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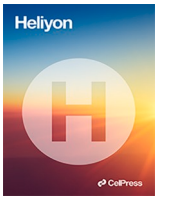

Research article

Blood levels of cytokines highlight the role of inflammation in Alzheimer's disease

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ABSTRACT

Inflammation and angiogenesis have been defined as potential mechanisms associated with clinical progression from a cognitively normal state to Alzheimer's disease (AD). In this observational case-control study, we aimed to determine plasma levels of cytokines as indicators of inflammation involved in cognitive decline. We measured 30 plasma proteins in 49 controls

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(CTL), 36 individuals with mild cognitive impairment (MCI) and 52 patients diagnosed with probable AD. After applying strict filters for quantification limits, only 13 analytes were included in the analysis. Kruskal–Wallis tests showed significant differences between diagnostic groups for nine cytokines (IL-16, IL-7, VEGF, IL-8, eotaxin, MCP-1, MCP-4, MDC and TARC). Non-parametric MANCOVA showed that sex and diagnosis affected cytokine levels in the blood. To determine the sensitivity and specificity of the markers, we performed receiver operating characteristic (ROC) curve analysis. Only those analytes that showed an area under the curve (AUC) $\geq$ 0.70 were included in the multivariate logistic regression models to better understand the contribution of cytokines to clinical status. Three models: 1) CTL vs. AD; 2) CTL vs. MCI, and 3) MCI vs. AD were developed, with sex and age as covariates. In each model, two cytokines remained significantly different (model 1: IL-16 and MDC; model 2: eotaxin and MDC and model 3: IL-7 and VEGF). Taken together, this report identifies a set of plasma markers of inflammation and strengthens the role of glial biology in different clinical stages of AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of slow onset and progressive deterioration, neuropathologically characterized by progressive brain atrophy and death of specific neuronal populations. The neuritic plaques, composed mainly of the aggregation of amyloid beta (Aβ) peptides [1], as well as neuronal cytoskeletal changes due to the accumulation of hyperphosphorylated tau [2] and neurogliosis, particularly in the form of microglia and astrocytic activation [3], are hallmarks of the disease. The role of glial cells, particularly microglia and astrocytes, in the pathophysiology of AD has been extensively studied, with evidence suggesting that they play a pathogenic role and activate inflammatory pathways [4]. A considerable body of evidence indicates that AD may be conceived of as a chronic inflammatory condition affecting the central nervous system (CNS). Early reports highlighted the presence of inflammation in the CNS of individuals with preclinical AD [5,6], proposing that this inflammation may contribute to disease progression. Later, the role of CNS inflammation in AD was explored suggesting that it evolves over time and may be a promising therapeutic target [7]. Furthermore, evidence indicates that aberrant cytokine levels in distinct brain regions may contribute to the onset and progression of AD [8]. The hypothesis that these inflammatory markers may permeate from the brain into the periphery, resulting in systemic inflammation, has been proposed as a potential mechanism for exacerbating neurodegenerative processes and cognitive decline [9].

A common feature of AD is dysfunction of the blood-brain barrier (BBB), which can result in the leakage of inflammatory markers from the brain into the periphery [10]. This bidirectional communication between the brain and the periphery, mediated by the BBB, highlights the potential role of peripheral biomarkers in reflecting neuroinflammation in AD [11]. In conclusion, the immune system plays a crucial role in AD by contributing to its aetiology and pathogenesis [12,13]. Furthermore, the importance of the immune system in AD has also been confirmed by the contribution of rare genetic variants in the microglial genes TREM2 (Triggering Receptor Expressed on Myeloid cell), PLCG2 (Phospholipase C Gamma 2) and ABI3 to the susceptibility for developing sporadic AD (Sims et al., 2017; Dalmasso et al., 2019).

In addition, it was shown that the meningeal immune system plays a significant role in maintaining the CNS in both health and disease [14]. The meninges, which envelop the CNS, are home to a diverse range of immune cells (T lymphocytes, B lymphocytes, neutrophils, dendritic cells, macrophages, mast cells) under healthy conditions and in inflammatory and neurodegenerative diseases [15]. These meningeal immune cells are also involved in CNS immune surveillance, with meningeal lymphatics serving as a conduit for CNS-derived antigens to reach the peripheral immune system [16].

Research on the relationship between cytokines plasma levels and AD staging has yielded mixed results. Several case-control studies have identified potentially relevant blood biomarkers, but the reproducibility of these results has not been confirmed [17]. In addition, a number of reports have shown that changes in circulating markers may be associated with progression from mild cognitive impairment (MCI) to AD [18]. MCI refers to a state of cognitive impairment that is below the normal level for an individual's age, but above that observed in individuals with AD. The role of inflammatory mechanisms in AD has been highlighted [8] and several circulating inflammatory proteins have been shown to be associated with cognitive domain scores [19]. Nevertheless, inconsistent findings regarding peripheral cytokine dysregulation have been noted, suggesting the need for methodological standardization and longitudinal sampling to clarify the impact of cytokines in AD progression [20].

Recently, a task force of the Alzheimer's Association (USA) reviewed the criteria for diagnosis and staging of AD, taking into account the development of plasma-based biomarkers, some of which have demonstrated diagnostic capacity. According to this report [21], biomarkers of inflammatory and immune processes in AD are divided into two groups depending on the type of reactive cells, either astrocytes or microglia. In this context, studies have shown that blood GFAP levels are higher in individuals with AD or MCI compared to healthy cognitively normal individuals suggesting that astrocytic damage or activation may begin in the pre-symptomatic stage of the disease [22,23]. In particular, plasma GFAP has been found to be a more sensitive biomarker than CSF GFAP in indicating Aβ pathology in the early stages of AD [24]. Taken together, these findings suggest that blood levels of GFAP may be a valuable tool for the early detection and monitoring the progression of AD.

In terms of microglial reactivity, different studies have consistently shown an association between CSF levels of soluble TREM2 (sTREM2) and neuroinflammation in AD. It was found that sTREM2 levels were associated with age-related neuroinflammation, tauopathy and markers of neurodegeneration [25,26]. However, a plasma cytokine signature specific for microglial and/or meningeal

immune system reactivity was not defined.

The aim of this work was determine the plasma levels of cytokines related to neurogliosis, using an ultrasensitive high throughput platform (multiplex electrochemiluminescence ELISA), in cognitively normal subjects and individuals with MCI or probable AD for a better understanding of the role of glial biology in different clinical stages of the disease.

2. Materials and methods

2.1. Ethics and consent

This observational study was conducted in accordance with the Code of Ethics of the World Medical Association for studies involving humans (Declaration of Helsinki) and the STROBE guidelines [27]. The protocols CBFIL#22 and #0681-N-21-dated April 14, 2021 received the approval from the ethical committee of Fundación Instituto Leloir (Argentina) and of Hospital Regional Universitario de Málaga (Spain), respectively. All participants and/or family members gave their informed consent.

2.2. Volunteers recruitment, sample collection and handling protocol

The samples intended for this study were obtained from two independent cohorts, one from Argentina and the other one form Spain. The Argentine samples were recruited at the following centers: Hospital de Clínicas "José de San Martín" (Buenos Aires City), Hospital HIGA-Eva Perón (General San Martín), Hospital "El Cruce" (Florencio Varela), and Neuropsychiatric Clinic "Nuestra Señora de Las Nieves" (Buenos Aires City). The Spanish samples were recruited at the Neurology Service of the Hospital Regional Universitario de Málaga (Málaga). All participants included in this study were subjects of both sexes, literates and 60 years of age or over. All enrolled subjects underwent clinical and neuropsychological examinations, and blood draw for chemical profile determination including cholesterol and glucose measurement, thyroid function (T3, T4, TSH), Vit B12 and folic acid, calcium, phosphorus, homocysteine, VDRL and HIV, Hepatitis B, Hepatitis C and complete blood count.

