Chapter 3.1

Brain volume and white matter hyperintensities as determinants of cerebral blood flow in Alzheimer's disease

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Abstract
To better understand whether decreased cerebral blood flow (CBF) in patients with Alzheimer’s disease (AD) reflects neurodegeneration or cerebral small vessel disease (SVD), we investigated the associations of normalized brain volume (NBV) and white matter hyperintensity (WMH) volume with CBF. We included 129 patients with AD (66±7 years, 53% female) and 61 age-matched controls (64±5 years, 43% female). CBF was measured with pseudo-continuous arterial spin labelling (pcASL) at 3T in whole brain and partial volume corrected (PVC) cortical maps. When NBV and WMH were simultaneously entered in age and sex adjusted models, smaller NBV was associated with lower whole brain (Stβ: 0.29; p<0.01) and cortical CBF (Stβ: 0.28; p<0.01) in patients with AD. Larger WMH volume was also associated with lower whole brain (Stβ:-0.22; p<0.05) and cortical CBF (Stβ: -0.24; p<0.05) in AD. Additional adjustments did not change these results. In controls, neither NBV nor WMH was associated with CBF. Our results indicate that in AD, lower CBF as measured using pcASL, reflects the combined disease burden of both neurodegeneration and SVD.
Introduction

Alzheimer’s disease (AD) is essentially regarded as a neurodegenerative disease, characterized by the accumulation of amyloid plaques and neurofibrillary tangles, that eventually leads to brain atrophy.\(^1\) Increasing evidence indicates that AD patient not only have brain volume loss, but also often have an altered cerebral blood flow (CBF). Although some studies report relative regional increases in (early) AD,\(^2,3\) the most consistent finding is a decrease in absolute CBF in patients with AD.\(^4-6\) This decreased CBF is in general assumed to be a reflection of the neurodegenerative process.\(^7\)

Lower CBF in AD patients may not only relate to neurodegeneration, but may also be associated with small vessel disease (SVD). White matter hyperintensities (WMH) of presumed vascular origin are a commonly used MRI marker to indicate the presence of SVD.\(^8\) WMH are assumed to result from ischaemia and they are more prevalent in AD patients compared to the general elderly population.\(^9\) Previous studies have shown that WMH are associated with lower CBF as well.\(^10-12\)

CBF can be measured by arterial spin labelling (ASL); a functional MRI technique that uses magnetically labelled arterial blood water as an endogenous tracer.\(^13\) The pseudo-continuous variant of ASL (pcASL) uses a multitude of millisecond-long pulses in order to achieve a high labelling efficiency and effective compensation of magnetization transfer effects.\(^14\)

Neurodegeneration and SVD are common in patients with AD, but to our knowledge, no previous studies have investigated how both processes relate to the generally described decreased CBF. Our aim was to explore whether independent relationships exist between normalized brain volume (NBV) or WMH on the one hand and CBF on the other hand. We hypothesized that the lower CBF in AD is not only reflective of the neurodegenerative process, but that CBF may be even further decreased when SVD is present. The well-characterized Amsterdam Dementia Cohort with pcASL measurement allowed us to investigate the determinants of CBF in AD patients and controls.

