesic and multi-domain MCI were as detailed in our previous study.<sup>[13]</sup> Inclusion as MCI mandated

non-conversion into AD/dementia at the time of initiation of study on follow-up in the cognitive disorders clinic. Exclusion criteria in the patient groups included presence of major medical (including uncontrolled diabetes, hypothyroidism, renal disease, peripheral vascular disease), psychiatric comorbidity (e.g., psychosis, significant anxiety, or depression), cardiovascular disease, prior history of cerebrovascular accidents or pre-existing major neurological deficit prior to evaluation and presence of Fazeka's grade 2-3 white matter (superficial and or deep) on MRI scans or brain infarcts. The study protocol is summarized in Figure 1.

### Imaging protocol

A 3T scanner (GE healthcare, Milwaukee, Wisconsin) with a 32- AQC: F channel phased array head coil was used to acquire structural and ASL. Structural images were obtained using a high resolution reference axial 3D brain volume imaging sequence (3D Bravo) with TR/TE = 7/2.98 ms, slice thickness = 1 mm, flip angle = 12°, matrix size = 256 × 256, and voxel size = 1 mm × 1 mm × 1 mm. ASL images were acquired by employing 3D fast spin echo pseudo-continuous ASL sequence with acquisition parameters: TR/TE = 4852/10.70 ms, flip angle = 111°, voxel size = 1.875 x 1.875 x 4, image reconstruction matrix = 128 x 128, field of view (FOV) = 24 cm, slice thickness = 4 mm, NEX = 3 and post label delay = 2025 ms.

### MRI Data Analysis (Supplementary Data file 1)

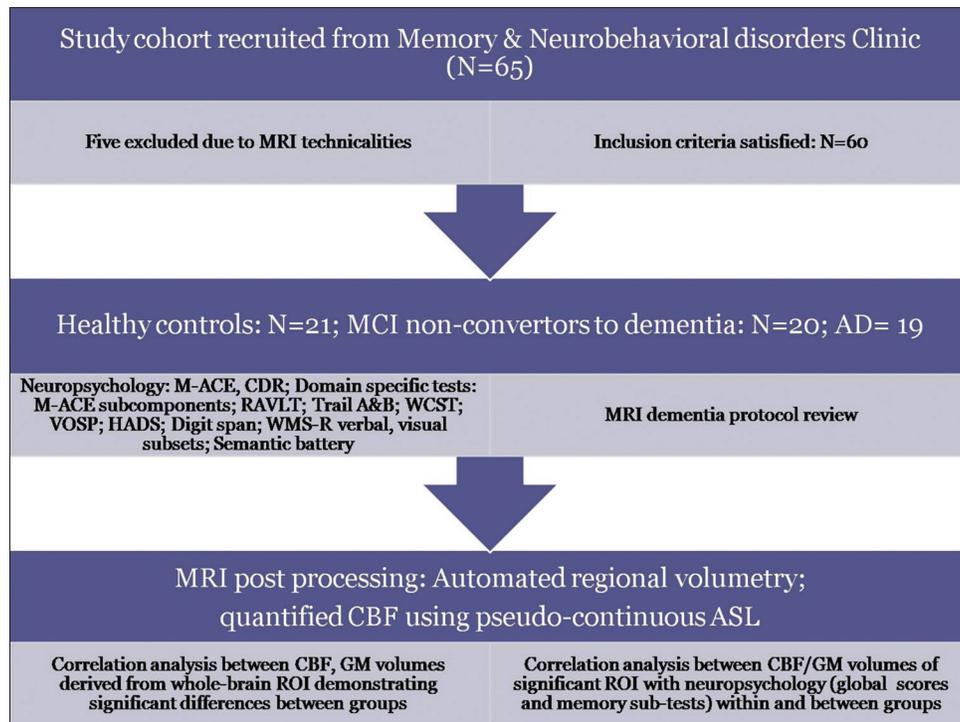
All the structural images and CBF maps were oriented in the AC-PC line using Statistical Parameter Mapping software (SPM12- After data acquisition and reconstruction, the images were transferred to a personal computer for further analysis. For ASL processing, quantitative CBF maps in units of ml/100 gm/min, generated automatically based on ASL data for each patient was used. ASL was quantified using the quantification algorithm as follows:

$$CBF = 6000 * \lambda \frac{\left[ 1 - \exp \left[ -\frac{ST(s)}{T_{1r}(s)} \right] \right] \exp \left[ \frac{PLD(s)}{T_{1b}(s)} \right]}{2T_{1b}(s) \left[ 1 - \exp \left[ -\frac{LT(s)}{T_{1b}(s)} \right] \right] \epsilon * NEX_{PW} \left[ \frac{PW}{SF_{pw} PD} \right]}$$

where T1b is T1 of blood and is assumed to be 1.6s for 3T. The partial saturation of the reference image (PD) is corrected for by using a T1t of 1.2s (typical of gray matter. ST is saturation time and is set to 2s. The partition coefficient λ is set to the whole brain average, 0.9. The efficiency, ε, is an overall efficiency 0.6. PLD is the post labelling delay used for the experiment. LT is the labelling duration. PW is the perfusion weighted or raw difference image. SPPW is the scaling factor of PW sequence. NEXPW is the number of excitation for PW images.

### Statistical analysis

In order to identify the group difference observed between



**Figure 1:** Algorithm of the study (patient recruitment and imaging analysis; abbreviations- MRI- Magnetic Resonance Imaging; M-ACE- Malayalam version of Addenbrooke's Cognitive Examination battery; CDR- Clinical Dementia Rating Scale; RAVLT- Rey Auditory Verbal Learning Test- Malayalam; WCST- Wisconsin Card Sorting Test; HADS- Hospital Anxiety-Depression Scale; WMS-R: Weschler's Memory Scale- Malayalam; CBF- Cerebral Blood Flow; ASL- Arterial Spin Labelling; ROI- Regions of interest; GM- Gray matter)

groups, the demographic, neuropsychological, GM volume, and CBF measures were compared across the three groups (HC, MCI, and AD) using multivariate analysis of variance with post-hoc test using Bonferroni method. All CBF and GM volume values were entered into general linear model in order to establish significance of observed differences ( $p < 0.05$ ). Pearson's correlation analysis was used to study correlation between ROI CBF and GM volumes with diagnosis as the controlling variable. For determining the relationship of CBF and GM volume with global cognitive measures and learning-recall, a linear regression model was created using the neuropsychological test score as dependent variable, MRI parameters as independent variables and group membership (MCI/AD) as a covariate. As whole brain measures were used and given the wider dispersion of values likely with limited sample size, we relied on a  $P$  value of  $<0.01$  to establish significant predictive utility of CBF and GM volume on neuropsychology test scores within and between groups.

## RESULTS

### Demographics, clinical data

Table 1 depicts demographics and neuropsychological assessment test scores in patients and controls. Patients with amnesic MCI and early AD were significantly impaired on ACE total, Rey auditory verbal learning test (RAVLT) total learning score and RAVLT recall after 20 min compared to

HC despite no significant differences noted in age and gender distribution.

### Voxel-wise ANOVA

With regard to whole brain gray matter perfusion measures, ANOVA models following FDR correction revealed that compared with controls, AD patients showed hypoperfusion in bilateral posterior cingulate cortices (PCC), bilateral lingual gyri, right precuneus, right fusiform, right middle temporal lobe and right superior temporal lobe, even after FDR corrections for multiple comparisons [Figure 2 b]. In AD compared to MCI, lower perfusion was noted in the AD group in the right inferior temporal lobe and left fusiform [Figure 2 c].

Considering whole brain GM volume difference, the subjects exhibited atrophy in most of the areas reported with change in perfusion [Figure 3]. Regions such as superior frontal and middle frontal gyri were detected to have hypoperfusion without atrophy [Supplementary Tables 1 and 2].