Inclusion Criteria for Controls (CTL): subjects with a normal neurological examination, no cognitive changes affecting daily living, and normal cognitive status as determined by the Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR). The maximum score on the MMSE is 30, and a score of 27 or higher is considered normal. A CDR = 0 indicates no dementia.

Inclusion criteria for subject with probable Alzheimer's disease (AD): diagnosis of probable AD followed diagnostic criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [28,29]. Subject showed a MMSE $\geq$15 and $\leq$ 26 and a CDR of 1–3. All of them with a clear history of cognitive and functional impairment for at least 1 year that was documented in medical records or documented by an informant who knows the participant well.

Inclusion criteria for subjects with mild cognitive impairment (MCI): participants were classified by the Andalusian Network for Clinical and Translational Research in Neurology [NEURO-RECA] on the basis of concerns about cognitive decline reported by the patient and/or informant and assessment by a neuropsychologist following guidelines previously reported [30] (a score of 1–1.5 standard deviations below the mean on cognitive tests and a CDR = 0.5). In addition, a quantitative analysis of brain neurodegeneration, identified by measuring glucose metabolism in the brain with FDG-PET (Neuroclaud PET), and expressed as the percentage deviation from the normal population, was performed (Supplementary Material Table S1).

Exclusion criteria: subjects with a history proven by computed tomography with focal organic lesions, brain tumor, history of clinical diseases with neuronal involvement, malformations of the central nervous system, history of HIV or Hepatitis B infection or Syphilis, history of epileptic disorders, brain surgery or psychiatric disorders. It is of note that none of the participants included in this report suffer chronic conditions that could affect levels of peripheral inflammation such as gastrointestinal inflammation, chronic obstructive pulmonary disease, rheumatoid arthritis, dermatitis, chronic fatigue syndrome, or myalgic encephalomyelitis as previously reported [31]. The description of the samples is shown in Table 1. Pre-analytical processing and storage of samples was similar between centers. Briefly, a maximum of five mL of whole blood was collected by venipuncture into 6 mL plastic tubes spray-coated with sodium citrate. The tubes were allowed to stand at room temperature (RT) for 30 min, centrifuged at 1800×*g* for 10 min at RT, immediately divided into 250 μL aliquots in 0.5 mL polypropylene storage tubes and stored at −80 °C until processing. Frozen samples were shipped from Malaga (Spain) to the City of Buenos Aires (Argentina), where the determinations were performed, with a sufficient amount of dry ice and placed in the shipping packaging provided by the international courier, in accordance with dangerous goods regulations. A total of 137 peripheral blood samples were processed to obtain plasma from 49 cognitively normal (CTL), 36 MCI and 52

Table 1
Descriptive characteristics of the samples across clinical categories.

Clinical categories	Total number of subjects (ARG/SPN)	Sex (% Female) (Female/Male)	Age (years) Mean (SD)
CTL	49 (49/0)	77.5 (38/11)	72.8 (6.3)
MCI	36 (2/34)	55.6 (20/16)	70.9 (6.7)
AD	52 (52/0)	62.5 (32/20)	76.4 (6.5)

Abbreviations: CTL, cognitively normal subjects; MCI, mild cognitive impairment; AD, Alzheimer's disease; ARG, Argentine; SPN, Spain; SD, standard deviation.

probable AD subjects.

2.3. Determination of cytokine plasma levels

The ultrasensitive instrument SECTOR Imager 6000 (MesoScale Discovery, Gaithersburg, MD, USA) was used to analyze 137 plasma samples with three multiplexed assays covering 30 analytes, namely the 10-plex Chemokine Panel 1 (MSD, cat#K15047D), the 10-plex Human Cytokine Panel 1 (MSD, cat#K15050D) and the 10-plex Human Proinflammatory Panel 1 (MSD, cat#K15049D). All assays were performed according to manufacturer's instructions as previously reported [32]. Data acquisition and analysis were performed using MSD Discovery workbench 4.0 software. When more than 10 % of the subjects displayed concentration values that were below of the Lower Limit of Quantification (LLoQ) and/or above the Upper Limit of Quantification (ULoQ), the analyte was excluded from the analysis. In the remaining analytes, values that were out of the LLoQ-ULoQ range were replaced by LLoQ/2 or ULoQ [33].

2.4. Statistics analysis

The SAMPL guidelines for reporting statistical analysis have been followed [34]. The Kolmogorov-Smirnov test was used to assess the assumption of normal distribution. Since no cytokine showed a normal distribution ($p < 0.05$ for all cases), Kruskal-Wallis tests followed by Dunn's multiple comparison tests were performed in order to compare the concentration of analytes between the three diagnostic groups (CTL, MCI and AD). Next, to study the influence of diagnostic group, age and sex on cytokine concentrations, a non-parametric multivariate analysis of covariance (MANCOVA) was performed. Cytokine concentrations were used as dependent variables, while diagnostic group, age and sex were used as independent variables. The non-parametric MANCOVA analysis allows assessment of the combined relationship of multiple independent variables with multiple dependent variables, without assuming specific distributions of the data. The Monte Carlo permutation test was used to obtain corrected p-values and ensure the validity of the results.

Next, to determine the specificity and sensitivity of the markers, receiver operating characteristic (ROC) curves were conducted. For the certainty of the ROC analysis and based on the literature [35], an a priori sample size calculation was performed (alpha = 0.05; beta = 93–0.20; power = 0.80; expected AUC = 0.70 (70 %); null value of AUC = 0.50 (50 %); ratio of negative to positive cases = 1). The minimum sample size was 31 positive and 31 negative cases (N = 62).

Finally, three multivariate binary logistic regression models were performed with diagnostic group (model 1: CTL vs.AD, model 2: CTL vs. MCI and model 3: MCI vs. AD) as dependent variables and each of the analytes as independent variables, adding age and sex as covariates. Only those analytes whose plasma concentration levels were significantly different between diagnostic groups and in which the area under the curve (AUC) was equal or greater than 0.70 in the ROC curve were included in the multivariate models.

All statistical tests and models were performed with R (version 4.3.3), Graphpad Prism 9.1.0 (San Diego, CA, USA) and SPSS software V.28 (IBM Corporation, Somers, NY, USA). Custom procedures were used for non-parametric MANCOVA analysis. A $p \leq 0.05$ (two-tailed) was considered statistically significant.

