

## CSF/serum albumin ratio in dementias: a cross-sectional study on 1861 patients

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**Abbreviations:**

AD – Alzheimer's disease

A $\beta$ 42 – Amyloid beta 42

BBB – Blood brain barrier

CNS – Central nervous system

CSF – Cerebrospinal fluid

EAD – Early onset Alzheimer's disease

FTD – Frontotemporal dementia

LAD – Late onset Alzheimer's disease

LBD – Lewy body dementia

MIX – Mixed AD and vascular dementia

MMSE - Mini mental state examination

NFL – Neurofilament light protein

NOS – Dementia not otherwise specified

Other – Other dementia diagnoses

PDD – Parkinson's disease dementia

P-tau – Phosphorylated tau

SveDem – Swedish Dementia Registry

T-tau – Total tau

VaD – Vascular dementia

WM – White matter

## **Abstract**

A connection between dementias and blood-brain barrier (BBB) dysfunction has been suggested, but previous studies have yielded conflicting results. We examined CSF/serum albumin ratio in a large cohort of patients diagnosed with Alzheimer's disease (AD, early onset [EAD, n=130], late onset AD [LAD, n=666]), vascular dementia (VaD, n=255), mixed AD and VaD (MIX, n=362), Lewy body dementia (DLB, n=50), frontotemporal dementia (FTD, n=56), Parkinson's disease dementia (PDD, n=23), other dementias (Other, n=48) and dementia not otherwise specified (NOS, n=271). We compared CSF/serum albumin ratio to two healthy control groups (n=292, n=20), between dementia diagnoses, and tested biomarker associations. Patients in DLB, LAD, VaD, MIX, Other and NOS groups had higher CSF/serum albumin ratio than controls. CSF/serum albumin ratio correlated with CSF neurofilament light in LAD, MIX, VaD and Other, but not with AD biomarkers. Our data shows that BBB leakage is common in dementias. The lack of association between CSF/serum albumin ratio and AD biomarkers suggests that BBB dysfunction is not inherent to AD but might represent concomitant cerebrovascular pathology.

## **1. Introduction**

Dementia is a major health concern with more than 47 million affected patients worldwide and an increasing prevalence as the population ages. The most common causes of dementia are Alzheimer's disease (AD) and vascular dementia (VaD) (Wimo et al., 2017). An increasing body of research suggests that there is a connection between dementia and vascular pathology (Nelson et al., 2016), including molecular and epidemiologic evidence that vascular disease is a risk factor for dementia (Beydoun et al., 2014, Hughes and Ganguli, 2009, Iadecola, 2013, McAleese et al., 2016, Wiesmann et al., 2013). Many risk factors for AD and reduced cognitive abilities, such as stroke, hypertension, hyperlipidemia, diabetes and atrial fibrillation, affect the vasculature (Gorelick, 2004, Hayden et al., 2006, Kilander et al., 1998, Kivipelto et al., 2001, Ott et al., 1996, Skoog et al., 1996). In addition, increased permeability of the blood brain barrier (BBB) has been indicated in several of these conditions (Hovsepian et al., 2004, Hsu and Kanoski, 2014, Starr et al., 2003, Tang et al., 1992).

There is great diversity in the vascular mechanisms that may underlie dementia, including diffuse white matter lesions, hypoperfusion, oxidative stress and inflammation (Iadecola, 2013). Many of these have effects on the brain vasculature causing endothelial damage, BBB breakdown and activation of the innate immune response (McAleese et al., 2016). BBB damage may lead to disruption of the tightly controlled metabolic balance between vascular and brain cells and suboptimal control of exposure of the brain tissue to blood-associated substances, which ultimately may result in demyelination, axonal loss and cognitive impairment (Fornari et al., 2012, Iadecola, 2013, Ryu et al., 2015). In the light of these findings, there is a need to further evaluate the contribution of BBB dysfunction in different dementias.

