**Title:** Correlation of lobar cerebral microbleeds with amyloid, perfusion and metabolism in Alzheimer’s disease

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**Running title:** Lobar microbleeds and Alzheimer’s disease

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NSB, RM, SAS, ANP, JTO and JHG contributed to conception and design of study

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**Abstract:**

**Background:** Despite the well-documented relationship between lobar CMBs (lCMB) and Alzheimer’s disease (AD), there is limited knowledge about the role of lCMB in AD pathology.

**Objective:** To understand the nature of this relationship, we investigated the association between lCMB, amyloid load, perfusion and metabolism.

**Methods:** Participants with AD, mild cognitive impairment (MCI) and healthy controls were recruited and scanned with 11C-Pittsburg-Compound B (PiB), Fluorodeoxyglucose (FDG) PET and susceptibility-weighted MRI. Early PiB-PET frames were used to estimate perfusion. The association between lCMB and PET uptake in each anatomical lobe was measured using multiple regression models.

**Results:** The presence of lCMB predicted increased total (p<0.001) and regional (p=0.0002) PiB uptake, as well as decreased cerebral perfusion (p=0.03). Cases with lCMB had hypometabolism in their temporal lobe (p=0.04).

**Conclusion:** There are significant relationships betweenlCMBs and various markers of AD pathology. lCMB has a spatial association with Aload and a complex effect on perfusion and metabolism.

**Keywords:**

Lobar cerebral microbleeds, Alzheimer’s disease, FDG-PET, Susceptibility weighted imaging (SWI), cerebral perfusion, cerebral metabolism, PiB-PET

**Introduction:**

Patients with Alzheimer’s disease (AD) express various degrees of vascular pathologies including lobar cerebral microbleeds (lCMBs), white matter changes, and microinfarcts. Vascular changes can reduce the threshold of dementia detection and result in accelerated cognitive decline independent of the level of AD pathology [1]. A growing body of evidence shows that the role of vascular pathologies in AD might be more important than what was known previously, and most patients over the age of 80 present with mixed AD and vascular pathologies [2-5]. lCMBs particularly have attracted significant interest in recent years because of similarity in pathogenesis (presence of amyloid (A)in both AD and lCMB) and their potential role in triggering adverse reaction to immunotherapy [6]. Deep CMB on the other hand is related to hypertension and not A pathology.

Patients with AD have a much higher prevalence of lCMB compared to age matched healthy controls (HC), and between 82-98% show deposition of Ain cerebral vessel walls in histopathology studies[7]. Amyloid PET can detect vascular A as well as senile plaques, and patients with multiple lCMBs have shown higher total cortical amyloid PET uptake compared to HC [8, 9]. More recently, it has also been shown that lCMB load correlates with the level of CSF A and tau, suggesting that lCMB could be used as a potential imaging biomarker in AD [10-12].

Despite evidence showing the association between lCMB and AD, we still have a poor understanding about the nature of this relationship, particularly the role of lCMB in the neurodegenerative process and their association with clinical symptoms. To explain the mechanism through which lCMB might affect cognition or behavior, it is important to investigate the relationship between lCMB load and neurodegenerative biomarkers. The association between lCMB and cerebral perfusion in AD is another under-investigated topic in this field. Although perfusion and metabolism in AD are usually considered interchangeable markers, many studies have shown that in some regions and also in early/preclinical AD they do not follow the same pattern [13, 14].

There is also limited knowledge regarding the spatial association between lCMB distribution and regional A deposition in AD.

In this study, we investigated the relationship between lCMB and glucose metabolism measured by 18F-FDG-PET to explore the association between lCMB and neurodegeneration. Also, we explored the association between cerebral perfusion, estimated from early 11 C-Pittsburgh Compound B (PiB) PET images, and lCMB.