3. Results

3.1. Cytokine levels in plasma of CTL subjects, MCI or probable AD patients

Here we report the evaluation of 30 plasma analytes in MCI and probable AD patients and age-matched cognitively normal subjects. All participants were part of a multicenter case-control study performed in cooperation between Argentina and Spain. Collecting samples from different centers broaden the relevance of the results to different groups. However, pre-analytical procedures, including the timeframe between sample collection and separation of supernatant from cells, are known to influence cytokine levels [36]. In this regard, key procedural recommendations [37] were followed at all recruitment sites to minimize factors that could affect cytokine levels. The accuracy of analyte measurements was assessed on the basis of reproducible technical replicates (maximum coefficient of variation: 12.95 %). From the total of 30 analytes measured only 13 showed no more of 10 % of its values out of the LLoQ-ULoQ range (Supplementary Material, Table S2). As a first approach, we performed a univariate analysis to describe how each analyte varied between groups. Non-parametric Kruskal-Wallis tests followed by Dunn's multiple comparison tests showed that out of a total of 13 analytes examined, the concentration of nine showed significant differences between diagnostic groups (IL-16, IL-7, VEGF, IL-8; eotaxin, MCP-1, MCP-4, MDC and TARC) (Fig. 1). On the contrary, four analytes showed no significant differences in their concentration levels between the different clinical groups (IL-12_23p40, TNF-α, IP-10 and MIP-1β) (Supplementary Material, Fig. S1). All exact p-values are given in Information, Table S3.

3.2. Diagnostic group and sex influence plasma levels of cytokines and chemokines

To study the influence of diagnostic group, sex and age on cytokine concentrations, a non-parametric multivariate analysis of covariance (MANCOVA) was performed. The results of the MANCOVA test showed that the diagnostic groups had a significant impact on cytokines plasmatic concentrations (Pillai's Trace = 0.68152, $F(2, 232) = 4.6124$, $p < 0.001$). The differences in cytokine concentrations between the CTL, MCI and probable AD groups were statistically significant, reinforcing the role of inflammation on disease onset and progression.

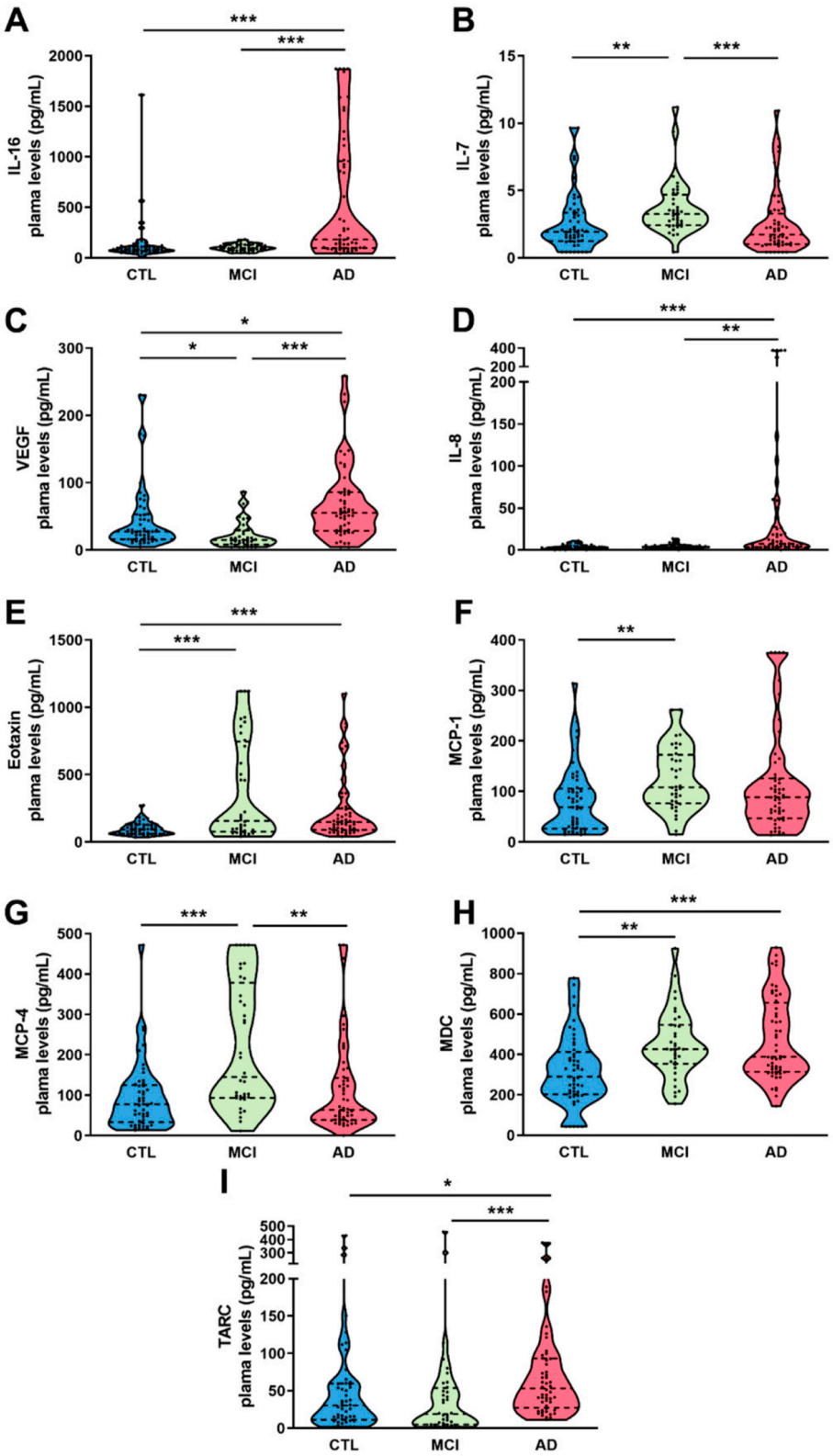

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Fig. 1. Cytokine plasma concentration levels between clinical categories. Only the nine cytokines whose concentration levels were significantly different between clinical categories are displayed. Violin plots show the frequency distribution of the data with individual data points superimposed. Dotted lines indicate the median and quartiles Q1 and Q3. *p < 0.05; **p < 0.01; ***p < 0.001 (see Table S2 for detailed statistics and exact p-values). Abbreviations: CTL, cognitively normal subjects; MCI, mild cognitive impairment; AD, Alzheimer's disease.

In addition, the sex variable showed a significant relationship with cytokine concentrations (Pillai's Trace = 0.22994, F(1, 114) = 2.6184, p = 0.0032), while the age variable did not (Pillai's Trace = 0.07681, F(1, 115) = 0.7360, p = 0.7244). These results indicate that both disease status and sex have a significant effect on plasmatic cytokine concentrations.

3.3. Multivariate logistic regression analysis to select cytokines associated with clinical diagnosis

Prior to performing the multivariate models, we carried out univariate ROC curves to select those analytes that will be included in the models. Only those analytes with an AUC ≥0.70, and that were significant different between diagnostic groups, were selected. These criteria were met by 6 analytes when discriminating between CTL and MCI subjects, 4 analytes when discriminate between CTL and AD subjects and 6 analytes when discriminating between MCI and AD subjects (Table 2). Next, three multivariate logistic regression models were developed (model 1: CTL vs. MCI, model 2: CTL vs. AD, and model 3: MCI vs. AD) with sex and age as covariates. We found that 2 analytes were informative in each one of the models. The results are summarized in Table 3. It is notable that the OR values in the regression models indicate that the two predictor variables in model 1 (IL-16 and MDC) and in model 2 (eotaxin and MDC) are associated with the risk of developing the disease. In contrast, in model 3, IL-7 is associated with protection, whereas VEGF is linked with the risk of developing dementia.

4. Discussion

Based on the evidence that inflammation is a key component of AD pathogenesis and that neurogliosis is indicative of the progression of the disease, in this report we measured 30 proteins including growth factors, chemoattractants and interleukins to position biomarkers of neuroinflammation and meningeal immune processes as a possible tool for disease characterization and prognosis.