The BBB is the interface between the blood and the brain, regulating the transport of molecules between the blood and the central nervous system (CNS). Its primary function is to maintain the tightly controlled microenvironment of the brain, which is a critical part in sustaining a healthy nervous system (Obermeier et al., 2013). A standard measure of BBB function in clinical laboratory practice is the CSF/serum albumin ratio (Tibbling et al., 1977). Proteins will pass from blood to CSF across the BBB at different rates, depending on their hydrodynamic radii, with passage of larger proteins being more restricted than that of smaller proteins (Felgenhauer and Renner, 1977). This limits the movement of albumin from blood to CSF. As albumin is not produced in the CNS, CSF/serum albumin ratio can be used to assess the integrity of the BBB (Reiber and Peter, 2001). There are other candidate methods for investigating BBB integrity. These include measurements of fluid biomarkers such as blood occludin (Pan et al., 2017) and other tight junction proteins (Sweeney et al., 2015), serum MMP-9 (Waubant et al., 1999), plasma fibrinogen (Bridges et al., 2014) and markers related to pericyte breakdown (Halliday et al., 2016), as well as non-fluid biomarker methods such as dynamic contrast-enhanced magnetic resonance imaging (MRI) (Montagne et al., 2015, Taheri et al., 2011, van de Haar et al., 2016). Although contrast-enhanced MRI can provide improved spatial and temporal resolution, CSF/serum albumin ratio has the advantage of being a readily available test in automated clinical chemistry analyzers that are standardized for routine use in general clinical laboratory practice around the world. It is also the only fluid biomarker that has been validated for clinical use.

Most studies investigating CSF/serum albumin ratio in dementia patients have included a relatively small number of individuals and have primarily focused on AD {Elovaara, 1986 #127; Wada, 1998 #129; Wada, 1998 #129; Farrall, 2009 #133; Alafuzoff, 1983 #126; Kay, 1987 #135; Hampel, 1997 #128; Blennow, 1990 #132; Hermann, 2014 #30}. However, there is a more comprehensive study of CSF/serum albumin ratio in dementia patients focusing mainly

on Creutzfeldt-Jakob disease {Karch, 2013 #213}. There have been conflicting reports as to whether BBB damage can be linked to AD, and reports of higher incidence of BBB damage in AD compared to controls have been published (Elovaara et al., 1986, Farrall and Wardlaw, 2009, Janelidze et al., 2017, Skoog et al., 1998, Wada, 1998), as well as reports where no difference in BBB integrity compared to controls could be found (Alafuzoff et al., 1983, Blennow et al., 1990, Hampel et al., 1997, Kaye et al., 1987). A recent meta-analysis suggests that there is no significant change in CSF/serum albumin ratio in AD (Olsson et al., 2016a). In animal models, most studies report that cerebrovascular changes and BBB alterations are part of the AD pathology (Blair et al., 2015, Gama Sosa et al., 2010, Giannoni et al., 2016, Kumar-Singh et al., 2005, Park et al., 2013), whilst one study reports no change in BBB permeability for several AD models (Bien-Ly et al., 2015).

Conflicting results have also been reported in studies of the prevalence of BBB damage in patients with VaD compared to AD (Alafuzoff et al., 1983, Blennow et al., 1990, Blennow et al., 1991, Farrall and Wardlaw, 2009, Skoog et al., 1998, Wada, 1998). Considering the contradictory findings in this area, we set out to investigate BBB integrity in a large population of dementia patients using data from the Swedish Dementia Registry (SveDem). We hypothesized that a subgroup of dementia patients have increased CSF/serum albumin ratio, as a marker of BBB dysfunction, and that this is partly linked to cerebrovascular disease. Since BBB integrity is tightly linked to the important homeostasis of the CNS, we also hypothesized that increased CSF/serum albumin ratio would be related to worse disease severity.

## **2. Methods**

### ***2.1 Data sources and clinical criteria***

Two sources of patient information were combined and used for this study. The first was a

complete set of archived data on all CSF/serum albumin ratios, A $\beta$ 42, total tau (T-tau), phosphorylated tau (P-tau) and neurofilament light protein (NFL) measurements performed in clinical practice at the Mölndal site of the Sahlgrenska University Hospital, Sweden from January 1, 2005 to June 1, 2012. The inclusion criteria for this study were an age of sampling above 30, and a maximum of 24 months between dementia diagnosis and lumbar puncture.