We also investigated the spatial correlation between lCMB and total amyloid burden (which includes vascular amyloid and senile plaques), determined from late PiB PET images, in each cerebral lobe to see if lCMB could be an indicator of total amyloid burden in each region. A correlation between lCMB presence/load on susceptibility-weighted imaging (SWI) and PiB-PET could potentially imply a role for lCMB as a surrogate amyloid biomarker, when amyloid PET is not available.

**Materials and Methods:**

Participants: 29 participants, comprising of 10 with AD, 11 with mild cognitive impairment (MCI) and 8 HC were recruited through well-established memory services at Cambridge University Hospitals and a National Registry for Dementia (Join Dementia Research) supported by the National Institute for Health Research (NIHR), UK. Only mild to moderate AD participants, with mini mental state examination (MMSE) > 17, and capacity to consent were included in the study. Ethical approval was obtained from the National Research Ethics Service. After giving written informed consent, all participants were assessed clinically by an expert cognitive neurologist. Participants with significant psychiatric and medical illnesses were excluded. Cognitive function was evaluated using the Addenbrooke’s Cognitive Examination-Revised (ACE-R), which incorporates the MMSE. Cardiovascular (CV) risk factors including hypertension, hypercholesterolemia, diabetes, ischemic heart disease, and stroke/transient ischemic attacks (TIA) were recorded.

PET imaging: 11C-PIB and 18F-FDG PET/CT scans were performed on a GE Discovery 690 (GE Healthcare, Waukesha, WI) either on the same day or within a week from each other at Addenbrooke’s Hospital, Cambridge, UK.

11C-PIB: Immediately following a low dose CT scan for attenuation correction, a median 460.4 MBq 11C-PIB was administered to each participant. Emission data from 1-8 min [15] and 40-70 min[16] post injection were reconstructed into a 192×192×47 matrix with 2.08×2.08×3.27 mm voxels, using 3D-filtered back-projection [17] with a ramp filter cut-off at the Nyquist frequency. Corrections for randoms, dead time, normalization, attenuation, scatter and sensitivity were applied as implemented on the scanner, together with an isotropic 2 mm FWHM Gaussian filter post reconstruction. The CT-AC acquisition parameters were: 120 kV; 50 mA; 0.8 s tube rotation time; pitch of 1.375; slice thickness 3.75 mm reconstructed to 3.27 mm.

18F-FDG: 18F-FDG-PET emission data were acquired 60-90 minutes following injection of a median 189.3 MBq 18F-FDG. Subjects fasted for at least 8 hours prior to scanning. 18F-FDG images were reconstructed using the same parameters as for 11C-PIB.

MRI: SWI and T1-weighted (T1w) images were obtained using a 3T GE Discovery MR750 (GE healthcare, WI) at Addenbrooke’s Hospital, Cambridge, UK.

 T1w images were acquired using a 3D inversion-prepared fast spoiled gradient-echo pulse sequence with these acquisition parameters: echo time (TE) 4.2 ms; repetition time (TR) 9.9 ms; inversion time 450 ms; flip angle 20 degrees; acquisition matrix 352×224×124, field of view (FoV) 22×22 cm; parallel imaging (ASSET) acceleration factor 2.

 SWI images were acquired with a customized pulse sequence using the following acquisition parameters: TR 32ms; with 4 different TEs: 6.5/13.2/20.0/26.7 ms; flip angle 15 degrees; ASSET acceleration factor 2; acquisition matrix 288 × 288 × 110; FoV: 18.7cm × 18.7cm; slice thickness: 1.4 mm resulting in acquired resolution of 0.65mm × 0.65mm x 1.4 mm and reconstructed resolution of 0.48 mm x 0.48 mm × 0.7 mm with a reconstructed matrix of 384 × 384. For TEs 13.2/20.0/26.7 ms, the phase images were high-pass filtered before combination with the magnitude images to produce an SWI image. A combined-echo SWI image was also produced from the average magnitude and filtered-phase images.