Cytokine levels in the blood of healthy individuals are known to be low, and some cytokines are not detected [38]. In this report we applied a highly sensitive multiplex technique that allowed us the rapid and cost-effective measurement of a broad panel of analytes. It is noteworthy that the 17 excluded analytes were included in the list of not detected cytokines in blood of aged subjects [38].

Table 2
Univariate receiver operating characteristic (ROC) models.

CTL vs. MCI					
Analyte	AUC	SE	CI (95 %)		P value
			Lower	Upper	
IL-7	0.7279	0.0555	0.6190	0.8368	0.0003
VEGF	0.7029	0.0577	0.5898	0.8161	0.0014
Eotaxin	0.7115	0.0600	0.5938	0.8291	0.0009
MCP-1	0.7120	0.0562	0.6018	0.8222	0.0008
MCP-4	0.7511	0.0538	0.6455	0.8568	<0.0001
MDC	0.7166	0.0561	0.6065	0.8266	0.0006
CTL vs. AD					
Analyte	**AUC**	**SE**	**CI (95 %)**		**P value**
			Lower	**Upper**	
IL-16	0.7814	0.0453	0.6924	0.8704	<0.0001
IL-8	0.7945	0.0436	0.7090	0.8800	<0.0001
Eotaxin	0.7370	0.0490	0.6410	0.8331	<0.0001
MDC	0.7162	0.0506	0.6169	0.8156	0.0002
MCI vs. AD					
Analyte	**AUC**	**SE**	**CI (95 %)**		**P value**
			Lower	**Upper**	
IL-16	0.7556	0.0505	0.6565	0.8547	<0.0001
IL-7	0.7420	0.0538	0.6364	0.8475	0.0001
VEGF	0.8216	0.0457	0.7320	0.9112	<0.0001
IL-8	0.7147	0.0530	0.6083	0.8212	0.0006
MCP-4	0.7217	0.0550	0.6137	0.8297	0.0004
TARC	0.7297	0.0576	0.6166	0.8428	0.0003

Only analytes in which the AUC were equal or higher than 0.70 are shown. Abbreviations: CTL, cognitively normal subjects; MCI, mild cognitive impairment; AD, Alzheimer's disease; AUC, area under the curve; SE, standard error; CI (95 %), 95 % confidence interval.

Table 3
Multivariate logistic regression models.

CTL vs. AD			
Age + Sex + IL-16 + MDC ($R^2 = 0.42$)			
Analyte	OR	CI (95 %)	P value
IL-16	1.003	1.001–1.005	0.003
MDC	1.006	1.003–1.010	0.001
CTL vs. MCI			
Age + Sex + Eotaxin + MDC ($R^2 = 0.38$)			
Analyte	**OR**	**CI (95 %)**	**P value**
Eotaxin	1.006	1.001–1.011	0.01
MDC	1.005	1.001–1.009	0.006
MCI vs. AD			
Age + Sex + IL-7 + VEGF ($R^2 = 0.47$)			
Analyte	**OR**	**CI (95 %)**	**P value**
IL-7	0.563	0.392–0.807	0.002
VEGF	1.067	1.033–1.103	0.001

Abbreviations: CTL, cognitively normal subjects; MCI, mild cognitive impairment; AD, Alzheimer's disease; OR, odd ratio; CI (95 %), 95 % confidence interval.

Moreover, most of these analytes were also eliminated in a previous report from a large case-control study of inflammatory biomarkers in AD plasma [39], reinforcing the validity of our results. The conclusions of the univariate analysis showed that the nine analytes exhibit statistical differences among groups. In particular, significant increases were observed in the levels of pro-inflammatory cytokines (IL-16 and IL-8), compounds that promote vascularity at the site of inflammation (VEGF), neuronal toxicity and ROS production (eotaxin), and those that regulate immune cell homeostasis (TARC and MDC) in AD samples compared to CTL.

As markers of early cognitive impairment, significantly elevated levels of IL-7, eotaxin, MCP-1, MCP-4 and MDC were observed in MCI subjects compared to CTL, while reduced levels of VEGF were accounted. To gain further insight into the progression from MCI to AD, we conducted a comparative analysis of individual analyte levels and observed alterations in six, including significant increases in AD vs. MCI in plasma levels of IL-16, IL-8, VEGF, and TARC, while decreases in MCP-4 and IL-7 were noted. It is noteworthy that MCP-4 has been identified by immunohistochemistry in human atherosclerotic lesions [40]. While there are no published reports linking plasma MCP-4 levels to cognitive performance, it is important to acknowledge that MCP-1 (which shares high similarity with MCP-4) has been reported to be a discriminating analyte between MCI and CTL subjects [39].

Recent studies have underscored the intricate interrelationship between sex, age, and inflammatory markers in AD. It was observed that women exhibited stronger verbal memory than men, and this advantage was moderated by IL-1β levels [41]. Additionally, sex differences in CSF and plasma inflammatory markers were reported [42]. Published reports displayed that age is a significant factor influencing plasma cytokine levels, with notable age-related alterations observed in inflammatory markers. Several studies have demonstrated that pro-inflammatory cytokines tend to increase with age [42–45]. Conversely, some anti-inflammatory cytokines, such as IL-2, have been observed to decrease with age [43]. These age-related changes in cytokine profiles may contribute to the development of age-restricted conditions and chronic low-grade inflammation in older adults [46].

In this study, we employed MANCOVA to evaluate the influence of two covariates, namely sex and age, on the statistically significant intergroup differences observed in the clinical groups under analysis. The findings indicated that sex is a significant covariate that should be controlled for. In this regard, our multivariate logistic regression analysis, which employed sex and age as covariates, revealed that a distinct set of analytes are involved within each clinical group. Increments in chemokines (eotaxin and MDC) are associated with early cognitive impairment. It is of note that MDC has been shown to play a role in suppressing Aβ-induced neurotoxicity [47]. These chemokines are produced by microglia and astrocytes and their receptors are significantly altered in AD patients, suggesting a potential role in the pathology of the disease [48]. Elevated levels of eotaxin (CCL11), originally identified for its role in immune response, have been consistently observed in the plasma and CSF of individuals diagnosed with AD [49]. Furthermore, this chemokine has been linked to a delayed onset of MCI and dementia, indicating a potential role in modifying the age at onset of AD [50]. Previous reports have indicated that elevated eotaxin levels are associated with alterations in brain homeostasis, synaptic plasticity, and cognition during the aging process [51]. Recently, eotaxin was identified as one of the 10 analytes associated with MCI and AD [39]. It is noteworthy that age-related increases in eotaxin have been associated with impairments in executive functions and episodic and semantic memory. Consequently, this chemokine is known as an "endogenous cognition deteriorating chemokine" or an "accelerated brain-ageing chemokine" [52].