The second source of data was the Swedish Dementia Registry, SveDem, which was started in May 2007 to improve the quality of the diagnostic workup, treatment and care for dementia patients throughout Sweden, and which presently covers 100% of all memory clinics and 75% of all primary care units in Sweden (Religa et al., 2015, SveDem, 2015). From SveDem information on clinical diagnoses, medications, date of diagnosis, and mini mental state examination (MMSE) scores were drawn. In SveDem, each patient is assigned to a single diagnosis group out of nine preset options in the report form: early onset AD (EAD, < 65 years of age), late onset AD (LAD, > 65 years of age) and vascular dementia (VaD) according to ICD-10 (World Health Organization., 1993), FTD according to Manchester criteria (The Lund and Manchester Groups, 1994), dementia with Lewy bodies (DLB) according to McKeith criteria (McKeith et al., 2005), Parkinson's with dementia (PDD) according to Movement Disorder Society Task Force criteria (Martinez-Martin et al., 2011), mixed AD and vascular dementia (MIX), dementia not otherwise specified (dementia NOS) and a group for the collected remainders of named dementia diagnoses called "Other" (including for example, Creutzfeldt-Jakob disease, HIV-associated neurocognitive disorder and Huntington's disease).

Information from the two data sources was cross-referenced using the unique Swedish personal identity number. Multiple CSF analyses on the same individual were excluded and only the measurement closest to the date of diagnosis was left in the dataset. 1,861 individuals were matched between the CSF data file and SveDem and used for the analyses in this study.

Reference data on the CSF/serum albumin ratio from healthy age matched control subjects without symptoms of cognitive dysfunction were obtained from two previously published sources from our laboratory. Data for 292 healthy controls (HC1) were obtained from a study conducted at the Memory Clinic of Skåne University Hospital in Malmö, Sweden (Janelidze et al., 2017) and data for 20 healthy controls (HC2) were obtained from a study conducted at a memory clinic in Falköping, Sweden (Johansson et al., 2011).

## ***2.2 Biochemical measurements***

All CSF analyses were performed in clinical practice by board-certified laboratory technicians using procedures accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC; the national accreditation body for laboratory medicine in Sweden). INNOTEST enzyme-linked immunosorbent assays were used to measure CSF T-tau, P-tau and A $\beta$ 42 concentrations (Fujirebio, Ghent, Belgium). CSF NFL concentration was measured as previously described (Skillback et al., 2014). Serum and CSF albumin concentrations were measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). The CSF/serum albumin ratio was calculated as CSF albumin (mg/L)/serum albumin (g/L). Longitudinal stability in the measurements over years was ascertained using an elaborate system of internal quality control samples and testing of incoming reagents and intra- and inter-day coefficients of variation were below 5%.

All patients were classified as having either a presence or absence of a pathological CSF/serum albumin ratio according to the clinical reference limits at the Sahlgrenska University hospital. The cutoffs used were  $> 6.8$  for study participants 30-45 years of age and  $> 10.2$  for study participants  $> 45$  years of age. The AD group was further sub-classified into biochemically positive or negative AD according to IWG-2. Biochemically positive patients

had low A $\beta$ 42 and high T-tau or P-tau (Dubois et al., 2014). The following cutoffs were used: A $\beta$ 42  $\leq$  550 pg/mL, T-tau  $\geq$  400 pg/mL, P-tau  $>$  60 pg/mL for patients  $<$  60 years, and P-tau  $>$  80 pg/mL for patients  $\geq$  60 years. When specified, the LAD, EAD and MIX groups were analyzed together as an AD group. Reporting clinicians were instructed to follow diagnostic guidelines as specified in ICD-10 to secure a unified basis for diagnosis (Sorbi et al., 2012).

**2.3 Data analysis** Age differences between groups were tested by Kruskal-Wallis analysis. CSF/serum albumin ratio and MMSE scores across diagnosis groups and subgroups (classifications according to biochemical profiles, presence of pathological CSF/serum albumin ratio, and presence of prescriptions for vascular medicine) were tested in age and sex corrected ANCOVA models. Age corrected linear regression models were fitted for A $\beta$ 42, T-tau, P-tau and NFL concentrations and CSF/serum albumin ratio in AD. Chi<sup>2</sup> statistics were used to analyze differences in proportions of patients with pathological vs. normal CSF/serum albumin ratios in each diagnosis group. Logarithmic transformations were applied to correct for significantly skewed data distributions. All statistics, charts and tables were produced in SPSS version 20 (IBM, New York).