### Quantified ROI analysis

The multivariate model with CBF values gave significant results for all multivariate tests. (Wilks lambda = 0.241, partial eta squared = 0.509,  $P < 0.001$ ). The model with ROI GM volume values was significant only in the Roy's largest root test. However, the model with all CBF and ROI GM volume values retained significance for all multivariate tests. (Wilk's lambda = 0.108, partial eta squared = 0.671,

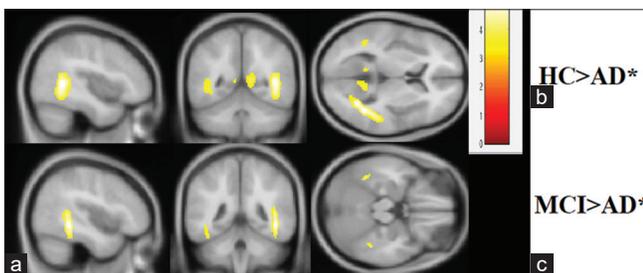
**Table 1: Comparison of demographic and neuropsychological measures between subjects**

Character-istic	HC (n=21)	MCI (n=20)	AD (n=19)	Bonferroni corrected P		
				MCI versus NC	AD versus NC	AD versus MCI
Sex (male/female)	11/10	11/9	11/8	0.87	0.77	0.88
Age (mean±SD in years)	64.57±5.74	66.75±4.08	66.68±5.31	0.37	0.66	0.898
ACE	93.38±4.11	81.12±11.09	72.00±11.09	<0.001	<0.001	0.020
RAVLT cumulative learning score	48.52±8.46	33.47±10.07	25.21±7.15	<0.001	<0.001	0.035
RAVLT 20 min recall score	10.00±2.82	5.41±3.74	1.36±1.73	<0.001	<0.001	0.001
Median CDR (range)	0	0.5 (0-0.5)	1 (0.5-1.5)	0.020	<0.001	0.01
Hypertension#	5	7	9	0.33	0.11	0.32
Diabetes Mellitus#	5	8	10	0.22	0.06	0.32

#Fisher's Exact test

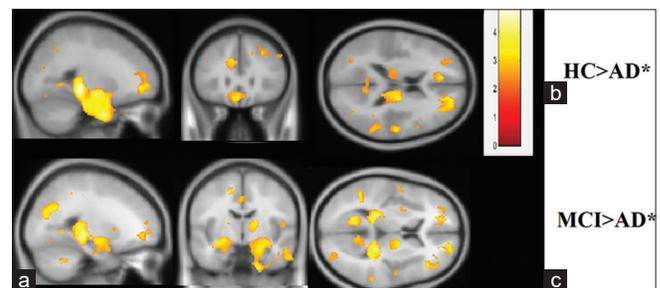
**Table 2: Multiple comparison results of region of interest (ROI) based CBF measurements using Bonferroni correction among HC, MCI and AD (Significant results in bold font)**

ROI for CBF analysis (mean±SD)	HC CBF (ml/100 g/min)	MCI CBF (ml/100 g/min)	AD CBF (ml/100 g/min)	Effect size Partial Eta Squared	Bonferroni corrected P		
					MCI versus HC	AD versus HC	AD versus MCI
Superior frontal	39.21±8.16	36.34±7.40	33.81±8.36	0.074	0.762	0.111	0.982
Middle frontal	35.20±8.44	32.52±8.79	33.81±8.36	0.017	0.956	1.000	1.000
Posterior Cingulate	54.43±10.59	44.26±8.64	39.11±7.72	0.341	<b>0.002</b>	<b>&lt;0.001</b>	0.250
Superior temporal	37.21±4.86	35.71±4.74	32.84±6.32	0.107	1.000	<b>0.036</b>	0.296
Middle temporal	32.57±5.46	31.77±5.52	29.52±6.93	0.046	1.000	0.341	0.738
Inferior temporal	27.90±6.78	27.05±7.05	24.47±8.97	0.036	1.000	0.484	0.888
Para-hippocampus	37.44±5.58	34.20±4.22	33.76±7.32	0.079	0.241	0.151	1.000
Lingual Gyrus	39.96±8.01	33.42±5.83	31.73±9.23	0.181	<b>0.028</b>	<b>0.005</b>	1.000
Fusiform	34.53±5.88	31.13±4.80	29.55±7.48	0.109	0.242	<b>0.039</b>	1.000
Hippocampus	39.19±6.62	32.69±7.31	34.13±9.72	0.118	<b>0.034</b>	0.147	1.000
Precuneus	38.84±8.75	33.89±5.90	33.89±11.20	0.070	0.236	0.247	1.000
Supramarginal	35.79±7.51	34.72±5.64	32.13±7.22	0.050	1.000	0.289	0.724