Our results suggest that levels of IL-7 and VEGF may be indicative of progression from MCI to AD. A potential association between peripheral levels of IL-7 and AD has been identified previously [53], but the results are conflicting. Levels of IL-7 have been found upregulated in CSF of subject at early stages of AD [54] and minor changes were reported in plasma levels of patients with established dementia [55]. Noteworthy, it was recently reported that plasma IL-7 levels are reduced in AD patients carrying APOEε4, who showed a negative correlation between plasma IL-7 levels and the degree of hippocampal atrophy [56]. Given the known role of IL-7 in the development and modulation of innate lymphoid cells that control tissue homeostasis and antimicrobial immune responses [57],

dysregulation of this cytokine may indeed indicate the presence of active inflammatory processes in neurodegenerative dementias, including AD. In addition, a significant association between IL-7 levels and depression in AD patients has been reported [58], although the correlation between the two variables differed depending on the sex and blood fraction (plasma or serum) examined. In some cases, the correlation was positive, in others it was negative. The overexpression of VEGF in plasma of AD is in accordance with the hypothesis that vascular disorder is an important feature of chronic neurodegeneration conditions [53], however, there is great controversy about the relevance of plasma VEGF levels in MCI and AD patients [52]. Possible reasons for these conflicting findings include the occurrence of depression, which was associated with higher serum VEGF levels in AD compared cognitively normal subjects [59].

A comparison of the most disparate clinical groups (CTL vs. AD) revealed that IL-16 and MDC were significant discriminative variables. Activation of microglia results in the production of IL-16 [60] and in rat models, the expression of IL-16 by microglia varies across different disease conditions, indicating its potential as a marker for microglial activation in CNS diseases [61]. In the context of AD, a correlation between specific IL-16 gene polymorphisms and a reduced risk of developing dementia was identified [62,63]. Taken together, these results suggest that different combinations of cytokines, as well as sex and age, may be suitable candidates for the development of convenient screening tools for clinical diagnosis. Nevertheless, further research is required to corroborate our findings in larger and more diverse datasets.

This brief report is limited by several factors. First, the modest sample size limited our ability to include multiple adjustment variables, such as comorbidity risk factors, without compromising the statistical power of the analysis [64], and the lack of proportionality (with the majority of MCI subjects belonging to the Spanish cohort and probable AD and control participants belonging to the Argentinean cohort) constrained the ability to analyze the influence of center/country. Second, the lack of confirmation of AD by established biomarkers. Third, the unavailability of imaging data for the majority of participants and fourth, the lack of an independent cohort to replicate our findings. Moreover, we performed a cross-sectional analysis and therefore causality cannot be inferred.

However, this report highlights noteworthy aspects of neuroinflammation as an emerging focus in contemporary AD research and adds information to the fraction of studies that are reliable and useful to the field. After applying rigorous filters, we have identified a set of plasma markers that reflect glial reactivity. However, these analytes may also have a peripheral origin. It is well documented that a number of pathological conditions, including arthritis, diabetes mellitus, obesity, systemic lupus erythematosus and inflammatory bowel disease, result in a state of chronic peripheral inflammation. Locally produced proinflammatory cytokines activate primary afferent pathways, such as the vagus nerve, and circulating cytokines gain access to the brain [65], thereby activating resident microglia in the brain. In this context, seminal clinical observations suggesting a role for peripheral immune dysregulation and peripheral-central immune communication in AD point to promising translational mechanistic studies for therapeutic intervention [66]. Therefore, although none of the participants had diagnosis of chronic inflammatory diseases, we cannot rule out a peripheral origin of these cytokines as product of subclinical conditions Overall, our results reinforce the role of inflammation in the different clinical stages of AD and suggest the possibility of using the plasmatic glial signature as an interesting additional marker to assess AD progression.

CRediT authorship contribution statement

Lorenzo Campanelli: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Pablo Galeano:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Federico A. Prestia:** Writing – review & editing, Methodology, Data curation. **Carolina Cuesta:** Writing – review & editing, Methodology, Data curation. **Maria C. Dalmasso:** Writing – review & editing, Supervision, Investigation. **María Flores-López:** Writing – review & editing, Supervision, Investigation. **Cristian Gona:** Writing – review & editing, Methodology. **Nicolás Irureta:** Writing – review & editing, Supervision, Investigation. **Claudia Kairiyama:** Writing – review & editing, Methodology. **Julieta Lisso:** Writing – review & editing, Methodology. **Antonio Jesús López-Gambero:** Writing – review & editing, Methodology. **Ines Mintz:** Writing – review & editing, Methodology. **Nancy Medel:** Writing – review & editing, Methodology. **Karen S. Campuzano:** Writing – review & editing, Methodology. **Carolina Muchnik:** Writing – review & editing, Methodology. **Gisela V. Novack:** Writing – review & editing, Methodology. **Natividad Olivar:** Writing – review & editing, Methodology. **Ivana Quiroga:** Writing – review & editing, Methodology. **Nerea Requena-Ocaña:** Writing – review & editing, Methodology. **Jose Antonio Reyes-Bueno:** Writing – review & editing, Methodology. **Pedro Serrano-Castro:** Writing – review & editing, Methodology. **Zulma Sevillano:** Writing – review & editing, Methodology. **Patricia Solis:** Writing – review & editing, Methodology. **Juan Suárez:** Writing – review & editing, Methodology. **Ivana Villella:** Writing – review & editing, Methodology. **Nancy Wukitsevits:** Writing – review & editing, Methodology. **Eduardo M. Castaño:** Writing – review & editing. **Fernando Taragano:** Writing – review & editing, Methodology. **Silvia Kochen:** Writing – review & editing, Supervision. **Daniel G. Politis:** Writing – review & editing, Supervision, Methodology. **Luis I. Brusco:** Writing – review & editing, Supervision, Methodology. **Fernando Rodríguez de Fonseca:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Laura Morelli:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41725.

Abbreviations

AD	Alzheimer's disease
APOE	apolipoprotein E
AUC	Area under the curve
Aβ42	amyloid β
BBB	blood brain barrier
CNS	central nervous system
CR1	complement receptor-1
CSF	cerebrospinal fluid
CTL	cognitively normal
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GWAS	genome-wide association studies
IFN	Interferon
IL	Interleukin
IP	Interferon gamma-induced protein
MCP	Monocyte Chemoattractant Protein
MDC	Macrophage-derived chemokine
MIP	Macrophage Inflammatory Proteins
P-tau	hyperphosphorylated tau
R2	Cox & Snell R-squared coefficient
ROC	receiver operating characteristic
T-tau	total tau
TARC	thymus- and activation-regulated chemokine
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

References

[1] A. Serrano-Pozo, M.P. Frosch, E. Masliah, B.T. Hyman, Neuropathological alterations in Alzheimer disease, Cold Spring Harb Perspect Med 1 (2011), https://doi.org/10.1101/CSHPERSPECT.A006189.

[2] C. Bergeron, Alzheimer's disease–neuropathological aspects, Can. J. Vet. Res. 54 (1990), 58./pmc/articles/PMC1255607/?report=abstract. (Accessed 14 July 2024).

[3] T.G. Beach, R. Walker, E.G. McGeer, Patterns of gliosis in Alzheimer's disease and aging cerebrum, Glia 2 (1989) 420–436, https://doi.org/10.1002/GLIA.440020605.

[4] R. Cabezas, M. Ávila, J. Gonzalez, R.S. El-Bachá, E. Báez, L.M. García-Segura, J.C.J. Coronel, F. Capani, G.P. Cardona-Gomez, G.E. Barreto, Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease, Front. Cell. Neurosci. 8 (2014), https://doi.org/10.3389/FNCEL.2014.00211.

[5] P.L. McGeer, E.G. McGeer, The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases, Brain Res. Rev. 21 (1995) 195–218, https://doi.org/10.1016/0165-0173(95)00011-9.