## **2.4 Ethics**

Patients and caretakers were informed orally and in writing about SveDem and could opt to decline participation and withdraw consent. This study was approved by the regional ethical boards at the University of Gothenburg and Lund University.

## **3. Results**

### **3.1 Demographics of study cohort**

Demographics of the study cohort can be found in Table 1. The EAD subjects were younger ( $p < .001$ ) than all other groups, and the FTD subjects were younger ( $p < .001$  for NOS, LAD, VaD and MIX,  $p = .038$  for DLB) than all groups except EAD, PDD and other. The MIX

subjects were older ( $p < .001$ ) than all other groups except VaD and PDD, and the VaD subjects were older ( $p < .001$ ) than the LAD, EAD, FTD, NOS and other groups.

### ***3.2 CSF/serum albumin ratios across diagnosis groups***

CSF/serum albumin ratios across the diagnostic groups are shown in Figure 1. The VaD group had higher CSF/serum albumin ratio than HC1 (mean diff = .26,  $p < .001$ , SE = .046), HC2 (mean diff = .33,  $p = .001$ , SE = .099), LAD (mean diff = .09,  $p = .017$ , SE = .039), FTD (mean diff = .20,  $p = .002$ , SE = .062), EAD (mean diff = .28,  $p < .001$ , SE = .055), PDD (mean diff = .22,  $p = .02$ , SE = .089) and NOS (mean diff = .10,  $p = .016$ , SE = .043). The LAD group had higher ratios than HC1 (mean diff = .16,  $p < .001$ , SE = .041), HC2 (mean diff = .24,  $p = .015$ , SE = .096) and EAD (mean diff = .19,  $p < .001$ , SE = .051). The MIX group had higher ratio than HC1 (mean diff = .21,  $p < .001$ , SE = .044), HC2 (mean diff = .287,  $p = .003$ , SE = .098), EAD (mean diff = .24,  $p < .001$ , SE = .054) and FTD (mean diff = .16,  $p = .011$ , SE = .061). The NOS group had higher ratio than HC1 (mean diff = .15,  $p = .001$ , SE = .045), HC2 (mean diff = .23,  $p = .022$ , SE = .098) and EAD (mean diff = .18,  $p = .001$ , SE = .055). The Other group had higher ratio than HC1 (mean diff = .22,  $p = .001$ , SE = .067), HC2 (mean diff = .30,  $p = .007$ , SE = .110), EAD (mean diff = .25,  $p = .001$ , SE = .074) and FTD (mean diff = .17,  $p = .037$ , SE = .079). The DLB group had higher ratio than HC1 (mean diff = .27,  $p < .001$ , SE = .069), HC2 (mean diff = .35,  $p = .002$ , SE = .111), EAD (mean diff = .30,  $p < .001$ , SE = .075), FTD (mean diff = .21,  $p = .008$ , SE = .081) and PDD (mean diff = .23,  $p = .024$ , SE = .103). These comparisons were for logarithmic values and corrected for age and sex.

We also classified all subjects according to prevalence of pathologic CSF/serum albumin ratio. Proportions per diagnosis are shown in Figure 2. VaD had higher proportion of pathologic CSF/serum albumin ratio than HC1, EAD, FTD, LAD, MIX and NOS ( $p < .05$ ). LAD had a higher proportion than HC1 ( $p < .05$ ). MIX had higher proportion than HC1, EAD

and LAD ( $p < .05$ ). NOS had higher proportion than HC1 and LAD ( $p < .05$ ). Other had higher proportion than HC1, EAD, FTD and LAD ( $p < .05$ ). DLB had higher proportion than HC1, EAD, FTD and LAD. All other differences were non-significant. The HC2 group had lower mean CSF/serum albumin value than all of the dementia diagnosis groups but the differences failed to reach significance.