Methods

Subjects

Subjects for this study were drawn from the memory clinic based Amsterdam Dementia Cohort. We included 129 AD patients and 61 age-matched controls who visited our memory clinic between October 2010-June 2012. All subjects underwent an extensive dementia screening, including medical history, neurological and physical examination, cognitive assessment, and brain MRI. The diagnosis ‘probable AD’ was made according to
the NINCDS-ADRDA criteria, by consensus of a multidisciplinary team and all patients fulfilled the core clinical criteria of the NIA-AA.\textsuperscript{15,16} The control group consisted of age-matched control subjects, who presented with cognitive complaints, but for whom clinical investigations were normal and criteria for mild cognitive impairment,\textsuperscript{47} dementia, or any other neurologic or psychiatric disorder were not met. As subjective complaints may represent preclinical AD in a subgroup,\textsuperscript{48} we only included subjects with normal cerebrospinal fluid (CSF) Aβ\textsubscript{42} levels (see Mulder et al\textsuperscript{19} for a detailed description of CSF analyses). For all subjects, presence of hypertension, hypercholesterolaemia, and diabetes mellitus were determined based on self-reported medical history and medication use. Smoking status was defined as never, former or current. Blood pressures were measured manually using a sphygmomanometer. Exclusion criteria were the presence of structural brain lesions and failure of pre-processing of the MRI scans. The ethical review board of the VU University Medical Center approved the study. We obtained informed consent from all patients to use their clinical data for research purposes.

MRI protocol
MRI of the brain was acquired on a 3T whole body MR system (Signa, HDxt, General Electric Medical Systems, Milwaukee, WI, USA), using an 8-channel phased-array head coil. The MRI protocol included a sagittal 3D T1-weighted sequence (IR-FSPGR, repetition time [TR]: 7.8ms, echo time [TE]: 3ms, inversion time [TI]: 450ms, flip angle: 12°, voxel size: 1.0x0.9x0.9mm); a sagittal 3D fluid-attenuated inversion-recovery (FLAIR: TR: 8000ms, TE: 123.6ms, TI: 2350ms, voxel size: 1.0x1.0x1.0mm) an axial 2D T2* gradient-echo with an echo-planar read-out (EPI: TR: 5300ms, TE: 25ms, voxel size: 1.0x0.5x0.5mm); and an axial 2D proton density/T2-weighted fast spin echo (PD-T2: TE: 8680ms, voxel size: 1.0x0.5x0.5mm). pcASL perfusion images (3D-FSE acquisition with background suppression, post-label delay: 2.0s, TR: 4.8s, TE: 9ms, spiral readout: 8 arms x 512 samples; voxel size: 1.0x1.7x1.7mm) were calculated using a single compartment model\textsuperscript{26} after the subtraction of labelled images from control images. Binnewijzend et al.\textsuperscript{6} provides a more detailed description of the ASL sequence.

PcASL cerebral blood flow measures
After correcting T1-weighted and pcASL images for gradient non-linearities in all three directions, data-analyses were carried out using FSL (version 4.1.9; http://www.fmrib.ox.ac.uk/fsl). Pre-processing of T1-weighted images consisted of removal of non-brain tissue,\textsuperscript{21} linear registration to standard space\textsuperscript{22} and tissue segmentation\textsuperscript{23} yielding partial volume estimates. PcASL images were linearly registered to the brain-extracted T1-weighted images. Partial volume estimates were transformed to the ASL data space and used in a regression algorithm\textsuperscript{24} using a Gaussian kernel of
9.5mm full width at half maximum, to create partial volume corrected (PVC) cortical CBF maps. Mean whole brain CBF was calculated using the segmented brain mask. Mean cortical CBF was calculated using the partial volume estimates as a weighting factor. CBF was defined in ml/100gr/min.

**Normalized brain volumes**

NBV (in millilitre, ml) was estimated with the SIENAX software tool, part of FSL, using optimized brain extraction tool (BET) options as described previously. In order to avoid lesion-associated segmentation biases, prior to segmentation lesions were filled with intensities of the normal appearing white matter using the automated lesion-filling technique LEAP.

**White matter hyperintensities**

WMH were segmented using a locally developed k Nearest Neighbour algorithm based on previous work. In short, this algorithm uses FLAIR and T1 tissue intensity, spatial information, and tissue priors to compare the brain voxels of a newly presented dataset to a collection of manually labelled examples in a feature space. Based on the most similar examples, the probability of a voxel being a lesion is computed and thresholded to obtain a binary lesion segmentation. Importantly, the training set for automated lesion segmentation was generated on images acquired with the same scanner and pulse sequences as those in the present study. All segmentations were visually inspected. WMH volumes (ml) were normalized for head size by multiplying the volumes by a scaling factor, derived from the SIENAX estimation.

