Is cerebral glucose metabolism related to blood–brain barrier dysfunction and intrathecal IgG synthesis in Alzheimer disease? A $^{18}$F-FDG PET/CT study

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Abstract
The aim of this study was to investigate the relationships between blood–brain barrier (BBB) dysfunction, intrathecal IgG synthesis, and brain glucose consumption as detectable by means of serum/cerebrospinal fluid (CSF) albumin index ($Q_{alb}$) and IgG index ($[\text{CSF IgG/serum IgG}] \times [\text{Serum albumin/CSF albumin}]$) and 2-deoxy-2-($^{18}$F) fluoro-D-glucose ($^{18}$F-FDG) positron emission tomography/computed tomography (PET/CT) in a selected population affected by Alzheimer disease (AD). The study included 134 newly diagnosed AD patients according to the NINCDS-ADRDA criteria. The mean (±SD) age of the patients was 70 (±6) years; 60 were male and 64 were female. Mini mental State Examination was equal to 18.9 (±7.2). All patients underwent a CSF assay and magnetic resonance before $^{18}$F-FDG PET scanning. The relationships were evaluated by means of statistical parametric mapping (SPM8). We found a significant negative correlation between the increase of $Q_{alb}$ and $^{18}$F-FDG uptake in the Brodmann Area 42 and 22 that corresponds to the left superior temporal gyrus, with higher $Q_{alb}$ values being related to a reduced glucose consumption in these areas. No significant relationships have been found between brain glucose consumption and IgG index. The results of our study suggest that BBB dysfunction is related to reduction of cortical activity in the left temporal cortex in AD subjects.

Abbreviations: $^{18}$F-FDG = 2-deoxy-2-($^{18}$F) fluoro-D-glucose, AD = Alzheimer disease, BBB = Blood–brain barrier, CSF = Cerebrospinal Fluid, IgG index = $[(\text{CSF IgG/serum IgG}] \times [\text{Serum albumin/CSF albumin}])$, PET/CT = Positron Emission Tomography/Computed Tomography, $Q_{alb}$ = Serum/CSF albumin index, SPM = Statistical Parametric mapping.

Keywords: Alzheimer, blood brain barrier dysfunction, dementia, IgG, PET, SPM

1. Introduction
Alzheimer disease (AD) is a primary neurodegenerative disease pathologically characterized by cortical deposits, the extracellular senile plaques, and the intracellular neurofibrillary tangles. Since its first appearance in the early 90s, the “amyloid hypothesis” has been proposed as a pioneering explanation of its pathological basis,[1] although recent evidences criticized its validity and suggested the neurofibrillary pathology as the main responsible for cognitive decline of AD.[2] Changes observed in AD brains are mirrored by changes in the content of their constituent proteins in the cerebrospinal fluid (CSF). Assay of the microtubule Tau protein (total and its hyper phosphorylated form) and amyloid peptides (as $\beta_{42}$ peptide) in CSF are indeed routinely used as biochemical diagnostic markers of AD and of other neurodegenerative disorders.[3–5]

The blood–brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system (CNS). Albumin is not synthesized in the CNS but penetrates BBB from the plasma; therefore, the albumin concentration quotient ($Q_{alb}$, see below) is generally accepted as a method for estimating the function of the BBB.[6,7] Up to 42% of subjects with AD may show a dysfunction in BBB and it has been hypothesized that this could affect the clearance of potentially toxic substances across the BBB.[8–10] Recent evidences showed that $\beta\mathrm{B}$ pathology itself could be responsible for the occurrence of an endotheliopathy in AD patients, a condition that was suggested to potentially precipitate the rate of cognitive decline in these patients.[11]