### *3.2.1 Correlations with biomarkers of neurodegeneration and A $\beta$ 42*

CSF/serum albumin ratio correlated with A $\beta$ 42 concentrations in LAD ( $\beta = .11$ ,  $p = .003$ ,  $R^2 = .12$ ), with T-tau in MIX ( $\beta = .15$ ,  $p = .003$ ,  $R^2 = .14$ ), and with P-tau in MIX ( $\beta = .12$ ,  $p = .013$ ,  $R^2 = .14$ ) and VaD ( $\beta = -.13$ ,  $p = .030$ ,  $R^2 = .12$ ). CSF/serum albumin ratio correlated with NFL concentrations in LAD ( $\beta = .24$ ,  $p < .001$ ,  $R^2 = .17$ ), MIX ( $\beta = .21$ ,  $p < .001$ ,  $R^2 = .17$ ), NOS ( $\beta = .25$ ,  $p < .001$ ,  $R^2 = .21$ ), Other ( $\beta = .35$ ,  $p = .41$ ,  $R^2 = .13$ ) and VaD ( $\beta = .24$ ,  $p < .001$ ,  $R^2 = .15$ ). All biomarker levels were on logarithmic scale.

### *3.2.2 Disease severity*

To test associations between CSF/serum albumin ratio and cognitive impairment, we used MMSE as a proxy for clinical severity and tested associations between MMSE and presence of pathological CSF/serum albumin ratio. No significant differences between the two groups were found for any diagnosis, in age and sex corrected analyses.

## **3.3 CSF/serum albumin ratio in AD**

### *3.3.1 Biochemical profile*

The AD group (LAD + EAD + MIX) was split according to the IWG-2 guidelines into a biochemically positive AD group (IWG-2+,  $n = 613$ , median age = 75) and a biochemically undetermined group (IWG-2-,  $n = 545$ , median age = 75). There were no differences in CSF/serum albumin ratio between the groups in an age and sex corrected analysis ( $p = .72$ ).

### *3.3.2 Treatment for vascular risk factors*

We further sub-classified the AD group according to declared use of medications for cardiovascular co-morbidities (n = 724 on treatment vs. n = 434 without treatment).

Cardiovascular drugs comprised antihypertensives, anticoagulants, lipid-lowering drugs, anti-diabetics and anti-angina medication. Subjects with prescriptions had higher CSF/serum albumin ratio (p = .007, mean diff = .06). However, after age and sex correction, the presence of prescriptions no longer significantly predicted the CSF/serum albumin ratio (p = .60).

## **4. Discussion**

We tested CSF/serum albumin ratio, as a surrogate measure of BBB integrity, in patients with different dementia diagnoses to elucidate if certain diagnoses are associated with more BBB damage. We found that the VaD, LAD, MIX, NOS, Other and DLB groups had higher CSF/serum albumin ratio than healthy controls. VaD in particular, but also DLB and MIX, had higher CSF/serum albumin ratio than the other groups. EAD had the lowest CSF/serum albumin ratio, although it should be noted that age correction cannot fully remove age as a confounder in the case of EAD vs. LAD as the age distributions in these groups do not overlap. Nevertheless, EAD were indistinguishable from similarly aged controls in terms of CSF/serum albumin ratio. In addition, we investigated the relationship between CSF/serum albumin ratio and AD biomarkers and found that a positive biomarker profile for AD was not linked to more BBB damage. The CSF/serum albumin ratio correlated positively with CSF NFL concentrations in VaD, MIX, LAD, NOS and Other. This is consistent with the presence of leaking vessels, as reflected by increased CSF/serum ratio, and injury to myelinated axon, as reflected by increased NFL, in diseases with white matter changes. Taken together, these findings show that BBB damage is most evident in VaD but also occurs in LAD, MIX, NOS and Other.