**Other MRI measures**

Left and right hippocampal volumes (ml) were quantified using FSL FIRST (FMRIBs Integrated registration and segmentation tool). All segmentations were visually inspected. Hippocampal volumes were normalized for head size by multiplying the volumes by the SIENAX derived scaling factor. For analytical purposes, left and right hippocampal volumes were summed. Cerebral microbleeds were visually assessed and defined as small round foci of hypointense signal, up to 10mm in brain parenchyma on T2*-weighted images. Microbleed count was dichotomized as present or absent. Lacunes (of presumed vascular origin) were defined as deep lesions (3-15mm), with CSF-like signal on all sequences; they were scored as present or absent.
Data analysis
Statistical analyses were performed using SPSS (version 20; SPSS, Chicago, Ill., USA). As WMH volumes were not normally distributed, we used log-transformed values. Differences in baseline characteristics between groups were investigated with student t-test for continuous variables and $\chi^2$ test for dichotomous variables. Differences in CBF between groups were analysed using one-way ANOVA, corrected for age and sex. Linear regression analysis was carried out to investigate the associations of NBV and WMH (independent) with CBF (dependent). All models were adjusted for age and sex. In model I we investigated the univariate associations of NBV or WMH with CBF. In model II, NBV and WMH were simultaneously entered. Model III consisted of model II, with additional adjustment for hippocampal volume, microbleed presence and lacune presence. Finally, we repeated the analyses with additional adjustment for hypertension, hypercholesterolaemia, diabetes mellitus, current smoking, and systolic and diastolic blood pressure. Linear regression analyses were stratified for diagnosis, to estimate the effects for controls and AD patients separately.

Results
Table 1 gives the patient characteristics by group, showing effective matching for age and sex. As expected, AD patients had a lower MMSE score than controls ($p<0.01$). Vascular risk factors were comparable for AD patients and controls. AD patients had smaller NBVs, smaller hippocampal volumes, and larger WMH volumes compared to controls (all $p<0.01$). There were no differences in the prevalence of microbleeds or lacunes. Whole brain CBF (ml/100gr/min) was lower in AD patients compared to controls ($27.3\pm5.8$ vs. $31.5\pm5.3$, $p<0.01$). AD patients also had a lower PVC cortical CBF compared to controls ($41.8\pm9.2$ vs. $47.0\pm7.8$, $p<0.01$).

Table 2 gives the associations of NBV and WMH with CBF by group. In AD patients, age and sex adjusted models (model I) showed that smaller NBV was associated with lower whole brain CBF ($St\beta 0.28$; $p<0.01$) and PVC cortical CBF ($St\beta 0.27$; $p<0.01$). In addition, in AD patients, larger WMH volume was associated with lower whole brain CBF ($St\beta -0.21$; $p<0.05$) and PVC cortical CBF ($St\beta -0.23$; $p<0.05$). These results remained essentially unchanged when NBV and WMH were simultaneously entered (model II), or when additional adjustment for MRI measures was performed (model III). Repeating the analyses with adjustment for vascular risk factors did not change these results (data not shown). Examples of whole brain CBF maps of four AD patients with different grades of atrophy and WMH are shown in figure 1.
### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls N= 61</th>
<th>AD patients N=129</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>64±5</td>
<td>66±7</td>
</tr>
<tr>
<td>Sex, % female</td>
<td>26 (43%)</td>
<td>69 (53%)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28±2</td>
<td>21±5*</td>
</tr>
<tr>
<td><strong>Vascular risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (23%)</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>5 (8%)</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>4 (7%)</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>26 (44%)</td>
<td>60 (48%)</td>
</tr>
<tr>
<td>Former</td>
<td>28 (48%)</td>
<td>46 (37%)</td>
</tr>
<tr>
<td>Current</td>
<td>5 (8%)</td>
<td>20 (16%)</td>
</tr>
<tr>
<td>Systolic BP, mmHG</td>
<td>141±19</td>
<td>144±19</td>
</tr>
<tr>
<td>Diastolic BP, mmHG</td>
<td>85±11</td>
<td>88±11</td>
</tr>
<tr>
<td><strong>MRI characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized brain volume, mL</td>
<td>1424.3±81.3</td>
<td>1368.5±72.3*</td>
</tr>
<tr>
<td>Hippocampal volume (left &amp; right), mL</td>
<td>9.9±1.1</td>
<td>8.7±1.2*</td>
</tr>
<tr>
<td>WMH volume, median (inter-quartile range), mL</td>
<td>4.8 (3.7;7.5)</td>
<td>10.9 (6.7;19.8)*</td>
</tr>
<tr>
<td>Microbleed presence</td>
<td>13 (21%)</td>
<td>40 (32%)</td>
</tr>
<tr>
<td>Lacune presence</td>
<td>3 (5%)</td>
<td>7 (5%)</td>
</tr>
</tbody>
</table>