[6] N.L. Monson, S.J. Ireland, A.J. Ligocki, D. Chen, W.H. Rounds, M. Li, R.M. Huebinger, C. Munro Cullum, B.M. Greenberg, A.M. Stowe, R. Zhang, Elevated CNS inflammation in patients with preclinical Alzheimer's disease, J. Cerebr. Blood Flow Metabol. 34 (2014) 30–33, https://doi.org/10.1038/JCBFM.2013.183.

[7] A.C. Cuello, Early and late CNS inflammation in alzheimer's disease: two extremes of a continuum? Trends Pharmacol. Sci. 38 (2017) 956–966, https://doi.org/10.1016/J.TIPS.2017.07.005.

[8] K.S. Lee, J.H. Chung, T.K. Choi, S.Y. Suh, B.H. Oh, C.H. Hong, Peripheral cytokines and chemokines in Alzheimer's disease, Dement. Geriatr. Cogn. Disord 28 (2009) 281–287, https://doi.org/10.1159/000245156.

[9] F.A.J. Gigase, E. Smith, B. Collins, K. Moore, G.J.L.J. Snijders, D. Katz, V. Bergink, M.M. Perez-Rodriquez, L.D. De Witte, The association between inflammatory markers in blood and cerebrospinal fluid: a systematic review and meta-analysis, Mol. Psychiatr. 28 (2023) 1502–1515, https://doi.org/10.1038/S41380-023-01976-6.

[10] G. Nehra, B. Bauer, A.M.S. Hartz, Blood-brain barrier leakage in Alzheimer's disease: from discovery to clinical relevance, Pharmacol. Ther. 234 (2022), https://doi.org/10.1016/J.PHARMTHERA.2022.108119.

[11] F. Angiulli, E. Conti, C.P. Zoia, F. Da Re, I. Appollonio, C. Ferrarese, L. Tremolizzo, Blood-based biomarkers of neuroinflammation in alzheimer's disease: a central role for periphery? Diagnostics 11 (2021) https://doi.org/10.3390/DIAGNOSTICS11091525.

[12] K.M. Wu, Y.R. Zhang, Y.Y. Huang, Q. Dong, L. Tan, J.T. Yu, The role of the immune system in Alzheimer's disease, Ageing Res. Rev. 70 (2021), https://doi.org/10.1016/J.ARR.2021.101409.

[13] S. Jevtic, A.S. Sengar, M.W. Salter, J.A. McLaurin, The role of the immune system in Alzheimer disease: etiology and treatment, Ageing Res. Rev. 40 (2017) 84–94, https://doi.org/10.1016/J.ARR.2017.08.005.

[14] K. Alves De Lima, J. Rustenhoven, J. Kipnis, Meningeal immunity and its function in maintenance of the central nervous system in health and disease, Annu. Rev. Immunol. 38 (2020) 597–620, https://doi.org/10.1146/ANNUREV-IMMUNOL-102319-103410/CITE/REFWORKS.

[15] A. Abbaoui, O. Fatoba, T. Yamashita, Meningeal T cells function in the central nervous system homeostasis and neurodegenerative diseases, Front. Cell. Neurosci. 17 (2023), https://doi.org/10.3389/FNCEL.2023.1181071.

[16] Z. Papadopoulos, J. Herz, J. Kipnis, Meningeal lymphatics: from anatomy to central nervous system immune surveillance, J. Immunol. 204 (2020) 286–293, https://doi.org/10.4049/JIMMUNOL.1900838.

[17] J.C. Park, S.H. Han, I. Mook-Jung, Peripheral inflammatory biomarkers in Alzheimer's disease: a brief review, BMB Rep 53 (2020) 10–19, https://doi.org/10.5483/BMBREP.2020.53.1.309.

[18] V. Van Giau, E. Bagyinszky, S.S.A. An, Potential fluid biomarkers for the diagnosis of mild cognitive impairment, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/IJMS20174149.

[19] J. Chen, M.F. Doyle, Y. Fang, J. Mez, P.K. Crane, P. Scollard, C.L. Satizabal, M.L. Alosco, W.Q. Qiu, J.M. Murabito, K.L. Lunetta, Peripheral inflammatory biomarkers are associated with cognitive function and dementia: framingham Heart Study Offspring cohort, Aging Cell 22 (2023), https://doi.org/10.1111/ACEL.13955.

[20] F. Brosseron, M. Krauthausen, M. Kummer, M.T. Heneka, Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview, Mol. Neurobiol. 50 (2014) 534–544, https://doi.org/10.1007/S12035-014-8657-1.

[21] C.R. Jack Jr., J. Scott Andrews, T.G. Beach, T. Buracchio, B. Dunn, A. Graf, O. Hansson, C. Ho, W. Jagust, E. McDade, J. Luis Molinuevo, O.C. Okonkwo, L. Pani, M.S. Rafii, P. Scheltens, E. Siemers, H.M. Snyder, R. Sperling, C.E. Teunissen, M.C. Carrillo, L. Ryan, Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup, Alzheimer's Dementia (2024), https://doi.org/10.1002/ALZ.13859.

[22] K.Y. Kim, K.Y. Shin, K.A. Chang, GFAP as a potential biomarker for alzheimer's disease: a systematic review and meta-analysis, Cells 12 (2023), https://doi.org/10.3390/CELLS12091309.

[23] P. Chatterjee, S. Pedrini, E. Stoops, K. Goozee, V.L. Villemagne, P.R. Asih, I.M.W. Verberk, P. Dave, K. Taddei, H.R. Sohrabi, H. Zetterberg, K. Blennow, C. E. Teunissen, H.M. Vanderstichele, R.N. Martins, Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease, Transl. Psychiatry 11 (2021), https://doi.org/10.1038/S41398-020-01137-1.

[24] A.L. Benedet, M. Milà-Alomà, A. Vrillon, N.J. Ashton, T.A. Pascoal, F. Lussier, T.K. Karikari, C. Hourregue, E. Cognat, J. Dumurgier, J. Stevenson, N. Rahmouni, V. Pallen, N.M. Poltronetti, G. Salvadó, M. Shekari, G. Operto, J.D. Gispert, C. Minguillon, K. Fauria, G. Kollmorgen, I. Suridjan, E.R. Zimmer, H. Zetterberg, J. L. Molinuevo, C. Paquet, P. Rosa-Neto, K. Blennow, M. Suárez-Calvet, Differences between plasma and cerebrospinal fluid glial fibrillary acidic protein levels across the alzheimer disease continuum, JAMA Neurol. 78 (2021) 1471–1483, https://doi.org/10.1001/JAMANEUROL.2021.3671.

[25] B.S. Rauchmann, T. Schneider-Axmann, P. Alexopoulos, R. Perneczky, CSF soluble TREM2 as a measure of immune response along the Alzheimer's disease continuum, Neurobiol. Aging 74 (2019) 182–190, https://doi.org/10.1016/J.NEUROBIOLAGING.2018.10.022.

[26] A.B. Knapskog, K. Henjum, A.V. Idland, R.S. Eldholm, K. Persson, I. Saltvedt, L.O. Watne, K. Engedal, L.N.G. Nilsson, Cerebrospinal fluid sTREM2 in Alzheimer's disease: comparisons between clinical presentation and AT classification, Sci. Rep. 10 (2020), https://doi.org/10.1038/S41598-020-72878-8.

[27] J.P. Vandenbroucke, E. Von Elm, D.G. Altman, P.C. Gøtzsche, C.D. Mulrow, S.J. Pocock, C. Poole, J.J. Schlesselman, M. Egger, Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration, Epidemiology 18 (2007) 805–835, https://doi.org/10.1097/EDE.0B013E3181577511.