PET image analysis: To reduce the impact of head motion during PET acquisition, frames from the PiB and FDG image series were re-aligned with the Advanced Normalization Tools package (<http://picsl.upenn.edu/software/ants>). Early and late phase PIB summed images were generated by averaging the registered emission series from the 1-8 min (e-PiB) and 40-70 min acquisition time windows respectively (l-PiB). For each subject, the mean re-aligned PiB and FDG images were rigidly co-registered to the T1w MRI with mutual information as the similarity metric. To allow the use of standard-space regions-of-interest (ROIs), the T1w MRI of each subject was normalized to the Montreal Neurological Institute (MNI) ICBM152 T1w brain atlas using symmetric normalization (SyN) driven by normalized cross-correlation similarity[18]. The quality of all registrations was carefully assessed by an experienced neuro-radiologist through visual inspection. Standard-space ROIs corresponding to the Microbleed Anatomical Rating Scale (MARS) were realized by combining regions identified in the Hammers atlas (<http://www.brain-development.org>) [19] (Figure 1). Grey matter (GM) from the following regions was analyzed: left and right frontal, temporal, parietal and occipital lobes, and insula. Regions for the anterior and posterior cingulate, averaged over the left and right cerebral hemispheres, cerebellar cortex and vermis were also included in the analysis. Transformation of the template ROIs to each participant’s T1w MRI was subsequently performed using the inverse of the transformation calculated for spatial normalization. GM radioactivity concentration within all ROIs was estimated using linear regression of the PET radioactivity concentration as a function of voxel-wise GM probability using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>) probability maps. e-PiB and 18F-FDG PET values were quantified by SUVR through normalization of the GM signal in each lobar region by the mean radioactivity in the cerebellar vermis[20, 21]. l-PiB SUVR values were obtained for the same ROIs by normalization to the mean radioactivity concentration in the cerebellar cortex.

SWI images were assessed by neuroradiologist with 10 years experience in reading SWI/Gradient echo images. The number and size of the microbleeds in each anatomical region were measured using the validated MARS scale [22]. Images from three echo times (TE) were assessed separately in addition to the combined echo images for better delineation of CMB since lower echo SWI has better signal-to-noise ratio while higher echo images had more pronounced susceptibility effects. Subjects with >1 CMB in any lobar distribution were considered CMB-positive (CMB+). In addition, we classified each of the 5 anatomical regions of cerebral hemispheres derived from MARS scale (frontal, temporal, parietal, occipital and insula regions) individually into CMB+/- based on presence of lCMB in that region.

Statistical analysis: JMP pro V13 was used for analysis. The association between dichotomized CMB and both early and late PiB and also FDG PET SUVR were assessed using multiple regression modelling adjusting for sex, age, education, and all above-mentioned CV risk factors in univariate models and consequently multivariate containing only significant factors. t-tests or non-parametric tests were used for comparison between two groups, Kruskal-Wallis and ANOVA was used when more than two groups were compared and chi-squared tests where nominal variables were compared. For correlation between two PET SUVRs we used Pearson correlation.

**Results:**

Demographic data, cognitive assessment results, and frequency of CV risk factors are summarized in table 1. There was no significant difference in sex, age, and level of education across three groups. The number of cases with lCMB in each group and prevalence of lCMB in each anatomical region are shown in table 2. Prevalence of lCMB was significantly different across three groups (p=0.009). Bonferroni corrected between group comparisons showed lCMBs were more frequent in AD compared to MCI (p=0.01) and control participants (p=0.03).

In univariate logistic regressions, there was no association between the presence of lCMB and age, sex, education, or any of the CV risk factors. Clinical diagnosis of AD, however, was significantly associated with increased odds of lCMB compared to HC (OR: 4.2, CI: 1.1-16.2, p=0.03) and MCI (OR: 10.0, CI: 2.4-41.2, p=0.001).