Data are represented as mean±SD, number of patients with variable present (%), or median (inter-quartile range). Group comparisons used Student t-test for continuous variables and χ²-test for categorial variables.


*: p<0.01.

In controls, NBV was not associated with whole brain (Stβ -0.07; n.s.) or PVC cortical CBF (Stβ -0.09; n.s.) in age and sex adjusted models. These results remained essentially unchanged when NBV and WMH were simultaneously entered (model II), or after additional adjustment for MRI measures (model III) or vascular risk factors (data not shown). Similarly, we found no association between WMH volume and whole brain CBF (Stβ -0.20; n.s.) or PVC cortical CBF (Stβ -0.22; n.s.) in controls.
These results did not change after additional adjustments in model II and model III. Repeating the analyses with adjustment for vascular risk factors did also not alter these results (data not shown). Figure 2 shows the association of NBV with whole brain CBF and the association of WMH with whole brain CBF by group.

### Table 2. Associations of normalized brain volumes and white matter hyperintensities with cerebral blood flow

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBF (ml/100gr/min)</td>
<td>CBF (ml/100gr/min)</td>
</tr>
<tr>
<td></td>
<td>Uncorrected whole brain</td>
<td>Uncorrected whole brain</td>
</tr>
<tr>
<td></td>
<td>PVC cortical</td>
<td>PVC cortical</td>
</tr>
<tr>
<td><strong>Model I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBV</td>
<td>-0.07</td>
<td>0.28**</td>
</tr>
<tr>
<td>WMH</td>
<td>-0.20</td>
<td>0.27**</td>
</tr>
<tr>
<td><strong>Model II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBV</td>
<td>-0.03</td>
<td>0.29**</td>
</tr>
<tr>
<td>WMH</td>
<td>-0.20</td>
<td>0.28**</td>
</tr>
<tr>
<td><strong>Model III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBV</td>
<td>-0.04</td>
<td>0.28**</td>
</tr>
<tr>
<td>WMH</td>
<td>-0.18</td>
<td>0.27**</td>
</tr>
</tbody>
</table>


Standardized regression coefficients are displayed to allow for direct comparison of each variable’s contribution.

Model I: NBV or WMH univariate; adjusted for age and sex.
Model II: NBV and WMH simultaneously; adjusted for age and sex.
Model III: additional adjustment for hippocampal volume, presence of microbleeds, and presence of lacunes.

**: p<0.01; *: p<0.05.

### Discussion

In the present paper we combined quantitative measurement of NBV and WMH volume with quantification of CBF as measured using pcASL. In AD patients, smaller NBVs and larger WMH volumes were both, independently, associated with a lower CBF. This indicates that CBF as measured using pcASL may be a final common pathway that reflects total disease burden in patients with AD.
Figure 1. Examples of Flair scans and uncorrected whole brain cerebral blood flow maps of four patients with Alzheimer’s disease with different degrees of atrophy and white matter hyperintensities. Abbreviations: CBF: cerebral blood flow; WMH: white matter hyperintensities; MMSE: Mini mental state examination.