**Figure 2:** Analysis of variance (ANOVA) (three groups, covariates: TIV, age and gender): Comparison of areas of hypoperfusion between (b) AD-HC and (c) AD-MCI with parametric maps of T values for group differences. \* FDR corrected ( $p < 0.05$ ). Significant results after FDR correction were obtained only for the group comparisons HC >AD and MCI >AD and not for HC >MCI. (Color bar represents T scores)

$P < 0.001$ ). Table 2 summarizes the results of ROI CBF analysis of the regions reported with hypoperfusion in the whole brain analysis. Compared to HC, patients with MCI had significantly lower perfusion in the PCC, lingual gyrus, hippocampus in MCI; additionally, over superior temporal gyrus (STG) and fusiform gyrus in AD as compared to



**Figure 3:** Analysis of variance (ANOVA) (three groups, covariates: TIV, age and gender): areas of gray matter atrophy with parametric maps of T values for the group difference in patients with AD as compared with HC and MCI. \* FDR corrected ( $p < 0.05$ ); Significant results after FDR correction were obtained only for the group comparisons (b) HC >AD and (c) MCI >AD and not for HC >MCI. (Color bar represents T scores)

HC with no significant differences between MCI and AD. Supplementary Table 3 depicts the regional GM volume for the areas reported with cerebral blood flow changes even after adding total intracranial volume as a covariate. Correlation analysis between ROI for CBF and VBM values revealed a moderate positive correlation only over the superior

**Table 3: Linear regression model to assess association between regional cerebral blood flow, regional brain volume with neuropsychological test performances; significant values shown in table;  $P < 0.01$  highlighted**

Variable	Standardized coefficients (beta)	t	Sig. (P)	95% confidence interval	
				Lower Bound	Upper Bound
ACE-M total score					
STG CBF	1.847	4.29	<b>0.001</b>	2.098	5.988
Precuneus CBF	1.595	3.589	<b>0.001</b>	0.934	3.562
Hippocampal CBF	-1.516	-3.415	<b>0.002</b>	-4.108	-1.013
Fusiform CBF	-1.543	-3.518	<b>0.002</b>	-4.592	-1.196
Supramarginal CBF	-0.827	-2.708	0.012	-2.872	-0.387
Precuneus Vol.	0.591	3.947	<b>0.001</b>	3.176	10.008
(Adjusted $R^2$ for CBF=0.529; for GM volume=0.327; $P < 0.001$ )					
RAVLT total learning score					
SFG CBF	1.135	2.156	0.04	0.066	2.665
MFG CBF	-1.374	-2.64	0.014	-2.673	-0.335
Mid-temporal Vol.	0.959	2.242	0.034	0.407	9.835
Fusiform Vol.	0.735	2.272	0.032	0.640	13.362
(Adjusted $R^2$ for CBF=0.198; $P=0.03$ ; for GM volume $R^2=0.347$ ; $P=0.01$ )					
RAVLT delayed recall score					
SFG CBF	2.01	4.47	<b>0.001</b>	0.486	1.313
MFG CBF	-1.774	-4.022	<b>0.001</b>	-1.092	-0.353
Hippocampal CBF	-0.606	-2.976	<b>0.006</b>	-0.526	-0.096
MTG CBF	0.458	2.734	0.011	0.067	0.474
(Adjusted $R^2$ for CBF=0.433; $P=0.011$ )					

temporal (STG) ( $r = 0.323$ ,  $P = 0.013$ ) and inferior temporal gyri (ITG) ( $r = 0.343$ ;  $P = 0.008$ ) with weak correlations over the middle frontal gyri ( $r = 0.293$ ;  $P = 0.024$ ).