[28] C.R. Jack, M.S. Albert, D.S. Knopman, G.M. McKhann, R.A. Sperling, M.C. Carrillo, B. Thies, C.H. Phelps, Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease, Alzheim. Demen. 7 (2011) 257–262, https://doi.org/10.1016/J.JALZ.2011.03.004.

[29] G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Clinical diagnosis of alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on alzheimer's disease, Neurology 34 (1984) 939–944, https://doi.org/10.1212/WNL.34.7.939.

[30] A.E. Budson, P.R. Solomon, New criteria for Alzheimer disease and mild cognitive impairment: implications for the practicing clinician, Neurol. 18 (2012) 356–363, https://doi.org/10.1097/NRL.0B013E31826A998D.

[31] Y. Sun, Y. Koyama, S. Shimada, Inflammation from peripheral organs to the brain: how does systemic inflammation cause neuroinflammation? Front. Aging Neurosci. 14 (2022) https://doi.org/10.3389/FNAGI.2022.903455.

[32] P. Galeano, M. de Ceglia, M. Mastrogiovanni, L. Campanelli, D. Medina-Vera, N. Campolo, G.V. Novack, C. Rosell-Valle, J. Suárez, A. Aicardo, K. Campuzano, E. M. Castaño, S. Do Carmo, A.C. Cuello, S. Bartesaghi, R. Radi, F. Rodríguez de Fonseca, L. Morelli, The effect of fat intake with increased omega-6-to-omega-3 polyunsaturated fatty acid ratio in animal models of early and late alzheimer's disease-like pathogenesis, Int. J. Mol. Sci. 24 (2023) 17009, https://doi.org/10.3390/IJMS242317009/S1.

[33] J. Herbers, R. Miller, A. Walther, L. Schindler, K. Schmidt, W. Gao, F. Rupprecht, How to deal with non-detectable and outlying values in biomarker research: best practices and recommendations for univariate imputation approaches, Compr Psychoneuroendocrinol 7 (2021), https://doi.org/10.1016/J.CPNEC.2021.100052.

[34] T.A. Lang, D.G. Altman, Basic statistical reporting for articles published in biomedical journals: the "statistical analyses and methods in the published literature" or the SAMPL guidelines, Int. J. Nurs. Stud. 52 (2015) 5–9, https://doi.org/10.1016/J.IJNURSTU.2014.09.006.

[35] A. Negida, N.K. Fahim, Y. Negida, Sample size calculation guide - Part 4: how to calculate the sample size for a diagnostic test accuracy study based on sensitivity, specificity, and the area under the ROC curve, Adv J Emerg Med 3 (2019) e33, https://doi.org/10.22114/AJEM.V0I0.158.

[36] A. Valaperti, P. Bezel, M. Vonow-Eisenring, D. Franzen, U.C. Steiner, Variability of cytokine concentration in whole blood serum and bronchoalveolar lavage over time, Cytokine 123 (2019), https://doi.org/10.1016/J.CYTO.2019.154768.