Increased concentrations of free immunoglobulins in CSF indicating an immune response within the CNS are commonly found in neuro-inflammatory diseases as multiple sclerosis. About two-thirds of the patients with this type of disease have increased CSF immunoglobulin (IgG) index (see below), as an indicator of intrathecal class G production and more than 90% of the patients display oligoclonal IgG bands on electrophoretic separation of CSF.[11] Plasma cells from compartmentalized lymphoid tissue (CLT) in CNS are the main source of intrathecal-
synthesized immunoglobulins. However, despite the solid evidence of the presence of Ig synthesis in multiple sclerosis (MS), the antigens targeted by intrathecal immunoglobulins are still largely unknown and many different epitopes have been demonstrated. As a direct consequence, the pathogenic potential of these immunoglobulins is still elusive and a hypothetical role of intrathecal synthesis in non-MS neurological diseases cannot be excluded. On the other side, the identification of intrathecal-synthesized Ig could represent only the epiphenomenon of the presence of deranged immune cells inside the CNS, and these cells contributing to neuropathology with Ig-independent mechanisms. Contrarily to Qalb, the percentage of AD subjects showing alterations in IgG index is small, with intrathecal IgG synthesis being detectable in ~4% of subjects with AD. Whether BBB changes could represent a possible marker of vulnerability during AD has not been explored yet. To do this, we investigated the relationship between BBB function markers, IgG index, and 18F-FDG uptake, in a population of clinically diagnosed AD.

The usefulness of 2-deoxy-2-[18F] fluoro-D-glucose ([18F]-FDG) positron emission tomography/computed tomography (PET/CT) in the diagnosis of AD has been widely investigated showing a good sensitivity, specificity, and diagnostic accuracy in the detection of hypometabolism associated with AD.

2. Materials and methods

2.1. Patients

We examined 134 newly diagnosed probable AD patients according to the NINCDS-ADRDA criteria. An overview of the population examined is provided in Table 1.

All patients underwent a complete clinical investigation, including medical history, neurological examination, minimal state examination (MMSE), a complete blood screening (including routine examinations, thyroid hormones, level of B12), mental state examination (MMSE), a complete blood screening (including medical history, neurological examination, mini-

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Table 1

| General overview of the AD population examined, including sociodemographic variables. | Whole population (n = 134; ± SD) | Male (n = 60; ± SD) | Female (n = 74; ± SD) | P |
|---|---|---|---|---|
| **Age** | 70 (±6) | 71 (±6) | 70 (±7) | F = 0.88; P = 1 |
| Education: BUL | 85 | 40 | 45 | P = 1 |
| Education: ULoA | 49 | 29 | 20 | |
| Occupation: M | 88 | 40 | 48 | P = 1 |
| Occupation: S | 46 | 22 | 24 | |
| **MMSE** | 18.9 (±7.2) | 19 (±6.2) | 18.4 (±8) | P = 1 |
| Ap42, pg/mL | 358.8 (±141.9) | 354.2 (±129.8) | 375.7 (±134.9) | 0.73 |
| t-Tau, pg/mL | 84.26 (±66.11) | 81.75 (±40.69) | 86.53 (±77.15) | 0.41 |
| IgG index | 652.7 (±361.6) | 690.2 (±401.2) | 654.1 (±392.3) | 0.38 |
| IgG index × Serum albumin (CSF albumin) | 0.46 (±0.07) | 0.47 (±0.07) | 0.46 (±0.05) | 1 |
| Qalb (Serum/CSF albumin index) | 6.79 (±2.8) | 7.9 (±3.15) | 6.5 (±2.59) | <0.001 |

BUL = below university level, M = manual, S = skilled, ULoA = university level or above.
specimens were also obtained at the same time of LP procedure. CSF samples were collected in polypropylene tubes using standard sterile techniques. The first 4 mL CSF sample was used for biochemistry routine analysis including total cell count and lactate levels. A second 4 mL CSF sample was centrifuged to eliminate cells and cellular debris and immediately frozen at -80°C until the analysis to assess t-Tau, p-Tau, and Aβ42 amounts, performed as previously described. Chemistry assays were carried out using commercially available kits following the manufacturer’s specifications (Flex reagent cartridge, Dimension Vista System, Siemens Healthcare Diagnostics GmbH, Munich, Germany).

Concentrations of IgG and albumin in serum and CSF were measured by means of a nephelometer BN ProSpec (Siemens Healthcare Diagnostics). The Qalb was calculated by the formula \([\frac{[\text{IgG}]_{\text{CSF}}}{[\text{Albumin}]_{\text{CSF}}]/[\text{IgG}]_{\text{SERUM}}/\text{Albumin}]_{\text{SERUM}}\times 1000\). IgG index was calculated by the formula \([\frac{[\text{IgG}]_{\text{CSF}}/[\text{Albumin}]_{\text{CSF}}]}{[\text{IgG}]_{\text{SERUM}}/\text{Albumin}]_{\text{SERUM}}\).

