

The Relationship between Markers of Inflammation and Degeneration in the Central Nervous System and the Blood-Brain Barrier Impairment in Alzheimer's Disease

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Abstract.

Background: It is known that YKL-40—a marker of glial inflammation, and VILIP-1—a marker of neuronal injury, reflect functional and structural changes in AD brains, although there is limited data concerning their potential influence on blood-brain barrier (BBB) homeostasis.

Objective: Therefore, the aim of our study was to investigate the relationship between markers of inflammation and degeneration in the central nervous system (CNS) of patients with AD and mild cognitive impairment (MCI) as well as immunological response in CNS and BBB function.

Methods: Cerebrospinal fluid (CSF) concentrations of proteins tested were determined in 45 AD patients, 18 MCI subjects, and 23 non-demented controls using ELISA method.

Results: CSF concentrations of YKL-40 were significantly higher in MCI and AD patients, whereas CSF levels of VILIP-1 were statistically higher in the AD group as compared to the subjects without cognitive deficits. Elevated concentrations of YKL-40 correlated significantly with increased albumin quotient and decreased $A\beta_{42/40}$ ratio in AD patients and with IgG quotient in the total study group. We did not find a relationship between VILIP-1 and immunological parameters reflecting BBB dysfunction and humoral immune response.

Conclusion: Our findings indicate that YKL-40 may contribute to decreased stability and increased permeability of BBB in AD patients. It is assumed that YKL-40 is implicated in the development of brain barriers, although its precise mechanism of action in the BBB disruption remains unrevealed. Further studies on larger groups of patients are required to confirm our hypothesis.

Keywords: Alzheimer's disease, blood-brain barrier disruption, cerebrospinal fluid, humoral immune response in the CNS, mild cognitive impairment, neuroinflammation, neurodegeneration

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INTRODUCTION

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disease with a variety of pathological changes within neuronal tissue which begins even 10–15 years before the onset of very mild dementia [1, 2]. The major histological hallmarks of AD are neurofibrillary tangles (NFTs) and senile plaques within the brain structures, especially in regions responsible for cognition [3, 4]. A gradual accumulation of amyloid- β (A β) within brain tissue induces a complex sequence of events including inflammatory changes, gliosis, neuritic and synaptic changes, loss of transmitters, and formation of NFTs [5], which cause not only the pathophysiological changes in the brain tissue but also lead to the release of various proteins reflecting pathological processes occurring in the brain during the development of AD [5]. It is possible that these proteins may have an impact on the blood–brain barrier (BBB) disruption and intensified humoral immunity in the central nervous system (CNS) in some AD patients.

In recent years, investigations concerning the altered BBB homeostasis and immunological response of the CNS in demented patients have aroused researchers' attention [6–9]. BBB is composed of a widespread network of endothelial cells, neurons, astrocytes, and glial cells, including microglia [10, 11]. Interactions between these cells guarantee a proper environment for brain function. Disturbances in the function of these structures may cause a loss of the BBB homeostasis, resulting in an overproduction of pro-inflammatory proteins and inhibitors of the cerebral blood flow by endothelial cells, which exacerbates neuronal damage as well as accumulation and activation of microglia [10]. Microglia cells and astrocytes are primarily responsible for the activation of immunological response within neuronal tissue and their accumulation has been found in the vicinity of A β deposits [12]. Pathological deposits of insoluble A β induce the activation of microglia and trigger an inflammatory process in the CNS with the release of inflammatory mediators [13]. Recent studies suggest that microglia activation may be associated with the BBB disturbance [10]. Literature data indicates that CSF YKL-40 may reflect the activity of glial inflammation in AD [14], and more importantly, is involved in the development of brain barriers [15]. However, there is limited data concerning its influence on the BBB disruption.

It is believed that the main cause of the BBB disruption in AD is the pathological process guided by A β , but some recent investigations imply that neurodegeneration can also be involved in the BBB damage [16, 17]. According to available studies, neuronal loss and degeneration in the CNS may be reflected by tau proteins (t-tau and pTau) as well as visinin-like protein 1 (VILIP-1) [18–20]. VILIP-1 belongs to the neuronal calcium sensor (NCS) proteins family, and is involved in the signaling pathways within neuronal tissue, synaptic plasticity as well as the degeneration and loss of neurons [21–23]. Thus, VILIP-1 is perceived as a helpful biological marker of neuronal injury [24].