1. Lobar CMB and l-PiB:

Comparing the mean of l-PiB SUVRs between CMB+ and CMB– cases (including all 5 anatomical regions) showed a significant increase in amyloid uptake in CMB+ participants (p<0.001)(Figure 2A). When each anatomical region was investigated individually, CMB+ subjects had higher l-PiB SUVR in the frontal (p=0.03) and parietal (p=0.03) lobes compared to CMB– individuals.

Multiple regression analyses investigating the association between the presence of lCMB in whole brain and total cortical l-PiB SUVR, adjusting for significant covariates showed association between the two (estimate= 6.7, p<0.001). More importantly, there was a significant association between presence of lCMB in each anatomical region and its corresponding cortical l-PiB SUVRs (estimate= 0.3, p=0.0002).

1. Lobar CMB and e-PiB:

e-PiB SUVR was lower in the CMB+ group compared to CMB– group (p=0.03). Analyzing each region individually showed a significant reduction in e-PiB SUVR in the temporal lobe between CMB+ and CMB– groups (p=0.01). In addition, comparing e-PiB SUVR between CMB+ and CMB– individuals in each clinical diagnostic group showed that in the AD group, CMB+ subjects had significantly lower e-PiB compared to CMB– cases (p= 0.03). In the MCI group, however, the result was reversed, and CMB+ participants had higher e-PiB SUVR than CMB– participants (p=0.02) (Figure 2B).

In a multiple regression model, there was significant association between e-PiB SUVR and presence of lCMB after adjusting for clinical diagnosis and l-PiB SUVR (estimate=– 0.04, p=0.04).

1. Lobar CMB and FDG-SUVR:

Overall, cases with lCMB in the temporal lobe had lower FDG SUVR in that region compared to CMB– subjects (p=0.04). Comparing FDG SUVR between CMB+ and CMB– groups in each clinical diagnosis demonstrated a significant difference only in the MCI group (p=0.03), with CMB+ subjects showing increased FDG uptake compared to CMB- ones (Figure 2C).

However, in a multiple regression model, the association between FDG SUVR and presence of lCMB was not significant (estimate= – 0.02, p=0.2).

1. e-PiB and FDG SUVR:

There was a significant correlation between e-PiB and FDG SUVRs (p<0.0001, R2 = 0.36). When cases with and without lCMB were assessed independently, the correlation was numerically stronger in CMB– (R2 =0.49, p<0.0001) compared to CMB+ (R2 =0.20, p=0.01).

1. Lobar CMB and Cognitive function:

Our data showed that both MMSE (p=0.003) and ACE-R (p=0.006) were significantly lower in the CMB+ group. Furthermore, there were significant associations between presence of CMB and both MMSE (estimate = –2.0, p= 0.002) and ACE-R (estimate= –7.2, p=0.005) in multiple regression models adjusting for FDG SUVR and e-PiB and SUVR.

**Discussion:**

In this cross-sectional study, we investigated the association between lCMB and PET markers of metabolism, perfusion and A load, provided by FDG, early and late PiB SUVR respectively. We found a significant association between lobar CMB and both total Aload detected by PET, and Aload in each anatomical region. Moreover, our data showed that lCMB had a different relationship with brain perfusion and metabolism at various stages of the disease, which could help provide some insight into the role of CMB in neurodegeneration, as well as potentially explain some of the conflicting results on early hyperperfusion and the association between perfusion and metabolism in AD.

Prevalence of lCMB in our AD cohort was 82%, which is in keeping with recent reports indicating prevalence of lCMB in AD in the range of 68-86% [10, 12]. Using high resolution SWI (instead of conventional GRE/T2\*) with higher field strength MRI may explain the increased detection rate of lCMB in recent MRI studies, which is closer to the prevalence of microbleeds in pathology reports [7].