### 2.4. 18F-FDG injection and PET/CT scan

The PET/CT system Discovery VCT (GE Medical Systems, Tennessee) has been used to assess 18F-FDG brain distribution in all patients by means of a 3D-mode standard technique in a 256 x 256 matrix. Reconstruction was performed using the 3-dimensional reconstruction method of ordered-subsets expectation maximization (OSEM) with 20 subsets and with 4 iterations. The system combines a high-speed ultra 16-detector-row (912 detectors per row) CT unit and a PET scanner with 13,440 bismuth germanate crystals in 24 rings (axial full width at half-maximum 1 cm radius, 5.2 mm in 3D mode, axial field of view 157 mm). A low-ampere CT scan of the head for attenuation correction (40 mA; 120 Kv) was performed before PET image acquisition. All the subjects fasted for at least 5 hours before intravenous injection of 18F-FDG; the serum glucose level was up to 95 mg/mL in all of them. All the subjects were injected intravenously with 185 to 210 MegaBequerels of 18F-FDG and hydrated with 500 mL of saline (0.9% sodium chloride). PET/CT acquisition was started 30 minutes after 18F-FDG injection.

### 3. Statistical analysis

Correlations among brain 18F-FDG uptake, clinical, and CSF data were analyzed using statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab R2012b (Mathworks, Natick, Massachusetts). MMSE scores, neuropsychological assessment scores, sex, age, and CSF biomarkers were used as covariates in each correlation analysis. 18F-FDG PET data have been subjected to affine and nonlinear spatial normalization into the Montreal Neurological Institute space. The spatially normalized set of images were then smoothed with a 8 mm isotropic Gaussian filter for individual variations in gyral anatomy and to increase the signal-to-noise ratio. Images have been globally normalized to 50 using proportional scaling to remove confounding effects to global cerebral glucose consumption changes, with a masking threshold of 0.8. The resulting statistical parametric maps, SPM \([t]\), have been transformed into normal distribution (SPM\([z]\) unit. Correction of SPM coordinates to match the Talairach coordinates was achieved by the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging). Brodmann areas (BAs) have been identified at a range from 0 to 3 mm from the corrected Talairach coordinates of the SPM output isocenters, after importing the corrected coordinates, by Talairach client (http://www.talairach.org/index.html). According to Bennett et al., SPM t-maps have been set at \(P < 0.05\), corrected for multiple comparisons with the False Discovery Rate option at voxel level, and at \(P < 0.01\) corrected for multiple comparison at cluster level. Only those clusters containing more than 100 \((5 \times 5 \times 5)\) voxels, i.e., \(11 \times 11 \times 11\) mm contiguous voxels have been accepted as significant. The voxel-based analyses have been performed using a ‘regression analysis’ design model using sex, age, MMSE, and CSF parameters presented in Table 1 as a covariate. In SPM maps, we searched the brain areas with a significant correlation using a statistical threshold of \(P = 0.01\), family-wise error-corrected for the problem of multiple comparisons, with an extent threshold of 100 voxels.

The cluster obtained by this comparison has been exported and further analyzed after a normalization process. In particular, the mean signal intensities computed of each cluster have been normalized within each subject to the average intensities of the cerebellar volume of interest as defined by Schmahmann et al. (2000). This choice was based on the knowledge that the cerebellum is poorly affected by AD pathological processes and on the evidence that, when using cerebellum instead of whole brain counts as the reference region, accuracy in distinguishing AD patients from controls increases (24). As proposed in another study of Pagani et al., a dataset including cerebellum-normalized 18F-FDG PET values relevant to the examined cluster has been exported. In order to assess that cerebellum-normalized 18F-FDG PET values for the cluster examined were of Gaussian distribution, D’Agostino K squared normality test has been applied (where the null hypothesis is that the data are normally distributed). Spearman correlation has been applied in order to investigate the relationships among cerebellum-normalized 18F-FDG PET values, CSF biomarkers (amyloid and Tau, see Table 1), albumin ratio, and IgG index. According to the results of correlation analyses (see below) and population characteristics, patients have been divided into groups according to Qalb values (i.e., <6 and >6, <9 and >9, etc.). In order to find an optimal Qalb cut-off value with the highest differences in cortical activity among groups, Mann–Whitney U test has been used in this comparison. A hypothesis was considered valid when \(P\) value was less than or equal to 0.05. Then, neuropsychological assessment scores of each patient from each group were evaluated to find differences among groups. No statistically significant differences were observed.