Considering that AD pathology is heterogeneous and a variety of mechanisms such as neurodegeneration, neuroinflammation, and the dysfunction of BBB may play important roles in it, it seems reasonable to assess the influence of the glial marker of CNS inflammation—YKL-40 and neurodegeneration marker—VILIP-1 on the BBB disruption in AD. According to our knowledge, there is no data in one study concerning the effects of both YKL-40 and VILIP-1 on the BBB. Therefore, this is the first study that analyzed a possible relationship between these two markers (YKL-40, VILIP-1) and parameters of neuroimmunological status, such as albumin and immunoglobulin quotients.

MATERIAL AND METHODS

Patients

We included in the study 45 patients with AD (36 females and 9 males), and eighteen subjects with MCI (10 females and 8 males) as well as 23 healthy, elderly individuals with no cognitive impairment as a control group (15 females and 8 males). All study subjects were recruited from the Department of Neurology of Jagiellonian University in Kraków, Poland as part of the BiomarkAPD – EU Joint Programme for Neurodegenerative Disease Research (JPND). The study was approved by the Ethics Committee of Jagiellonian University (No. KBET/288/B/2011). Informed consent was obtained from all study participants. A general physical, neurological, psychiatric, and extensive neuropsychological examination, routine blood and biochemical screening tests, and neuroimaging [magnetic resonance imaging (MRI)/computed tomography (CT)] were used in the clinical evaluation of the patients.

Table 1
Characteristics of study groups

Variable tested	Median (min-max values)			<i>p</i> (Kruskal-Wallis test)	<i>p</i> (Dwass-Steale-Critchlow-Fligner test)		
	AD (<i>n</i> = 45)	MCI (<i>n</i> = 18)	Controls (<i>n</i> = 23)		AD versus Controls	AD versus MCI	MCI versus Controls
MMSE	22.0 (0.0–28.0)	27.5 (20.0–30.0)	28.0 (24.0–30.0)	<0.001*	<0.001*	<0.001*	<0.001*
Aβ _{1–42} [pg/mL]	500 (197–994)	802 (382–1878)	924 (357–2122)	<0.001*	<0.001*	0.005*	0.859
Aβ _{42/40} ratio	0.033 (0.020–0.046)	0.045 (0.029–0.075)	0.068 (0.023–0.093)	<0.001*	<0.001*	<0.001*	0.004*
t-tau [pg/mL]	667 (331–1722)	389 (209–994)	222 (102–576)	<0.001*	<0.001*	<0.001*	0.001*
pTau ₁₈₁ [pg/mL]	83 (37–193)	57 (34–97)	37 (24–92)	<0.001*	<0.001*	0.001*	0.002*

*Statistically significant (*p* < 0.05).

AD and MCI diagnoses were established according to the recommendations of the National Institute on Aging – Alzheimer’s Association (NIA-AA) working groups [25, 26]. Moreover, the Erlangen Score based on commonly used AD biomarkers such as Aβ_{1–42}, Aβ_{42/40} ratio, t-tau, and pTau₁₈₁ was used to facilitate the classification of patients into appropriate study groups. The Erlangen Score interpretation algorithm had been described previously [27]. The characteristics of study groups are presented in Table 1.

The MCI criteria define the condition as a stage of AD preceding the development of fully symptomatic dementia. MCI subjects display changes in their functioning, documented as cognitive decline greater than that expected for the patients’ age and educational level in at least one cognitive aspect, although they may function independently in a social or occupational environment. Factors excluding demented subjects (AD and MCI) from the study were a history or presence of vascular diseases (i.e., stroke, aortic aneurysm, intracranial aneurysm, cerebral hemorrhage, or arteriovenous malformation), clinically significant central nervous system trauma, intracranial tumors and other malignant neoplasms, severe infections or systemic autoimmune disorders as well as neurological diseases and relevant cerebral abnormalities as assessed by magnetic resonance imaging (MRI) and/or computed tomography (CT). Moreover, the presence of metabolic/endocrine disorders and decreased levels of folic acid or vitamin B12 as well as alcohol and/or substance abuse or dependence (except nicotine use) constituted exclusion criteria. Deformity of the lumbosacral spine was a contraindication to lumbar puncture.