Our data showed that presence of lCMB was significantly associated with higher total cortical l-PiB SUVR independent of clinical diagnosis. Histopathology studies, though with modest sample sizes, have demonstrated that PiB-PET can bind to vascular A (mainly A40) in addition to A neuritic and diffuse plaques (mainly A42)[23-25]. Consequently, many studies focused on the diagnostic role of amyloid PET in CAA and most recruited non-demented CAA cohorts. These studies suggest that total cortical amyloid uptake is higher in subjects with CAA compared to HC, but lower than in individuals with AD [8, 9].

Our data, however, demonstrated that even among AD and MCI groups, the presence of lCMB is associated with increased Aβ burden as measured by l-PiB SUVR. More importantly, we found a significant spatial association between lobar CMB in each anatomical region and l-PiB uptake in the same area independent of the other possible confounding factors, such as clinical diagnosis, age, sex, and CV risk factors. Previous studies have shown high l-PiB uptake in foci of CMB in co-registered PET/MRI images in cases of CAA without dementia [26], and an association between total cortical l-PiB SUVR and the presence of lCMB in HC [27]. The latter study also assessed the regional PiB uptake in non-demented cases and found a relationship between lobar CMB and SUVR only in parieto-occipital regions, but not in frontal areas [27]. In a recent study investigating CMB and the distribution of amyloid burden in ICH, participants with lobar CMB had higher global and lobar amyloid uptake (frontal, parietal, temporal and occipital lobes) in comparison with subjects with mixed and deep CMB [28]. None of these studies, however, investigated the spatial association between presence of lobar CMB in each anatomical region and l-PiB uptake in the same area in AD and MCI.

The strong association between CMB and l-PiB uptake in each anatomical region suggests that vascular Apresenting as lobar CMB could potentially be a marker of total A burden (vascular and plaques). The higher l-PiB SUVR observed in the frontal and parietal lobe (regions known to have higher A plaque burden) of subjects with CMB compared to those without further corroborates this idea.

Utilizing early frames of PiB PET (1-8 min or 1-6 min) to estimate brain perfusion has been reported in many studies [15, 29, 30]. Our data showed that CMB+ participants had significantly lower perfusion in the whole brain and also in the temporal lobe compared to CMB– subjects. This was independent of their clinical diagnosis. In addition, we found a significant association between perfusion estimates and the presence of lCMB in a regression model adjusted for all cofounding factors including l-PIB SUVR.

Although previous studies have shown hypoperfusion in CAA measured by either e-PiB PET [20] or SPECT [31], very few studies have investigated the association between CMB and perfusion in cases with a diagnosis of MCI or AD. Farid et al reported whole cortex hypoperfusion in CAA cases compared to HC and a different pattern of lobar hypoperfusion in participants with CAA (mainly occipital) compared to cases with AD (mainly PCG). The cohort, however, was chosen in a way that none of the CAA cases had cognitive impairment and none of the AD cases had CMB on T2\* images [20]. Another study has also reported hypoperfusion in temporal and parietal regions in subjects with CAA compared to HC [31]; nevertheless, CAA participants in this study had mixed pathologies and different levels of cognitive function. In a more recent study on transgenic mice, vascular amyloidosis was found to be a major contributing factor to regional cerebral blood flow (rCBF) reduction. Using PET and multiparametric MRI in a longitudinal study, the authors demonstrated that the association between the presence of Aplaques and rCBF is mediated by vascular amyloidosis [32]. These results are in line with the findings of our study.

We also studied the association between presence of CMB and perfusion in each clinical diagnostic group. In the MCI group, CMB+ regions appeared to have higher perfusion but the reverse pattern was observed in AD.