To our knowledge, we are the first to investigate associations of NBV and WMH with CBF as measured using pcASL in a well-characterized set of AD patients and controls. In the present study our control group consisted of subjects who presented with subjective complaints at our memory clinic. This may be considered a limitation, as it has previously been shown that the presence of subjective memory complaints may predict incident AD. However, we only included subjects with normal CSF Aβ42, thereby limiting the change to have included subjects with preclinical AD. Another limitation is that we could not check the reliability of our CBF measurement in white matter. The reliability of measuring CBF in white matter is still being debated and the method that we used did not allow the performance of statistics to check measurement reliability. We were therefore not able to investigate white matter CBF. Moreover, a longitudinal design could have given more insight into the still largely unknown order in which neurodegeneration, SVD and decreased CBF occur.
In AD patients, we found that smaller NBVs and larger WMH volumes were both associated with lower CBF. We did not only find an association for NBV with whole brain CBF, but also with PVC cortical CBF, in which errors that have been induced by atrophy have been accounted for.\textsuperscript{24} To our knowledge, relatively little research has been performed on the determinants of decreased CBF in AD patients. In a previous perfusion weighted imaging (PWI) report, regional brain volume changes and regional CBF decreases appeared to be dissociated in early AD, suggesting different underlying pathogenetic mechanisms.\textsuperscript{33} Findings regarding an association between WMH volume and decreased CBF in AD are not straightforward. Previous ASL\textsuperscript{34} and positron emission tomography (PET)\textsuperscript{11} studies did not find an association between WMH and CBF in AD, whereas a single photon emission computed tomography (SPECT) study did find a decreased regional CBF in AD patients with WMH.\textsuperscript{35} The use of various methods limits comparison with previous reports, but our results suggest that NBV and WMH are both, independently, associated with pcASL-measured whole brain and cortical CBF in AD patients.
The independency of the associations that we found in AD patients indicates that in the presence of severe neurodegeneration, CBF is even lower when additional SVD is present. As we previously showed that CBF was associated with cognition in patients with AD, our findings have clinical relevance and may have several implications. In the first place, this study again underlines the importance of the prevention and treatment of modifiable risk factors for vascular disease in AD patients. In addition, efforts to improve CBF, for instance by means of exercise, may have beneficial effects. Most importantly, however, pcASL may provide a new measure for total disease burden in AD. Accumulating evidence suggests that cerebrovascular pathology interacts with AD pathology, not only affecting the risk of AD, but also its course and cognitive symptoms. The exact mechanisms are, however, still not well-understood. CBF measured with pcASL may be a final common pathway, that reflects the cumulative burden of neurodegeneration and SVD in patients with AD.

Contrary to our findings in AD patients, we found no associations between NBV or WMH with CBF in controls. To our knowledge no previous literature exists on the association of brain volume with CBF in healthy elderly. In patients that suffer from vascular pathology, however, total brain volume or atrophy were not found to be associated with whole brain CBF. Previous reports on the association between WMH and CBF in healthy elderly are not straightforward. Vernooij et al did report an association between lower perfusion and larger WMH volume in the general elderly population using phase-contrast MRI. Using ASL, however, Schuff et al did not find an association within their (small) group of controls. Our results suggest that in healthy elderly, variability in pcASL-measured CBF reflects normal variation, which is not determined by brain volume or the burden of SVD.

Overall our results indicate that independent processes contribute to a decreased CBF. We conclude that CBF as measured using pcASL may provide a bridge between neurodegeneration and SVD and offers opportunities for future research regarding both pathological processes in AD.

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References