Patients with subjective memory complains who did not fulfill the MCI criteria or suffered from

recurrent headaches were included in the control group. The detailed inclusion criteria for non-demented controls were: the ability to cooperate and to complete all aspects of the study, e.g., MRI/CT, lumbar puncture, and neuropsychological assessment, visual and auditory perception allowing for study participation (with permission to use hearing aids and eyeglasses), independence in domestic/social/professional functioning without any disturbances, no memory deficits defined by the Mini-Mental State Examination (MMSE) score above 24 and the Clinical Dementia Rating (CDR) scale score of 0.

Protein analyses

CSF samples for analysis were obtained by lumbar puncture. All samples were collected into polypropylene tubes, centrifuged, aliquoted, and frozen at –80°C until assayed. Concentrations of all the tested biomarkers in CSF were measured using an enzyme-linked immunosorbent assay (ELISA). YKL-40 levels were assessed with Quidel kits (San Diego, USA) in diluted CSF samples (1 : 5), whereas VILIP-1 concentrations were measured with BioVendor kits (Brno, Czech Republic) in undiluted CSF samples. The concentrations of tau proteins in CSF (t-tau and pTau₁₈₁) were determined using Fujirebio tests (Ghent, Belgium), while levels of Aβ_{1–40} and Aβ_{1–42} were assayed with IBL kits (Hamburg, Germany).

In all study subjects, the simultaneous nephelometric quantification (Immage 800 analyzer, Beckman Coulter, Krefeld, Germany) of albumin and immunoglobulins in serum and CSF was conducted to estimate albumin (Qalb) and immunoglobulins quotients (QIgG, QIgM, QIgA). Evaluation of these

quotients was conducted according to Reiber [28]. Individuals with Qalb values exceeding the reference range, according to the age-related equation $Qalb = 4 + (\text{age [years]}/15)$, were excluded from the study. Immunoglobulins (IgG and IgM) concentrations below the limit of detection were calculated as zero values.

Statistical analysis

CSF concentrations of the measured biomarkers did not follow a normal distribution in preliminary analysis with the Shapiro-Wilk test. Therefore, the non-parametric Kruskal-Wallis and Mann-Whitney tests were used for comparisons between three or two groups tested, respectively. Moreover, when significant differences were shown in the Kruskal-Wallis test, the *post hoc* Dwass-Steele-Critchlow-Fligner test was applied to determine which groups were different. Statistical significance was established at p value below 0.05. The obtained results are shown as medians and minimum-maximum ranges. We also performed the non-parametric Spearman rank correlation test for the analysis of correlations between the tested variables (patients' age, MMSE, NDD biomarkers, and CSF levels of YKL-40, VILIP-1, and immunological parameters).

RESULTS

CSF concentrations of YKL-40 and VILIP-1 in respect to AD CSF profile

Concentrations of YKL-40 and VILIP-1 in the CSF are shown in Fig. 1a, b. Statistically significant

differences were observed for CSF YKL-40 and CSF VILIP-1 levels between AD, MCI and controls in the Kruskal-Wallis test ($p=0.007$ and $p<0.001$, respectively). In the *post hoc* Dwass-Steele-Critchlow-Fligner test, CSF YKL-40 levels were significantly higher in MCI (418 ng/mL) and AD (387 ng/mL) patients as compared to controls (292 ng/mL), $p<0.030$ and $p<0.008$, respectively. However, the highest concentration of YKL-40 was observed in MCI individuals. CSF concentrations of VILIP-1 were significantly elevated only in AD group (118 pg/mL) in comparison to the subjects without cognitive deficits (41 pg/mL), $p<0.001$ in the *post hoc* Dwass-Steele-Critchlow-Fligner test.