#### **4.1 Differences in BBB permeability in patients with different diagnoses of dementia**

CSF/serum albumin ratios were highest in the groups VaD, MIX, DLB and Other. The prevalence of pathological CSF/serum albumin ratio was higher in DLB, LAD, MIX, NOS, Other and VaD compared to HC1. Among these diagnoses, the prevalence of patients with pathological CSF/serum albumin ratio ranged from 10.5% in LAD to 26% in DLB, which is in accordance with a previous study showing a prevalence of pathological CSF/serum albumin ratio in VaD of 25% (Brettschneider et al., 2005). Importantly, the same study found that about 15% of patients receiving diagnostic lumbar puncture, who showed no other evidence of neurological disease had increased CSF/serum albumin ratio. Our healthy control groups combined showed a lower prevalence of pathological CSF/serum albumin ratio of 5.4% (5.1% and 10.0% in HC1 and HC2, respectively). Although rare, this shows that it is possible to have a slight BBB dysfunction, at least as reflected by the CSF/serum albumin ratio, without having significantly impaired cognition. This is also an inherent consequence of how reference limits are established in clinical chemistry; normality is defined by cut-points that define 95% of the tested individuals.

The higher prevalence of pathological CSF/serum albumin ratio in DLB compared to AD was in accordance with a previous study (Llorens et al., 2015). The prevalence of cerebrovascular lesions in DLB (De Reuck et al., 2013), which may lead to reduced CSF flow rate, could contribute to increased CSF/serum albumin ratio. Other studies using the SveDem data found that, despite a high burden of cerebrovascular disease, DLB patients have less diabetes mellitus than other dementia patients (Cermakova et al., 2015). Taken together with our findings of high prevalence of BBB damage in DLB but no correlation between NFL and CSF/serum albumin ratio, this could indicate separate mechanisms of vascular pathology between DLB and VaD.

#### **4.2 Increased BBB permeability is more prevalent in VaD than other dementia diagnoses**

VaD patients had significantly higher CSF/serum albumin ratio than most other patients. This indicates that BBB disruption plays a part in, or reflects VaD pathophysiology, which has also been suggested in other studies (Blennow et al., 1990, Blennow et al., 1991, Farrall and Wardlaw, 2009, Skoog et al., 1998). A previous study showed that late stage PD patients without dementia had elevated CSF/serum albumin ratios (Pisani et al., 2012). However, we found no evidence of this, as the median CSF/serum albumin ratio did not increase with age in the PDD subjects in our cohort.

#### **4.3 Increased BBB permeability is more prominent in LAD than in EAD**

Post mortem neuropathological studies show degeneration of several important parts and functions of the BBB in AD (Halliday et al., 2016, Hultman et al., 2013, Sengillo et al., 2013). Similarly several other studies show increased occurrence of blood derived proteins in AD brains (Cortes-Canteli et al., 2010, Halliday et al., 2016, Hultman et al., 2013, Sengillo et al., 2013), and expression of the E4 isoform of apolipoprotein E, a strong genetic risk factor of developing AD (Hauser and Ryan, 2013, Michaelson, 2014), has been shown to lead to BBB breakdown in mice (Bell et al., 2012). Considering these reports, it is plausible that BBB integrity is compromised in some way in AD. Although the role of BBB damage in AD has been disputed, a recent meta-analysis found no significant association of CSF/serum albumin ratio with AD (Olsson et al., 2016a). We found higher CSF/serum albumin ratio in LAD than in the control groups but no difference between the control groups and EAD. This may suggest increased BBB damage in LAD, but not in EAD. Pathological CSF/serum albumin ratios did not correlate with lower MMSE scores or IWG-2 positivity, and the CSF/serum albumin ratio correlated positively to A $\beta$ 42 levels. Taken together, our results indicate that BBB damage in AD is not primarily linked to AD pathology but rather to pathological

vascular events that occur in LAD (Iturria-Medina et al., 2016). This view is further supported by the lack of difference in CSF/serum albumin ratio between EAD patients and age-matched controls in our study.