### 4. Results

Twenty-one out of the 134 subjects examined (15.6%) showed abnormal Qalb values (>9), while 3 subjects (2%) showed an abnormal IgG index (>0.7).

SPM analysis performed in AD patients documented a significant negative correlation between the increase of Qalb and 18F-FDG uptake. In particular, we documented a selective correlation linking CSF albumin levels to the reduced brain glucose consumption occurring in the BA 42 and 22 that corresponds to the left superior temporal gyrus (LSTG, Table 2, Fig. 1), with higher Qalb values being related to a reduced glucose consumption in these areas.

Cerebellum-normalized 18F-FDG PET values for LSTG (BA 42 and 22) resulted equal to 1.05 ± 0.12 (mean ± standard deviation) and were not normally distributed (K2 = 32.2 and \(P < 0.001\)). Spearman correlation analysis showed a good correlation between albumin ratio levels and normalized 18F-FDG uptake.
Positive correlation — — — — — — — —

Negative correlation cluster level Voxel level

Either analysis (positive or negative correlation).

Table 2
Multiple regression analysis showing the serum/CSF albumin index related areas of decreased \(^{18}\)F FDG brain uptake.

| Analysis | Cluster level | Voxel level |
|----------|---------------|-------------|
| Negative correlation | cluster | P (FWE-corr) | 0.001 | 0.000 | 5404 | L Temporal | Z score of maximum | Talairach coordinates | Cortical region | BA |
| | | | | | | | | — — | Superior temporal gyrus | 42 |
| | | | | | | | | — — | Superior temporal gyrus | 22 |
| | | | | | | | | 3.91 | — — | 26, 10 |
| | | | | | | | | 3.88 | — — | 46, 14 |

In the ‘cluster level’ section on left, the number of voxels, the corrected P value of significance, and the cortical region where the voxel is found, are all reported for each significant cluster. In the ‘voxel level’ section, all of the coordinates of the correlation sites (with the Z score of the maximum correlation point), the corresponding cortical region, and Brodmann area are reported for each significant cluster. In the case that the maximum correlation is achieved outside the grey matter, the nearest grey matter (within a range of 5 mm) is indicated with the corresponding BA. BA=Brodmann’s area, L=left.

Further showing that low levels of albumin ratio were related to lower levels of \(^{18}\)F-FDG uptake \((r=-0.22, P=0.009)\).

The highest difference in cortical \(^{18}\)F-FDG metabolism has been found in subjects with Q\(_{\text{alb}}\) ≤ 6 (n=61) as compared with those with a Q\(_{\text{alb}}\) > 6 (n=73). Cerebellum-normalized \(^{18}\)F-FDG PET values for LSTG resulted equal to 1.08±0.12 and 1.02±0.11, respectively \((P=0.005)\).

We did not find significant relationships between \(^{18}\)F-FDG uptake and IgG index at any explored statistical threshold in either analysis (positive or negative correlation).

Q\(_{\text{alb}}\) was not related to A\(_{\beta 42}\) \((P=0.81; r=0.02)\), t-Tau \((P=0.64, r=0.04)\), and p-Tau \((P=0.19, r=-0.11)\). IgG index was not related to A\(_{\beta 42}\) \((P=0.06; r=0.17)\), t-Tau \((P=0.70; r=-0.03)\), and p-Tau \((P=0.97; r=-0.02)\).