Correlations of CSF YKL-40 and VILIP-1 concentrations with neurochemical dementia diagnostics biomarkers

In the total study group, CSF levels of YKL-40 significantly correlated with age ($r=0.47$, $p<0.001$), Erlangen Score ($r=0.30$, $p=0.005$), $A\beta_{42/40}$ ratio ($r=-0.24$, $p=0.023$), t-tau ($r=0.48$, $p<0.001$), pTau₁₈₁ ($r=0.60$, $p<0.001$), and VILIP-1 ($r=0.47$, $p<0.001$) (Table 2). CSF concentrations of VILIP-1 were significantly associated with age ($r=0.32$, $p=0.003$), Erlangen Score ($r=0.50$, $p<0.001$), $A\beta_{42/40}$ ratio ($r=-0.43$, $p<0.001$), t-tau ($r=0.72$, $p<0.001$), pTau₁₈₁ ($r=0.74$, $p<0.001$), and MMSE scores ($r=-0.42$, $p<0.001$).

Furthermore, we assessed the relationships between concentrations of the tested biomarkers (YKL-40, VILIP-1), clinical data, and CSF NDD biomarkers such as $A\beta_{1-42}$, $A\beta_{42/40}$ ratio, t-tau, and

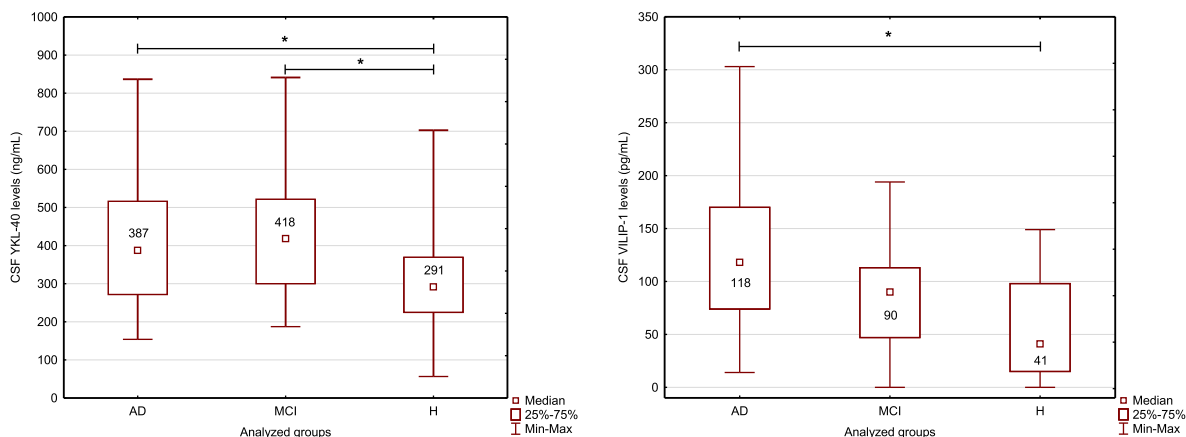


Fig. 1. (a) CSF YKL-40 levels in study groups. (b) CSF VILIP-1 levels in study groups. *Statistically significant ($p<0.05$).

Table 2
Spearman's correlations between tested variables in the total study group

Total study group	Erlangen Score	MMSE	A β_{1-42}	A $\beta_{42/40}$	t-tau	pTau ₁₈₁	VILIP-1	YKL-40	Qalb	QIgG
Age										
r	0.28	-0.14	0.02	-0.24	0.27	0.29	0.32	0.47	0.27	0.16
p	0.008*	0.213	0.848	0.028*	0.013*	0.007*	0.003*	<0.001*	0.013*	0.135
Erlangen Score										
r	1.00	-0.74	-0.58	-0.79	0.80	0.72	0.50	0.30	0.18	0.18
p		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.005*	0.092	0.095
MMSE										
r		1.00	0.53	0.59	-0.65	-0.52	-0.42	-0.10	-0.13	-0.13
p			<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.349	0.224	0.216
A β_{1-42}										
r			1.00	0.75	-0.24	-0.13	-0.03	0.15	0.11	0.05
p				<0.001*	0.027*	0.250	0.805	0.154	0.295	0.668
A $\beta_{42/40}$										
r				1.00	-0.58	-0.53	-0.43	-0.24	0.00	0.01
p					<0.001*	<0.001*	<0.001*	0.023*	0.985	0.904
t-tau										
r					1.00	0.94	0.72	0.48	0.31	0.25
p						<0.001*	<0.001*	<0.001*	0.004*	0.022*
pTau ₁₈₁										
r						1.00	0.74	0.60	0.33	0.23
p							<0.001*	<0.001*	0.002*	0.029*
VILIP-1										
r							1.00	0.47	0.20	0.13
p								<0.001*	0.062	0.243
YKL-40										
r								1.00	0.38	0.27
p									<0.001*	0.011*
Qalb										
r									1.00	0.94
p										<0.001*