Independent of vascular status or knowledge about the presence of CMB, a number of studies have shown hypoperfusion in AD cases compared to HC and MCI. Studies investigating the pattern of perfusion in MCI, on the other hand, have reported both hypo and hyperperfusion [33-35]. Previous reports have suggested that hyperperfusion precedes hypoperfusion during the course of the disease from preclinical stage to MCI to AD [36, 37]. This phenomenon can be explained by the capillary dysfunction theory, which posits that an increase in the capillary transit time heterogeneity at very early stage of the disease increases CBF to maintain tissue oxygenation. Based on this theory, once this compensatory mechanism is exhausted, the same process will act toward reducing CBF [38]. According to this model, any change in blood velocity, vessel wall deformity or loss of integrity could affect capillary transit time heterogeneity. Nevertheless, there is only very limited data on the effect of different vascular pathologies on perfusion in MCI and early AD. Very recently the role of white matter hyperintensities – one of the three main AD-related vascular pathologies – on perfusion was investigated, and results showed that the presence of white matter changes is positively correlated with capillary transit time heterogeneity in AD [39].

To our knowledge, there is no data in the literature on the association between lCMB and perfusion at different stages of AD. lCMB can increase capillary transit time heterogeneity as deposition of A in the wall of arteries or capillaries, affects vessel wall integrity, induces a local inflammatory reaction and alters the lumen diameter [40]. Therefore, the capillary dysfunction theory can also potentially explain our results, which indicate that the presence of CMB is associated with an increase in 11C-PiB PET-derived perfusion estimates during MCI and decrease in perfusion at later stages (dementia).

We found the effect of CMB on metabolism was partly similar to its effect on perfusion. Overall, CMB+ participants showed hypometabolism in the temporal lobe, and MCI cases with lCMB had higher FDG uptake in comparison to MCI without lCMB. Only one other clinical study has investigated the effect of lCMB on metabolism in AD, and reported similar hypometabolism patterns in the temporal lobe in CMB+ subjects [41]. Prior to this report, Merlini et al also suggested that the presence of CAA can impair brain metabolism in a study on transgenic mice [42].

There are different pathways through which CMB could alter metabolism in AD. Inflammatory processes at the site of CMB can directly cause local neuronal injury in the surrounding tissue and ultimately contribute to hypometabolism. However, areas of hypometabolism are usually more extensive than the exact site of CMBs. It has been suggested that CMB could also indirectly affect metabolism through the clearance mechanism. Deposition of Ain the vessel wall and failure of perivascular drainage canreduce the clearance of Aand other toxic metabolites from interstitial fluid, altering the neuronal environment and consequently resulting in neurodegeneration [43]. In addition, given the high association between perfusion and metabolism, it is believed that CMB could also indirectly impact neuronal injury and metabolism through capillary perfusion and widespread ischemia [41, 44].

Hypermetabolism in CMB+ MCI can be partially explained by the increased perfusion in those cases. It can also be justified by the brain reserve theory [45] in which MCI cases with lobar CMB (or higher Aload) might have higher basal cerebral metabolism at earlier stage, compared to MCI cases with no CMB.

Although FDG SUVR was lower in CMB+ AD individuals compared to CMB– AD in our study, the difference did not reach statistical significance. This could be explained by the fact that our AD cases were mild and at early stage of the disease (all had MMSE > 17), hence there was significant reduction in perfusion but not in metabolism. Our modest sample size could also be another contributing factor.

Our data confirmed that CMB has a significant association with cognitive decline even after adjusting for the effect of all other confounding factors. The role of CMB on cognition is most likely through the above-mentioned pathways affecting perfusion and metabolism.

In line with previous published results, we found significant correlation between e-PiB and FDG SUVR. For the first time, we also investigated the role of lobar CMB on this relationship and found that the presence of lCMB could weaken the correlation between perfusion and metabolism. Moreover, in AD subjects with lCMB, perfusion was decreased compared to CMB– AD cases, while metabolism was not significantly different between these two groups.

According to previous studies there is a close relationship between CBF and FDG-PET in the healthy brain except when vascular coupling is compromised, such as in stroke or other vascular pathologies [46, 47]. Our data suggests that presence of lCMB could also have the same effect in the later stage of the disease, either through vascular dysfunctioning or increase in AD pathology, which might explain the discrepancy between these two measures in some studies. Based on these results, though needed to be confirmed in future studies, it is very crucial to know the status of vascular pathology, particularly presence of lCMB in AD cases, before using perfusion and metabolism imaging techniques interchangeably.