### Correlation analysis between neuropsychology parameters with regional CBF and GM volume

The linear regression model revealed strong positive correlations ( $p < 0.01$ ) between ACE-M and CBF over STG, both CBF and GM volume of precuneus, RAVLT delayed-recall with CBF of superior frontal gyrus. Consistent negative correlations were noted for these measures with medial temporal and middle frontal gyri as noted in Table 3. Pearson correlation analysis within groups revealed a moderately positive correlation between total ACE-M scores and CBF in STG ( $r = 0.50$ ,  $P < 0.04$ ) in patients with MCI. Strong positive correlation was evident between ACE-M score and GM volumes of STG ( $r = 0.65$ ,  $P < 0.01$ ), lingual gyrus ( $r = 0.64$ ,  $P < 0.01$ ) and fusiform gyrus ( $r = 0.62$ ,  $P < 0.01$ ) of patients with AD. Moreover, cumulative learning trials score on RAVLT in patients with AD showed positive correlation with regional volumes of atrophic regions such as STG ( $r = 0.64$ ,  $P < 0.01$ ), lingual gyrus ( $r = 0.60$ ,  $P < 0.04$ ), fusiform gyrus ( $r = 0.60$ ,  $P < 0.02$ ), and hippocampus ( $r = 0.60$ ,  $P < 0.02$ ).

## DISCUSSION

Our study used a whole brain 3D FSE pCASL technique on 3T MRI to investigate perfusion difference abnormalities in patients with MCI and AD with NC and to analyze its correlation with the GM changes in ROI-based and voxel-wise analyses. The results of our study indicate that CBF estimation

was not able to consistently differentiate between MCI and AD, despite its ability to separate both patient groups from controls. However, on VBM, quantified middle temporal gyri and hippocampal volumes differentiate AD from MCI.

The most consistent finding in our study is that even after Bonferroni correction, robust ASL hypoperfusion were found in PCC and lingual gyrus [Table 2] in MCI and AD in comparison to controls without significant differences between the patient groups, except on voxel-wise ANOVA [Figures 2 and 3]. Partial volume correction on our data was done for each voxel by dividing its CBF value by the fraction of its GM content on the segmentation maps to separate perfusion from structural effects. Although these regions reported atrophy, no significant volume differences were apparent after Bonferroni correction between MCI and controls as opposed to multiple ROIs demonstrating differences between AD and controls as well as on middle temporal gyri and hippocampal volume comparisons between AD and MCI. Longitudinal studies have shown that CBF measurement distinguishes between controls and AD, identify those controls at risk for MCI and AD, and predict conversion of controls to MCI and AD, suggesting its usefulness as a preclinical marker of cognitive decline.<sup>[1,17]</sup> In our cross-sectional observational study, the regional mean CBF values were not distinctive between MCI and AD, despite the fact that our MCI group in itself may have been of diverse aetiologies and not exclusively due to AD pathology. This can only be established using biomarkers of amyloid accumulation or tau deposition and longitudinal follow-up.<sup>[15,18]</sup> This finding may be because hypoperfusion occurs early in the course of the illness, and this particular feature may be used as a reliable

marker to delineate patients with MCI and AD from cognitively normal HC. However, from MCI to AD transformation, the perfusion reduction in these regions may be less remarkable and hence may not have significant diagnostic utility as a stand-alone measure as opposed to volume measures.