*Statistically significant ($p < 0.05$).

pTau₁₈₁ in the group of AD patients. CSF YKL-40 levels were found to correlate significantly with A $\beta_{42/40}$ ratio ($r = -0.35$, $p = 0.019$), t-tau ($r = 0.41$, $p = 0.005$), pTau₁₈₁ ($r = 0.58$, $p < 0.001$), VILIP-1 ($r = 0.41$, $p = 0.005$), and age ($r = 0.41$, $p = 0.006$). VILIP-1 significantly correlated with t-tau ($r = 0.64$, $p < 0.001$), pTau₁₈₁ ($r = 0.70$, $p < 0.001$), and A β_{1-42} ($r = 0.41$, $p = 0.005$).

Similar observations were made in MCI subjects. YKL-40 and VILIP-1 concentrations in CSF significantly correlated with t-tau ($r = 0.78$, $p < 0.001$; $r = 0.56$, $p = 0.017$, respectively), pTau₁₈₁ ($r = 0.86$, $p < 0.001$; $r = 0.52$, $p = 0.028$, respectively), and each other ($r = 0.47$, $p = 0.048$). YKL-40 also correlated with A β_{1-42} ($r = 0.56$, $p = 0.016$). In addition, the levels of CSF YKL-40 and CSF VILIP-1 in cognitively normal subjects significantly correlated with age ($r = 0.53$, $p = 0.010$; $r = 0.56$, $p = 0.005$, respectively), t-tau ($r = 0.56$, $p = 0.006$; $r = 0.87$, $p < 0.001$, respectively), pTau₁₈₁ ($r = 0.62$, $p = 0.002$; $r = 0.79$, $p < 0.001$, respectively), and each other ($r = 0.43$, $p = 0.039$). VILIP-1 also correlated with Erlangen Score ($r = 0.69$, $p < 0.001$).

Associations between YKL-40, VILIP-1, and neuroimmunological status of study groups

We investigated the relationships between CSF YKL-40, VILIP-1, and immunological parameters reflecting pathological processes in the brain tissue, such as albumin and immunoglobulin quotients.

The status of ongoing neuroimmunological processes in the CSF of patients was established based on the CSF/serum albumin ratio (Qalb), reflecting the BBB integrity and immunoglobulins quotients (QIgG, QIgM, QIgA), indicating the intrathecal synthesis of proteins in CSF as a sign of intensified humoral immunity. All study subjects (AD, MCI, controls) had preserved BBB integrity based on values of the albumin quotient (established with regard to age-related norms). However, we observed significant differences in Qalb between the three tested groups analyzed using the Kruskal-Wallis test (Fig. 2). Additionally, in the Dwass-Steele-Critchlow-Fligner test (*post-hoc* test), we found a tendency to higher Qalb values in MCI as well as AD patients in comparison to non-demented

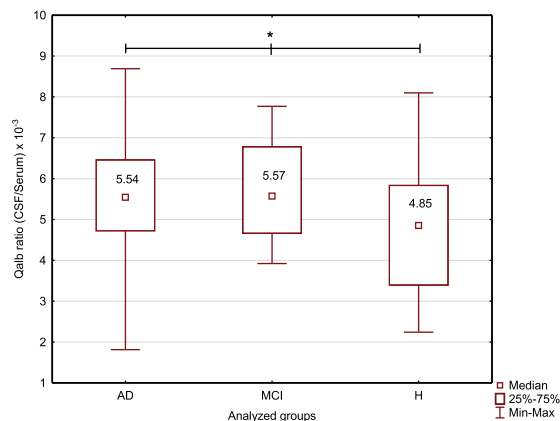


Fig. 2. Values of Qalb in study groups. * $p=0.046$ (Kruskal-Wallis test).