The limitation of our study is the modest sample size. Our results should be confirmed in future studies with larger sample size.

In summary, our data suggested that lCMBs could have extensive and significant role in AD pathology. In addition to the high spatial association with Aload on PiB PET, lCMB may affect perfusion and metabolism via complex mechanisms at different stages of the disease, potentially expediting the process of neurodegeneration and cognitive decline.

**Acknowledgements**

This work was funded by the National Institute for Health Research (NIHR)-Cambridge Biomedical Research Council (BRC)\*\*. We thank the DeNDRoN research network for help in recruitment; Sarah Hillborne, Jackie Mason, and the radiographers in the MRIS and PET/CT Units in Addenbrooke’s Hospital for their help in recruitment and imaging.

\*\*The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

**Conflict of interest/Disclosure:**

The authors have no potential conflict of interest to report

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**Tables:**

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | HC | MCI | AD |
| No (female) | 8(6) | 10(2) | 11(2) |
| Age + SD | 74.1 + 5.3 | 73.5 + 7.6 | 76.6 + 8.8 |
| Education ± SD  | 17 ± 3.2 | 16.8 ± 8.2 | 15.3 ± 5.5 |
| MMSE ± SD | 29.2 ± 1.1 | 27.4 ± 1.4 | 22.6 ± 3.5\*, † |
| ACE-R ± SD | 97 ± 3.9 | 84.5 ± 3.7‡ | 68.3 ± 11.1\*, † |
| Hypertension (%)  | 1/8 (13) | 5/10(50) | 3/11(27) |
| Hypercholesterolemia (%)  | 3/8 (38) | 4/10 (40) | 4/11(36) |
|  Ischemic heart disease (%)  | 0/8 (0) | 2/10 (20) | 1/11(9) |
| Stroke/TIA (%) | 1/8 (12) | 1/10 (10) | 3/11(27) |

Table 1: Sex, age, education, cognitive assessment tests and prevalence of cardiovascular risk factors across three groups. There was no significant difference in sex, age, and level of education across three groups. \* : significant difference between AD and HC (p<0.0001); † : significant difference between AD and MCI (p < 0.0004); ‡ : significant difference between MCI and HC (p= 0.005).

|  |  |  |  |
| --- | --- | --- | --- |
| Group | HC | MCI | AD |
| LCMB+ (%) | 2/8 (25%)\* | 2/10 (20%)† | 9/11 (82%)  |
| No cases with CMB +  | Frontal | 0 | 0 | 5 ‡ |
| Temporal  | 1  | 0  | 6 ‡ |
| Parietal  | 2  | 2 | 5 |
| Occipital  | 0 | 1 | 5 ‡ |
| Insula  | 1  | 0 | 3 |

Table 2: Number (percentage) of cases with lCMB in the whole brain and in each anatomical region across three diagnostic groups. \* : significant difference between HC and AD (p=0.01); † : significant difference between MCI and AD (p=0.004); ‡ : significant differences between AD and both MCI & HC (p<0.01).

**Figures’ legends:**

**Figure 1:** Susceptibility-weighted-image (SWI) (A) with multiple lobar CMBs (white arrows) in left and right frontal and parietal lobes in an AD case. Fludeoxyglucose (FDG)- (B) and Pittsburgh compound B (PiB)-PET (C) in the same individual showed hypometabolism in the parietal regions and widespread amyloid deposition in the frontal, parietal lobes.

**Figure 2:** Levels of late Pittsburgh compound B (l-PiB)(A), early PiB (e-PiB)(B) and Fludeoxyglucose (FDG) SUVRs (C) in AD and Mild cognitive impairment (MCI) participants with and without cerebral microbleeds (CMB). \* : p<0.05, \*\* : p<0.01