Significantly, the CBF and regional brain volumes correlated only in the temporal neocortex which cannot be fully explained with the available evidence from literature. We have recently reported temporal neocortical atrophy in MCI relative to controls with greater atrophy noted in AD and correlations between neuropsychology with hippocampal volumes in MCI and temporal neocortical volumes in AD.<sup>[19]</sup> While this finding is in line with our results, the consistent positive correlations noted between global cognitive measures and CBF over STG as well as both on CBF and GM volumes of the precuneus are significant. The role of the STG in auditory processing, language networks as well as long term memory is well established, with prior evidence that the postero-superior portion of the left temporal lobe is recognised as an important site for retrieval and long-term storage of, phonological and lexical representations.<sup>[20]</sup> Similarly, the positive correlations demonstrated in the precuneus are not surprising,<sup>[9]</sup> given its more global functional involvement in domains such as visuospatial perception, episodic memory retrieval and consciousness, with a variety of connections to higher associative cortical and subcortical structures.<sup>[21]</sup> Patients with aMCI also show reduced cortical thickness in the precuneus compared to the non-aMCI.<sup>[22]</sup> On the other hand negative correlations noted by us consistently between hippocampal, middle frontal gyri perfusion with global cognition as well as delayed recall scores were seen only on the whole-group analysis and were not apparent within groups. Similar findings of association between higher CBF and poorer memory performance among studies on amyloid-beta positive older adults and those with subjective cognitive impairment in other studies may indicate a cellular and/or vascular compensatory response to pathologic processes whereby higher CBF is needed to maintain normal memory abilities.<sup>[23,24]</sup> This is consistent with findings from our regression analyses that revealed significant interactions of CBF on memory performance independent of volume or cortical thickness, further suggesting that CBF may play a role in cognitive functioning independent of GM atrophy or neuronal loss.

Our study has certain limitations. Arterial transit time may vary among subjects, especially for AD patients, causing potential inaccuracy of CBF measurement. We could not screen for carotid artery disease in all subjects as our protocol did not include TOF MR Angiography which would have been ideal. To offset this partly, we also analyzed the prevalence of vascular risk factors like diabetes and hypertension in the cohort and showed that these were not significantly different in controls and patients. Multi T1 sequence would have been better, however multi-delay ASL was not available in our MRI scanner for clinical studies while conducting the investigations. As this was a cross-sectional design, we are

unable to compare the trajectories of volumetry and CBF estimations longitudinally on the rate of cognitive decline between MCI and AD. Again with the small sample size; the causal relationship between the neurovascular biomarkers for development of MCI due to AD could not be fully understood. The statistical analysis did not satisfy all nine assumptions of multivariate analysis of variance, particularly multivariate normality and the Mahalanobis distance for outliers and a larger cohort is required to validate the robustness of our findings. To demonstrate the temporal and causal relationship of cerebral perfusion with neuropsychological dysfunction and rates of regional volume loss, additional longitudinal studies with adequate sample size will be required in the future.

In conclusion our study primarily investigated the associations between neuropsychological, structural, and vascular biomarkers of MCI and AD from relevant anatomical ROIs after analysis of whole brain ROI. ASL enables us to understand early changes in perfusion in the MCI stage, which correlate with ROI topographic variability with cognition. On the other hand, with disease progression to AD, GM changes, rather than CBF, enable differentiation between MCI and AD. ASL is thus a complementary addition in multimodality MRI based assessment of early cognitive impairment prodromal dementia, especially in centres which are resource or logistically constrained to conduct substrate-based or metabolic imaging with FDG/amyloid-PET.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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## Supplementary data:

### Methodology of MRI Data Analysis

After data acquisition and reconstruction, the images were transferred to a personal computer for further analysis. For ASL processing, quantitative CBF maps in units of ml/100 gm/min, generated automatically based on ASL data for each patient was used. ASL was quantified using the quantification algorithm as follows;

$$CBF = 6000 * \lambda \frac{\left[ 1 - \exp \left[ -\frac{ST(s)}{T_{1r}(s)} \right] \right] \exp \left[ \frac{PLD(s)}{T_{1b}(s)} \right]}{2T_{1b}(s) \left[ 1 - \exp \left[ -\frac{LT(s)}{T_{1b}(s)} \right] \right] \epsilon * NEX_{pw}} \left[ \frac{PW}{SF_{pw} PD} \right]$$