subjects (Table 3). Indeed, it can be suspected that significant differences in the *post-hoc* test were not obtained due to the limited number of patients studied. Owing to the fact that these values were marginally close to the level of significance ($p=0.07$), we performed the Mann-Whitney test. The results of this test showed significantly higher Qalb values in AD and MCI patients in comparison with non-demented subjects ($p=0.031$, $p=0.027$, respectively). Furthermore, we demonstrated increased values of the immunoglobulins CSF/serum quotients (QIgG, QIgM, QIgA) in MCI and AD individuals as compared to healthy controls, although the differences were not significant (Table 3). QIgG values tended to differentiate between all study groups in Kruskal-Wallis test ($p=0.07$). In addition, in the Mann-Whitney test QIgG values achieved a significance level in demented patients (AD versus controls, $p=0.049$ and MCI versus controls, $p=0.041$).

It is well established that increased Qalb is related to the dysfunction of BBB, and hence we investigated whether Qalb alterations may correlate with levels

of the classical AD biomarkers and YKL-40 as well as VILIP-1 in the CSF of AD and MCI patients. In the total study group, we demonstrated a correlation between Qalb and t-tau, pTau₁₈₁ proteins ($r=0.31$, $p=0.004$; $r=0.33$, $p=0.002$, respectively) as well as YKL-40 ($r=0.38$, $p<0.001$) (Table 2). Furthermore, relationships between CSF QIgG and YKL-40 concentrations ($r=0.25$, $p=0.023$) as well as tau proteins (tau; $r=0.25$, $p=0.022$, pTau₁₈₁; $r=0.23$, $p=0.029$) were shown. Interestingly, we revealed an association between Qalb and YKL-40 in AD patients ($r=0.36$, $p=0.014$). In order to enhance our knowledge about the relationship between YKL-40 and Qalb, we established their quotient. The ratio provides more precise information since it is known to eliminate the influence of variations in blood concentrations of these proteins on their levels in the CSF. We found correlations between YKL-40/Qalb and A β _{42/40} ratio ($r=-0.43$, $p=0.003$) as well as tau proteins (t-tau; $r=0.31$, $p=0.038$ and pTau₁₈₁; $r=-0.39$, $p=0.007$, respectively) in AD group. We did not observe any associations between markers of neurodegeneration (VILIP-1, tau proteins) and neuroimmunological parameters in the CSF and serum such as Qalb and QIg in any study subjects (AD + MCI + healthy) (Table 2).

DISCUSSION

The etiology of AD is very heterogeneous, involving multiple pathological processes such as amyloid production and deposition, neurodegeneration, neuroinflammation, and BBB dysfunction appearing decades before disease onset. Understanding the exact neurobiological mechanisms underlying AD pathology may help to establish a biomarker for the early diagnosis or at least evaluation of susceptibility to dementia development. Considering the above,

Table 3
Neuroimmunological status of study groups

Immunological parameters	AD ($n=45$)	MCI ($n=18$)	Controls ($n=23$)	p (Kruskal-Wallis test)	p (Dwass-Steele-Critchlow-Fligner test)		
	Median (range)	Median (range)	Median (range)		AD versus Controls	AD versus MCI	MCI versus Controls
Qalb	5.54 (1.81–8.69)	5.57 (3.92–7.77)	4.85 (2.24–8.10)	0.046*	0.079	0.923	0.070
QIgG	2.72 (0.83–5.69)	2.98 (1.66–3.95)	2.46 (0.00–3.91)	0.072	0.121	0.885	0.101
QIgM	0.37 (0.00–5.30)	0.43 (0.00–1.46)	0.31 (0.00–0.91)	0.203	0.333	0.863	0.202
QIgA	1.29 (0.35–4.23)	1.39 (0.82–2.12)	1.18 (0.44–7.13)	0.407	0.509	0.957	0.417