[37] I.M.W. Verberk, E.J. Nossent, H.J. Bontkes, C.E. Teunissen, Pre-analytical sample handling effects on blood cytokine levels: quality control of a COVID-19 biobank, Biomarkers Med. 15 (2021) 987–997, https://doi.org/10.2217/BMM-2020-0770.
[38] G. Kleiner, A. Marcuzzi, V. Zanin, L. Monasta, G. Zauli, Cytokine levels in the serum of healthy subjects, Mediat. Inflamm. 2013 (2013), https://doi.org/10.1155/2013/434010.
[39] T.A. Berkhout, H.M. Sarau, K. Moores, J.R. White, N. Elshourbagy, E. Appelbaum, T.J. Reape, M. Brawner, J. Makwana, J.J. Foley, D.B. Schmidt, C. Imburgia, D. McNulty, J. Matthews, K. O'Donnell, D. O'Shannessy, M. Scott, P.H.E. Groot, C. Macphee, Cloning, in vitro expression, and functional characterization of a novel human CC chemokine of the monocyte chemotactic protein (MCP) family (MCP-4) that binds and signals through the CC chemokine receptor 2B, J. Biol. Chem. 272 (1997) 16404–16413, https://doi.org/10.1074/jbc.272.26.16404.
[40] T.A. Berkhout, H.M. Sarau, K. Moores, J.R. White, N. Elshourbagy, E. Appelbaum, T.J. Reape, M. Brawner, J. Makwana, J.J. Foley, D.B. Schmidt, C. Imburgia, D. McNulty, J. Matthews, K. O'Donnell, D. O'Shannessy, M. Scott, P.H.E. Groot, C. Macphee, Cloning, in vitro expression, and functional characterization of a novel human CC chemokine of the monocyte chemotactic protein (MCP) family (MCP-4) that binds and signals through the CC chemokine receptor 2B, J. Biol. Chem. 272 (1997) 16404–16413, https://doi.org/10.1074/jbc.272.26.16404.
[41] J.Z.K. Caldwell, J.W. Kinney, A. Ritter, A. Salazar, C.G. Wong, D. Cordes, G.M. Slavich, Inflammatory cytokine levels implicated in Alzheimer's disease moderate the effects of sex on verbal memory performance, Brain Behav. Immun. 95 (2021) 27–35, https://doi.org/10.1016/J.BBI.2020.12.001.
[42] P. Duarte-Guterman, A.Y. Albert, A.M. Inkster, C.K. Barha, L.A.M. Galea, Inflammation in alzheimer's disease: do sex and APOE matter? J Alzheimers Dis 78 (2020) 627–641, https://doi.org/10.3233/JAD-200982.
[43] A. Shortanbayev, B. Bizhigitova, A. Tarabayeva, A. Nurmuchanbetova, Features of cytokine profile in different age groups, Cent. Asian J. Global Health 3 (2014), https://doi.org/10.5195/CAJGH.2014.173.
[44] A. Larsson, L. Carlsson, A.L. Lind, T. Gordh, C. Bodolea, M. Kamali-Moghaddam, M. Thulin, The body mass index (BMI) is significantly correlated with levels of cytokines and chemokines in cerebrospinal fluid, Cytokine 76 (2015) 514–518, https://doi.org/10.1016/J.CYTO.2015.07.010.
[45] R.P. Stowe, M.K. Peek, M.P. Cutchin, J.S. Goodwin, Plasma cytokine levels in a population-based study: relation to age and ethnicity, J Gerontol A Biol Sci Med Sci 65 (2010) 429–433, https://doi.org/10.1093/GERONA/GLP198.
[46] L. Álvarez-Rodríguez, M. López-Hoyos, P. Muñoz-Cacho, V.M. Martínez-Taboada, Aging is associated with circulating cytokine dysregulation, Cell. Immunol. 273 (2012) 124–132, https://doi.org/10.1016/J.CELLIMM.2012.01.001.
[47] D. Raman, S.Z. Milatovic, D. Milatovic, R. Splittgerber, G.H. Fan, A. Richmond, Chemokines, macrophage inflammatory protein-2 and stromal cell-derived factor-1α, suppress amyloid β-induced neurotoxicity, Toxicol. Appl. Pharmacol. 256 (2011) 300–313, https://doi.org/10.1016/J.TAAP.2011.06.006.
[48] C. Liu, G. Cui, M. Zhu, X. Kang, H. Guo, Neuroinflammation in Alzheimer's disease: chemokines produced by astrocytes and chemokine receptors, Int. J. Clin. Exp. Pathol. 7 (2014) 8342. /pmc/articles/PMC4314046/. (Accessed 4 May 2023).
[49] A.K. Huber, D.A. Giles, B.M. Segal, D.N. Irani, An emerging role for eotaxins in neurodegenerative disease, Clin. Immunol. 189 (2018) 29–33, https://doi.org/10.1016/J.CLIM.2016.09.010.
[50] M.A. Lalli, B.M. Bettcher, M.L. Arcila, G. Garcia, C. Guzman, L. Madrigal, L. Ramirez, J. Acosta-Uribe, A. Baena, K.J. Wojta, G. Coppola, R. Fitch, M.D. De Both, M.J. Huentelman, E.M. Reiman, M.E. Brunkow, G. Glusman, J.C. Roach, A.W. Kao, F. Lopera, K.S. Kosik, Whole-genome sequencing suggests a chemokine gene cluster that modifies age at onset in familial Alzheimer's disease, Mol. Psychiatr. 20 (2015) 1294–1300, https://doi.org/10.1038/MP.2015.131.
[51] S.A. Villeda, J. Luo, K.I. Mosher, B. Zou, M. Britschgi, G. Bieri, T.M. Stan, N. Fainberg, Z. Ding, A. Eggel, K.M. Lucin, E. Czirr, J.S. Park, S. Couillard-Després, L. Aigner, G. Li, E.R. Peskind, J.A. Kaye, J.F. Quinn, D.R. Galasko, X.S. Xie, T.A. Rando, T. Wyss-Coray, The ageing systemic milieu negatively regulates neurogenesis and cognitive function, Nature 477 (2011) 90–96, https://doi.org/10.1038/NATURE10357.
[52] A. Custodia, M. Aramburu-Núñez, M. Rodríguez-Arrizabalaga, J.M. Pías-Peleteiro, L. Vázquez-Vázquez, J. Camino-Castiñeiras, J.M. Aldrey, J. Castillo, A. Ouro, T. Sobrino, D. Romaus-Sanjurjo, Biomarkers assessing endothelial dysfunction in alzheimer's disease, Cells 12 (2023), https://doi.org/10.3390/CELLS12060962.
[53] K. Govindpani, L.G. McNamara, N.R. Smith, C. Vinnakota, H.J. Waldvogel, R.L.M. Faull, A. Kwakowsky, Vascular dysfunction in alzheimer's disease: a prelude to the pathological process or a consequence of it? J. Clin. Med. 8 (2019) https://doi.org/10.3390/JCM8050651.
[54] S.M. de la Monte, M. Tong, A.J. Hapel, Concordant and discordant cerebrospinal fluid and plasma cytokine and chemokine responses in mild cognitive impairment and early-stage alzheimer's disease, Biomedicines 11 (2023) 2394, https://doi.org/10.3390/BIOMEDICINES11092394/S1.
[55] U. Kilic, B. Elibol, O. Uysal, E. Kilic, B. Yulug, A. Sayin Sakul, G. Babacan Yildiz, Specific alterations in the circulating levels of the SIRT1, TLR4, and IL7 proteins in patients with dementia, Exp. Gerontol. 111 (2018) 203–209, https://doi.org/10.1016/J.EXGER.2018.07.018.
[56] Y.J. Zhang, Y. Cheng, H.L. Tang, Q. Yue, X.Y. Cai, Z.J. Lu, Y.X. Hao, A.X. Dai, T. Hou, H.X. Liu, N. Kong, X.Y. Ji, C.H. Lu, S.L. Xu, K. Huang, X. Zeng, Y.Q. Wen, W.Y. Ma, J.T. Guan, Y. Lin, W. Bin Zheng, H. Pan, J. Wu, R.H. Wu, N.L. Wei, APOE ε4-associated downregulation of the IL-7/IL-7R pathway in effector memory T cells: implications for Alzheimer's disease, Alzheim. Demen. 20 (2024), https://doi.org/10.1002/ALZ.14173.
[57] U. Bank, K. Deiser, C. Plaza-Sirvent, L. Osbelt, A. Witte, L. Knop, R. Labrenz, R. Jänsch, F. Richter, A. Biswas, A.C. Zenclussen, E. Vivier, C. Romagnani, A. A. Kühl, I.R. Dunay, T. Strowig, I. Schmitz, T. Schüler, c-FLIP is crucial for IL-7/IL-15-dependent NKp46+ ILC development and protection from intestinal inflammation in mice, Nat. Commun. 11 (2020), https://doi.org/10.1038/S41467-020-14782-3.
[58] J.R. Hall, A. Wiechmann, M. Edwards, L.A. Johnson, S.E. O'Bryant, IL-7 and Depression: the importance of gender and blood fraction, Behav. Brain Res. 315 (2016) 147–149, https://doi.org/10.1016/J.BBR.2016.08.026.
[59] J. Jung, S. Kim, K. Yoon, Y. Moon, D. Roh, S. Lee, K. Choi, J. Jung, D. Kim, The effect of depression on serum VEGF level in Alzheimer's disease, Dis. Markers 2015 (2015), https://doi.org/10.1155/2015/742612.
[60] M.L. Zhao, Q. Si, S.C. Lee, IL-16 expression in lymphocytes and microglia in HIV-1 encephalitis, Neuropathol. Appl. Neurobiol. 30 (2004) 233–242, https://doi.org/10.1046/J.0305-1846.2003.00527.X.
[61] L.H. Guo, M. Mittelbronn, C. Brabeck, C.A. Mueller, H.J. Schluesener, Expression of interleukin-16 by microglial cells in inflammatory, autoimmune, and degenerative lesions of the rat brain, J. Neuroimmunol. 146 (2004) 39–45, https://doi.org/10.1016/j.jneuroim.2003.09.017.
[62] N.E. Anvar, K. Saliminejad, M. Ohadi, K. Kamali, P. Daneshmand, H.R.K. Khorshid, Association between polymorphisms in Interleukin-16 gene and risk of late-onset Alzheimer's disease, J. Neurol. Sci. 358 (2015) 324–327, https://doi.org/10.1016/J.JNS.2015.09.344.
[63] T. Khoshbakht, M. Soosanabadi, M. Neishaboury, K. Kamali, M. Karimlou, N. Bazazzadegan, H.R.K. Khorshid, An association study on IL16 gene polymorphisms with the risk of sporadic alzheimer's disease, Avicenna J. Med. Biotechnol. (AJMB) 7 (2015) 128. /pmc/articles/PMC4508336/. (Accessed 14 July 2024).
[64] M.A. Babyak, What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models, Psychosom. Med. 66 (2004) 411–421, https://doi.org/10.1097/01.PSY.0000127692.23278.A9.
[65] R. Dantzer, J.C. O'Connor, G.G. Freund, R.W. Johnson, K.W. Kelley, From inflammation to sickness and depression: when the immune system subjugates the brain, Nat. Rev. Neurosci. 9 (2008) 46–56, https://doi.org/10.1038/NRN2297.
[66] B.M. Bettcher, M.G. Tansey, G. Dorothée, M.T. Heneka, Peripheral and central immune system crosstalk in Alzheimer disease — a research prospectus, Nat. Rev. Neurol. 17 (11 17) (2021) 689–701, https://doi.org/10.1038/s41582-021-00549-x, 2021.