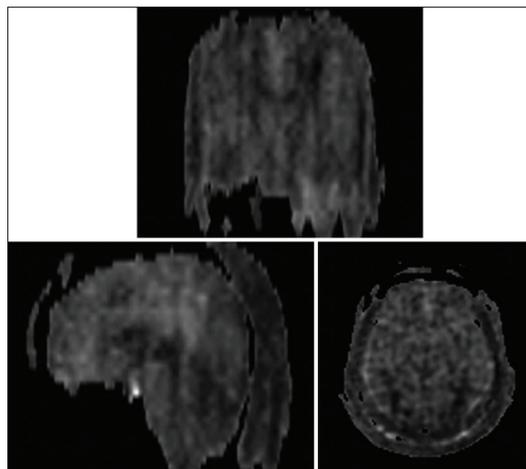
where T1b is T1 of blood and is assumed to be 1.6s for 3T. The partial saturation of the reference image (PD) is corrected for by using a T1t of 1.2s (typical of gray matter. ST is saturation time and is set to 2s. The partition coefficient  $\lambda$  is set to the whole brain average, 0.9. The efficiency,  $\epsilon$ , is an overall efficiency 0.6. PLD is the post labelling delay used for the experiment. LT is the labelling duration. PW is the perfusion weighted or raw difference image. SPPW is the scaling factor of PW sequence. NEXPW is the number of excitation for PW images.

All the structural images and CBF maps were oriented in the AC-PC line using Statistical Parameter Mapping software (SPM12- <http://www.fil.ion.ucl.ac.uk/spm/download/spm12/>). CBF maps of all the participants were then normalized to a standard stereotaxic space and spatially smoothed with an 8 mm isotropic Gaussian kernel to improve signal to noise ratio. For analyzing the cortical gray matter changes of the brain, Volume based morphometry (VBM) analysis was done using cat12 tool-box (<http://www.neuro.uni-jena.de/cat12/>) in SPM. This involved segmentation of the AC-PC aligned structural images into Gray Matter (GM), White Matter (WM) and CSF and GM image normalization to templates in stereotaxic space. After normalization, GM images were smoothed using an 8 mm isotropic Gaussian kernel. Partial volume correction was performed for each voxel by dividing its CBF value by the fraction of its gray matter content on the segmentation maps to separate perfusion from structural effects.

Second-level statistical procedures implemented in SPM12 was used to statistically analyze the CBF maps and GM images. CBF and GM volume differences between subject groups were estimated by means of Voxel-wise analysis of variance (ANOVA). Age, gender and total intra-cranial volume (TIV) were used as covariates. The following contrasts were performed: HC > MCI [1 -1 0], HC < MCI [-1 1 0], HC > AD [1 0 -1], HC < AD [-1 0 1], MCI > AD [0 1 -1], MCI < AD [0 -1 1]. The thresholds of the resulting parametric maps of t values were adjusted at  $P < 0.001$  uncorrected and at  $P < 0.05$ , corrected for multiple comparisons based on false discovery rate (FDR).

Information on regional perfusion values were extracted by means of a region of interest (ROI) analysis. Anatomic ROIs for the regions that showed perfusion changes in the whole brain analysis were defined by means of the WFU Pickatlas tool ([https://www.nitrc.org/projects/wfu\\_pickatlas/](https://www.nitrc.org/projects/wfu_pickatlas/)). Regional CBF was estimated using parameter extraction with MarsBar (<https://www.nitrc.org/projects/marsbar/>). Values within ROIs of both sides were then averaged for NC, patients with MCI and patients with AD. The regional GM volume for those regions reported with increased and decreased perfusion were analyzed using estimate ROI mean in cat12 toolbox. The significant areas with atrophy and difference in perfusion were overlaid on T1-weighted standard brain images and the regions were reported in MNI with the help of xjview toolbox.