*Statistically significant ($p<0.05$).

we firstly investigated CSF concentrations of YKL-40 and VILIP-1 in AD and MCI patients as well as in non-demented controls and evaluated their correlations with the classical AD biomarkers ($A\beta$ and tau proteins). Secondly, we verified if there were associations between CSF levels of neuroinflammation protein (YKL-40), marker of neurodegeneration (VILIP-1), and other neuroimmunological parameters, reflecting the activity of immunological response in CSF and BBB functioning. The levels of AD biomarkers such as $A\beta_{1-42}$, t-tau, and pTau₁₈₁ were used in the diagnosis of patients with AD and MCI, and to exclude these diseases in the healthy control group. In addition, albumin and IgG quotients are used in the routine diagnosis of AD and MCI. Hence, these levels are *a priori* biased toward the diagnostic groups and therefore caution in the interpretation of their correlation with the other measures should be taken.

The findings of our study confirmed significant differences in CSF concentrations of both YKL-40 and VILIP-1 in all study groups, similarly to the results obtained by other researchers [19, 29–32]. Some evidence suggests that both biomarkers are useful in the early diagnosis of AD, although CSF levels of YKL-40 seem to increase at an earlier stage in the brain during the development of dementia [33]. Our results support this hypothesis, as we revealed higher CSF YKL-40 levels in MCI and AD patients in comparison with cognitively normal individuals. However, YKL-40 concentration was the highest in the MCI group and reached a plateau in AD. Elevated levels of YKL-40 reflect an intensified activity of neuroinflammatory processes at the onset of early dementia stages, probably prior to the MCI phase, and continue increasing (to a lesser degree) during the symptomatic stages of AD. Similar results have been presented by Kester et al. and Antonell et al. [29, 34].

In this context, it is worth noting that the overproduction of YKL-40 by activated astrocytes and/or microglia is closely associated with amyloid plaques and with NFT pathology [35]. In agreement with that, we established a significant correlation between both biomarkers (YKL-40 and VILIP-1) in MCI and AD patients, thus confirming the fact that inflammatory and neurodegenerative processes already co-occur in the early stages of AD. However, a significantly higher concentration of VILIP-1, a marker of neuronal injury, and correlation with other CSF markers of neuronal damage (t-tau and pTau₁₈₁) was observed in full-blown AD, which indicates that VILIP-1 levels may increase as the clinical stage of the disease

is approached. Our results are in agreement with other studies [19, 32, 36, 37]. Both YKL-40 and VILIP-1 seem to be valuable markers in AD diagnosis as well as in the assessment of disease progression. As far as we are concerned, this is the first study to show the relationship between CSF levels of YKL-40, VILIP-1, and increased concentrations of t-tau and pTau₁₈₁ as well as decreased $A\beta_{1-42}$ based on the Erlangen Score in the whole study group. These findings may suggest the potential utility of the proteins in the evaluation of disease severity.

Despite the fact that some recent studies have demonstrated that neurodegeneration and neuroinflammation may be associated with increased BBB permeability during AD progression, little is known of which particular proteins related to these processes may have an influence on the BBB [38, 39]. Therefore, according to our best knowledge, this is the first study that analyzed the relationship between markers of neuroinflammation (YKL-40), neurodegeneration (VILIP-1), and deterioration of BBB as well as humoral intrathecal response. In our study, all subjects (AD and MCI patients) had a preserved BBB, although 76% subjects with AD and 72% participants with MCI revealed increased Qalb ratios in comparison to individuals without cognitive deficits. According to literature data, impaired BBB integrity occurs with increasing age, during normal aging, and it begins in the hippocampus, a region of the brain which is also affected in AD [8]. However, our and other findings [8] have shown that this process is accelerated in patients with dementia as compared to the subjects without cognitive deficits. Thus, we may speculate that increasing Qalb, reflecting decreased BBB integrity, can be associated with the development of early pathological changes leading to dementia.