In contrast to our findings, studies using imaging techniques have found increased BBB leakage in EAD patients (van de Haar et al., 2016) and BBB damage in patients with mild cognitive impairment (Montagne et al., 2015). Indeed, using different techniques to measure BBB permeability will affect disease correlation. The conflicting results in this area are a testament to that BBB changes are subtle and large cohort sizes are important. Considering the different scopes of fluid biomarkers and imaging techniques, it may be beneficial to include both techniques in future studies of BBB damage in dementia patients. In addition, our data highlights that analyzing AD patients as one group or separated into LAD and EAD can influence the outcome. The patient cohort in the study where HC1 was originally published is similar to our, however in that study LAD and EAD were analyzed as one AD group which was found to have increased BBB permeability compared to healthy controls (Janelidze et al., 2017). There are several similarities between our results and the results reported by Janelidze *et al.*; for example, the CSF/serum albumin ratio in DLB and VaD was found to be higher than in AD in both studies. In addition, the study by Janelidze et al. found that increased CSF/serum albumin ratio is independent of AD-related features such as amyloid pathology and *APOE* genotype. Similarly, we found that pathological CSF/serum albumin ratio in AD did not correlate with IWG-2 positivity. Our results suggest that BBB leakage in dementia may not be linked directly to AD pathology but rather to contaminant cerebral vascular pathology (Olsson et al., 2016b, Zhao et al., 2015).

CSF NFL concentrations correlated to the CSF/serum albumin ratio, as did declared use of medications for cardiovascular co-morbidities before correction for age and sex was

introduced. CSF NFL correlates with white matter (WM) lesions in several conditions, and while AD patients show significant WM atrophy, which could lead to both elevated CSF NFL concentrations and CSF/serum albumin ratio, WM atrophy is more prominent and CSF NFL concentrations are considerably higher in VaD (Blennow et al., 1991, Jonsson et al., 2010, Lycke et al., 1998, Sjogren et al., 2001, Skillback et al., 2014, Zanier et al., 2011). Taken together, these results might stem from contaminant cerebral vascular pathology and a relative rarity of biochemically pure AD, but could also indicate abundance of WM lesions (Attems and Jellinger, 2014, McAleese et al., 2016). Further studies are required to understand the extent of the overlap between AD and VaD. The MIX group had a large proportion of patients with pathological CSF/albumin ratios and a positive correlation between CSF NFL concentration and CSF/albumin ratio, consistent with the notion of concomitant VaD as a driving force of BBB damage in AD, corroborating previous studies (Hermann et al., 2014).

#### **4.4 Strengths and Limitations**

The main strength of this study was the large cohort size, the broad referral base and the wide range of dementia diagnoses represented. The main limitation is the lack of detailed clinical data, for example *APOE* genotype and post-mortem diagnosis confirmation. In addition, we did not test different subtypes of VaD, which have distinct effects on BBB integrity (McAleese et al., 2016). Further, some researchers caution against describing the CSF/serum albumin ratio as a BBB test and state that it actually reflects the blood-CSF barrier at the choroid plexus (Reiber and Peter, 2001). However, in for example stroke, leaving the choroid plexus intact but injuring cerebrovascular endothelial cells, the CSF/serum albumin ratio may be increased (Brouns et al., 2011), suggesting that albumin ratio probably is a marker of both barriers. Albumin levels may also be affected by other factors for which we lack information, such as cleavage, degradation or uptake by microglia, astrocytes, pericytes and NG2-positive cells, or presence of spinal stenosis or other deformations affecting the flow rate of CSF

(Braganza et al., 2012, Ivens et al., 2007, LeVine, 2016). Another limitation was the use of MMSE as a measure of disease severity, as MMSE is a test primarily designed to measure cognitive decline due to AD and hippocampal dysfunction, and is not an ideal test for cognitive decline due to VaD, FTD, DLB or PDD (Hodges et al., 1999, Palmqvist et al., 2009, Prieto et al., 2011). A previous study has indicated that measures of executive function might be more closely linked to BBB integrity in VaD, MIX and AD (Hermann et al., 2014).

#### **4.5 Conclusions**

Our results indicate that BBB permeability is increased in dementia patients compared to healthy controls, with increased CSF/serum albumin ratio primarily in DLB, LAD, VaD, MIX, Others and NOS. The influence of BBB function impairment is most prominent in VaD, DLB, MIX Other and NOS, and more prominent in LAD than in EAD. The results suggest that BBB leakage in AD may be a consequence of contaminant cerebrovascular pathology rather than the AD process *per se*.

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## **Disclosure statement**

The authors wish to confirm that there are no known conflicts of interest associated with the publication of this manuscript.