### Original perfusion map of a control to demonstrate quality of ASL maps:



**Supplementary Table 1: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and sex; only FDR corrected ROI with differences shown): areas of CBF variations in patients, cluster size threshold: 20**

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	Y	Z
HC > AD				
R Superior temporal*	4.14	50	-32	-2
R Middle temporal*	3.86	52	-32	2
R Posterior cingulate*	3.99	4	-36	28
R Precuneus*	3.14	6	-52	14
R Fusiform*	3.7	22	-64	-14
R Lingual gyrus*	3.6	-14	-72	-8
L Posterior cingulate*	3.46	-6	-40	14
L Lingual*	3.17	-14	-72	-8

\* $P < 0.05$  False Discovery Rate (FDR) corrected

**Supplementary Table 2: Analysis of variance (ANOVA) (three groups, covariates: TIV, age and sex; only FDR corrected ROI with differences shown): areas of gray matter atrophy in patients with mild cognitive impairment (MCI) and mild dementia in Alzheimer's disease (AD) compared with cognitively healthy controls (HC); cluster size threshold: 20**

Anatomic Location	Voxel EquivZ	MNI coordinates		
		x	Y	Z
HC > AD				
R Superior temporal*	2.85	57	-1.5	-6
R Middle temporal*	4.2	63	-28.5	-13.5
R Inferior temporal*	4.55	63	-25.5	-19.5
R Supramarginal*	3.17	64.5	-28.5	36
R Inferior parietal*	2.97	39	-46.5	52.5
R Posterior cingulate*	3.55	4.5	-48	30
R Precuneus*	3.12	12	-61.5	42
R Fusiform*	3.03	31.5	-33	-19.5
R Lingual gyrus*	2.57	16.5	-61.5	-6
R Parahippocampus	4.56	21	-34.5	-10.5
L Middle temporal*	3.85	-58.5	-25.5	-10.5
L Posterior cingulate*	3.14	-6	-46.5	30
L Lingual*	3.27	-16.5	-43.5	-6
MCI > AD				
R Middle temporal*	2.36	55.5	-24	-15

\* $P < 0.05$  False Discovery Rate (FDR) corrected

**Supplementary Table 3: Multiple comparison results of region of interest (ROI)-based voxel-based gray matter volume measurements using Bonferroni correction among HC, MCI, and AD. (Significant results in bold font)**

ROIS for VBM analysis	HC GM volume (ml)	MCI GM volume (ml)	AD GM volume (ml)	Effect size Partial Eta Squared	Bonferroni corrected <i>P</i>		
					MCI versus HC	AD versus HC	AD versus MCI
Superior frontal	26.59±2.07	26.49±3.47	24.85±2.30	0.083	1.000	0.135	0.185
Middle frontal	19.91±1.64	20.02±2.74	18.40±1.96	0.107	1.000	0.094	0.069
Posterior Cingulate	8.65±0.77	8.58±1.23	8.19±1.19	0.035	1.000	0.547	0.798
Superior temporal	14.30±1.21	13.67±1.78	13.03±1.59	0.106	0.582	<b>0.035</b>	0.605
Middle temporal	12.73±1.11	12.68±1.91	11.47±1.45	0.131	1.000	<b>0.034</b>	<b>0.047</b>
Inferior temporal	11.29±1.04	11.03±1.61	10.05±1.33	0.140	1.000	<b>0.015</b>	0.081
Parahippocampus	3.80±0.31	3.63±0.42	3.35±0.42	0.196	0.450	<b>0.001</b>	0.087
Lingual Gyrus	7.29±0.80	7.15±0.76	6.65±0.95	0.099	1.000	0.059	0.208
Fusiform	6.93±0.63	6.73±0.93	6.17±1.04	0.123	1.000	<b>0.024</b>	0.151
Hippocampus	3.81±0.32	3.53±0.58	2.98±0.66	0.300	0.299	<b>&lt;0.001</b>	<b>0.007</b>
Precuneus	6.07±0.71	6.18±1.03	5.63±0.96	0.063	1.000	0.415	0.204
Supramarginal	7.40±0.64	7.51±0.89	6.92±0.94	0.088	1.000	0.225	0.092