5. Discussion

The main finding of our study is a significant relationship between brain glucose consumption and Q\(_{\text{alb}}\) in a wide portion of the left temporal lobe (LSTG, left BA 42 and 22). Unexpectedly, the more Q\(_{\text{alb}}\) increased the worst glucose consumption appeared in that area. The gradient did not correlate with cognitive decline severity as measured with neuropsychological assessment. Mechanisms linked to the neurodegenerative process are likely to be important for such increase regional permeability. On one hand, physiological aging could alter the normal immune response of an individual promoting microglial activation and BBB disruption, contributing to neurodegeneration of the brain,\([32,33]\) whereas on the other hand, A\(_{\beta 42}\)-mediated pathology could be responsible for endothelial dysfunction responsible in turn for increase in BBB permeability.\([34,35]\) Left temporal lobe has been shown to be an important structure in the pathway involved in social cognition processes.\([36]\) Including the superior temporal gyrus, areas more anterior and dorsal within the temporal lobe have been linked to the ability of processing information.\([36]\) Temporal lobes are also considered preferential vulnerable sites in AD patients, whose degeneration is responsible for memory complaining of these patients.\([37,38]\) To the best of our knowledge, very few imaging studies have been carried out to date in order to investigate the relationships between BBB and brain functions. Brain magnetic resonance imaging (MRI) study in a group of mild cognitive impairment (MCI) patients compared with control subjects found that the temporal lobes had a lower vascular space owing to suppose that this area of the brain might represent a constitutional site of vulnerability, responsible for evolutionary aspects of AD.\([39]\) In another study, Starr et al.\([40]\) evaluated BBB dysfunction in AD and controls by means of dynamic contrast-enhanced MRI. The authors demonstrated that, in AD subjects, an initial rise in gray matter MRI signal intensity followed by a later increase was detectable thus suggesting a BBB permeability even at the early stages of AD.\([40]\)

Although the hypothesis of a BBB impairment as an evolutionary factor in AD is intriguing, the heterogeneous results
reported in literature lead to inconclusive hypothesis.\textsuperscript{[41]} A positron emission tomography (PET) study with [68Ga]EDTA did not find increased BBB permeability in AD\textsuperscript{[42]} and an MRI study performed to specifically examine white matter lesions in 10 demented patients (including 5 with elevated CSF/serum albumin ratios) found no evidence for BBB leakage.\textsuperscript{[43]} In particular, it has been suggested that BBB impairment could affect transport of trophic substances to the CNS and the removal of toxic substances as amyloid\textsuperscript{[43]} responsible for neurodegeneration. Unfortunately, BBB increased permeability was observed only in a small subgroup of AD patients, reducing the possibility to consider BBB disruption as a general mechanism of neurodegeneration.\textsuperscript{[44]}

The results of our study suggest that in a selected population of AD (\textasciitilde 16\%), BBB dysfunction may occur and that this pattern is related to a worse metabolic pattern, as shown in Figs. 1 and 2.

Interesting, the higher difference in brain glucose consumption has been found when a cut-off of 6 in Qalb was used, that is, lower than the standard used in other studies.\textsuperscript{[8]} This latter finding suggests that, in AD subjects, a functional correlate of BBB dysfunction appears for relatively low levels of Qalb. Thus, further studies would be performed to deeply explore the potential impact of BBB dysfunction at the early stages of the disease even in subjects with mild cognitive impairment.

Few literature evidences demonstrated that intrathecal synthesis of Ig could be documented in a small subgroup of AD patients,\textsuperscript{[41–47]} suggesting a potential role in neurodegeneration. In our study, however, we failed to find any significant correlation between glucose brain consumption and CSF/blood IgG index, a solid measure of intrathecal Ig synthesis. However, the low prevalence of clinically significant Ig synthesis in our study population did not allow a subanalysis of this distinct category of patients. Globally taken, our data support the hypothesis that the contribution of B-cell dependent CNS inflammation could be considered minimal, at least in the vast majority of AD patients, but no inferences could be made on those with demonstrated intrathecal Ig synthesis. On the other side, IgG index alone could be insufficiently sensitive to detect subtle abnormalities, such as intrathecal synthesis of disease-specific cytoskeletal proteins as recently demonstrated.\textsuperscript{[48]}

6. Conclusion

BBB impaired permeability in AD patients is restricted to a subset of patients not identifiable by means of neuropsychological, biomarkers, and MRI findings. Whether BBB changes might represent a site of vulnerability and a negative prognostic factor at the stage of our analysis could not be confirmed. Inconclusive and often conflicting results led us to suggest that BBB changes observed during AD might likely represent a pathological phenomenon, a marker of aging process associated with the course of neurodegenerative diseases such as AD.

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