Given the hypothesis that the deposition of $A\beta$ peptides in blood vessels induces pro-inflammatory and cytotoxic events and, as a consequence, may lead to increased BBB permeability [40], we analyzed the relationship between Qalb, YKL-40, $A\beta_{1-42}$, and the $A\beta_{42/40}$ ratio in MCI and AD patients. In line with the above hypothesis, we found correlations between YKL-40 and the $A\beta_{42/40}$ ratio and Qalb as well as an association between the YKL-40/Qalb quotient and the $A\beta_{42/40}$ ratio in AD patients. It seems that the overproduction of toxic $A\beta$ oligomers leads, in time, to the initiation of inflammatory processes in CNS and the release of pro-inflammatory proteins such as YKL-40, which contributes to decreased sta-

bility and increased permeability of the BBB. On the other hand, the correlation between YKL-40 and Qalb may also point to the conclusion that BBB alteration induced by the inflammatory process in AD may increase amyloid deposition and contribute to disease progression. An alternative explanation for the correlation between CSF YKL-40 and Qalb could be that more YKL-40 from the periphery leaks in across a slightly impaired BBB. YKL-40 seems to play a key role in the development of brain barriers [15]. Moreover, it is one of the factors regulating the migration of peripheral mononuclear blood cells across the BBB and is involved in controlling local angiogenesis and peripheral cell access to the forebrain [15]. Our study documented that increased CSF YKL-40 can be associated with impaired BBB function in demented patients, but its exact mechanism of action in BBB impairment remains unknown.

We also investigated the relationship between levels of the marker reflecting inflammatory processes in the CNS (YKL-40) and the activity of humoral immune response in the brain (based on immunoglobulin quotients). In contrast to chronic CNS inflammatory diseases, we observed mild intrathecal immunoglobulins synthesis in AD subjects. Additionally, we demonstrated a correlation between QIgG and YKL-40 in the whole study group. Literature data concerning intrathecal Ig synthesis in AD patients are conflicting [41–43]. However, some studies have demonstrated accelerated immune response within the CNS accompanied by the blood–CSF barrier dysfunction in AD patients without identifying any sound reasons for the intrathecal humoral response [6, 42, 43].

Some evidence from animal and human studies have suggested that also tau protein may initiate increased BBB permeability [16, 17, 44, 45]. In order to verify the hypothesis of whether neurodegeneration has an impact on the BBB stability, we investigated two groups of independent markers of neuronal damage such as tau proteins (t-tau, pTau₁₈₁) and VILIP-1, which are specific for the brain and have different mechanisms of action. We did not reveal any relationships between Qalb and markers of neurodegeneration in our demented patients (MCI and AD), although we found correlations between the marker of neuroinflammation and tau proteins as well as YKL-40/Qalb and tau proteins in MCI and AD cases. This fact may indicate that there is a more complex phenomenon also involving other mechanisms necessary to trigger the deterioration of the BBB function. Nowadays, CSF analyses of different

proteins associated with specific pathomechanisms underlying neurodegenerative disease are needed not only to improve their diagnosis but also to find effective treatment.

Conclusion

Summarizing, our findings suggest altered BBB function and slightly intensified humoral immune response in AD patients, which may imply that both phenomena could be involved in the development and, possibly, progression of dementia. Moreover, we confirmed significantly increased concentrations of glial inflammation (YKL-40) and neurodegeneration (VILIP-1) biomarkers in AD patients, but only YKL-40 seems to contribute to decreased stability and increased permeability of the BBB. It is suggested that YKL-40 is involved in the development of brain barriers, although its exact mechanism of action in the BBB damage is still unknown. Therefore, additional studies are needed to confirm our hypothesis and clarify molecular mechanism of YKL-40 in BBB leakage in patients with early AD. Understanding the exact neurobiological mechanisms underlying AD pathology may help to provide a biomarker for the early diagnosis or, at least, for evaluation of susceptibility to dementia development. Considering the fact that AD is a heterogeneous disease, a combination of various CSF biomarkers tracking different pathological processes in the AD brain might improve the classification of patients with neurodegenerative diseases. Furthermore, it may be important not only for routine clinical practice but also for clinical trials.

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