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## **Figure legends**

### **Figure 1.** CSF/serum albumin ratio per diagnosis

*Median and IQR levels of CSF/serum albumin ratio. Observed data are shown here; see main text for comparisons adjusted for age and sex. Dashed lines indicate thresholds for albumin quotient pathology for 30-49 year olds (orange) and >50 year olds (red). VaD, MIX and DLB have the highest CSF/serum albumin ratios while the healthy control groups and EAD have lower CSF/serum albumin ratios.*

### **Figure 2.** CSF/serum albumin ratio pathology proportions per diagnosis

*Pathologic albumin quotients are most common in DLB, Other, VaD and MIX.*

**Table 1.** Demographics and CSF/serum albumin ratio in all diagnostic groups

Diagnosis		HC1	HC2	LAD	EAD	MIX	VAD	FTD	DLB	PDD	Other	NOS	
<b>N</b>		292	20	666	130	362	255	56	50	23	48	271	
<b>Sex</b>	F (%)	60	50	448 (67%)	81 (62%)	210 (58%)	110 (43%)	29 (52%)	14 (28%)	6 (26%)	23 (48%)	144 (53%)	
	M (%)	40	50	218 (33%)	49 (38%)	152 (42%)	145 (57%)	27 (48%)	36 (72%)	17 (74%)	25 (52%)	127 (47%)	
<b>Age at sampling</b>	Mean (SD)	73 (5)	74 (5)	75 (6)	59 (4)	79 (7)	77 (8)	67 (10)	73 (7)	73 (8)	72 (10)	74 (9)	
	Mdn (IQR)	73 (69-76)	75 (71-78)	75 (71-80)	60 (56-62)	79 (74-84)	78 (72-83)	65 (60-74)	74 (69-79)	73 (68-78)	73 (65-80)	75 (68-80)	
<b>MMSE</b>	Mean (SD)	29 (1)	28 (2)	22 (5)	22 (5)	22 (5)	22 (4)	25 (5)	22 (5)	24 (4)	23 (5)	22 (5)	
	Mdn (IQR)	29 (29-30)	29 (27-29)	23 (20-26)	22 (18-25)	23 (19-25)	22 (20-25)	26 (23-28)	22 (20-25)	27 (22-28)	25 (20-27)	23 (20-26)	
<b>Albumin quotient</b>	Mean (SD)	5.9 (2.2)	5.9 (2.4)	6.8 (2.7)	6.8 (7.6)	7.7 (3.4)	8.9 (8.2)	6.5 (2.4)	8.4 (4.3)	6.6 (2.4)	8 (3.8)	7.4 (3.4)	
	Mdn (IQR)	5.8 (4.3-7.0)	5.5 (3.9-7.8)	6.3 (4.9-8.2)	5.4 (4.1-7.7)	7.3 (5.4-9.0)	7.6 (5.6-10.1)	6.5 (4.6-7.8)	7.4 (5.6-10.5)	6.1 (4.7-8.8)	7.3 (5.1-9.6)	6.6 (5.1-8.8)	
<b>Age stratified Alb quotients</b>	-50	Mdn (N)	(0)	(0)	(0)	5.1 (4)	(0)	(0)	7.3 (2)	(0)	(0)	9.9 (1)	5.2 (1)
	51-60	Mdn (N)	(0)	(0)	(0)	5.5 (68)	6 (3)	7.4 (13)	4.7 (13)	7.8 (2)	6.1 (1)	7.1 (4)	6.7 (17)
	61-70	Mdn (N)	5.3 (109)	5.8 (5)	5.9 (164)	5.3 (58)	6.5 (53)	7.1 (31)	6.2 (24)	8.2 (14)	6.5 (6)	5.1 (16)	6.2 (69)
	71-80	Mdn (N)	5.8 (157)	5.5 (14)	6.1 (353)	(0)	7.3 (149)	7.9 (116)	6.9 (12)	7 (27)	6 (13)	8.3 (18)	6.9 (120)
	81-90	Mdn (N)	6.3 (26)	2.8 (1)	7.5 (148)	(0)	7.4 (148)	7.5 (92)	7.8 (5)	7.6 (7)	5.8 (3)	6.7 (9)	6.7 (61)
	90+	Mdn (N)	(0)	(0)	4.4 (1)	(0)	9.6 (9)	6.9 (3)	(0)	(0)	(0)	(0)	6.4 (3)