Archivum Immunologiae et Therapiae Experimentalis (2022) 70:16
https://doi.org/10.1007/s00005-022-00653-w

ORIGINAL ARTICLE

Sex Differences in Innate Immune Response of Peripheral Blood Leukocytes of Alzheimer's Disease Patients

Marta Sochocka[1] · Michał Ochnik[1] · Maciej Sobczyński[2] · Beata Orzechowska[1] · Jerzy Leszek[3]

Received: 25 February 2022 / Accepted: 4 May 2022 / Published online: 16 June 2022

Abstract

Neurodegenerative disorders, including Alzheimer's disease (AD), are associated with a disruption of normal immune function that could potentially impact the brain. In AD sex and gender have been noted as relevant to disease prevalence or clinical manifestation. It is suggested that disease progression could vary as a result of the different inflammation state among males and females. The objective was to investigate sex-dependent difference in innate immunity of AD patients and healthy, age-matched controls. The level of innate immunity was measured with test based on peripheral blood leukocytes (PBLs) resistance to viral infection (vesicular stomatitis virus, VSV) ex vivo. Cytokine: TNF-α, IFN-γ, IL-1β, IL-10 production by uninfected and VSV-infected PBLs ex vivo with enzyme-linked immunosorbent assay were examined. In contrast to controls, women with AD exhibit lower average level of innate immunity than AD men. The mean level of TNF-α, IL-10 and IL-1β was higher in AD men than in AD women whereas such changes were not observed among controls. The level of IFN-γ was higher in AD than in controls. PBLs from AD did not increase IFN-γ production after viral infection in contrast to controls. Leukocytes from women with AD exhibited a weaker response to viral infection and much less cytokine production compared to men with AD. It is important to consider sex as a biological variable in AD as it shows promises to advance our understanding of mechanisms of AD pathology and may be the basis for future treatment of AD.

Keywords Alzheimer's disease · Innate immunity · PBLs · Cytokine production · Inflammation

Abbreviations

B12	Vitamin B12/cobalamin
BBB	Blood–brain barrier
CNS	Central nervous system
CRP	C-reactive protein
CT	Computed tomography
DGN	The diagnostic according to the guidelines of the German Society of Neurology
DSMV	Fifth edition of diagnostic and statistical manual of mental disorders classification (criteria for major neurocognitive disorder)
FBS	Fetal bovine serum
GSK3	Glycogen synthase kinase 3
IFN	Interferon
IL	Interleukin
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
NINCDS-ADRDA	National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association
PBLs	Peripheral blood leukocytes
PMA	Phorbol 12-myristate 13-acetate
$TCID_{50}$	Fifty-percent tissue culture infective dose
TNF	Tumor necrosis factor
TSH	Thyroid-stimulating hormone
VSV	Vesicular stomatitis virus

✉ Marta Sochocka
marta.sochocka@hirszfeld.pl

[1] Laboratory of Virology, Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, 53-114 Wrocław, Poland

[2] Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[3] Department of Psychiatry, Wroclaw Medical University, Wrocław, Poland

Introduction

Alzheimer's disease (AD) accounts for two-thirds of dementia cases globally. The disease affects cognition-associated areas of the brain—the cortex and hippocampus—resulting in progressive neurodegeneration. Neurodegeneration in AD, manifesting as in neuronal loss and brain injury has been found to be result of the presence of two main pathologies, amyloid-β plaques and neurofibrillary tangles. Nevertheless, the presence of chronic inflammation in the brain has become as a third core pathology in AD that possibly plays a role in onset and progression of the disease (Heneka et al. 2015; Kinney et al. 2018). Aging is often related with developing a chronic low-grade pro-inflammatory state (Bektas et al. 2018). In AD numerous of the immunological/inflammatory mechanisms related to, i.e., dysregulation of the immune system have been implicated in AD pathogenesis. Alterations which occur in both central and peripheral immune compartments that change over time have been underscored (Bettcher et al. 2021). Clinical studies of immune dysregulation showed that alterations (increased) in peripheral inflammatory markers, such as C-reactive protein (CRP), interleukin IL-6, tumor necrosis factor TNF-α and IL-1β may presage cognitive changes and/or symptomatic manifestations of AD. Next to plasma cytokine levels a phenotypic and functional assays of monocytes have also confirm a role for inflammatory dysregulation in AD dementia (Bettcher et al. 2021). It is suggested that systemic inflammation induced by different infectious etiologies may be an important feature of AD development next to amyloid/tau pathology, and neuroinflammation (Butler and Walker 2021). Every infection introduce systemic immune alterations and later in life infections may lead to alterations in peripheral immune markers and exacerbate cognitive decline in elderly adults (Bettcher et al. 2021). This presents AD as a complex and multifactorial disease with a strong immune component.

AD belongs to a group of diseases where age is the most important risk factor (Hanamsagar and Bilbo 2016). Currently, it is widely accepted that AD is a systemic disease and affects both the central nervous system (CNS) and periphery (Morris et al. 2014). Many years of research of AD biology, especially by genome-wide association studies, have provided convincing data that most of genetic variants influencing sporadic AD risk are involved in immune system functioning (Butler and Walker 2021; Giri et al. 2016). It was also noticed that inflammatory processes play a role in the modulation of cognitive function and age-related cognitive decline (Lim et al. 2013). Proper immune system signaling via brain resident macrophages (microglia) decides of maintaining brain homeostasis, neuronal integrity and synaptic plasticity. Nevertheless, injury by non-infectious as well as infectious factors may trigger microglial activation leading to neuroinflammation (Wang et al. 2018). Inflammation in the CNS is not restricted only to the brain of AD patients but is also influenced by the immune system activation and inflammatory reactions in the periphery (Le Page et al. 2018). Therefore, the peripheral immune system may strongly contribute to AD pathology. On the other hand the neuroinflammatory process pending in the brain also causes passage of the inflammatory molecules to the periphery where they act on peripheral immune cells and in turn recruit them back to the brain. Recirculation of peripheral immune cells as well as inflammatory mediators such as cytokines, chemokines and complement through the altered blood–brain barrier (BBB) establishes a vicious circle between these two compartments—the brain and periphery (Le Page et al. 2018; Schwartz and Deczkowska 2016). AD has been associated with a disruption of immune function that could harmfully impact the brain (Hanamsagar and Bilbo 2016). Therefore, function or rather dysfunction of immune response might be an important feature of AD pathogenesis (Giri et al. 2016).

In many neurological disorders such as depression or schizophrenia, sex differences in prevalence and outcome have been observed (Hanamsagar and Bilbo 2016). Although AD is diagnosed generally in aged individuals sex and gender have been noted as relevant to disease prevalence or clinical manifestation (Podcasy and Epperson 2016). It has been highlighted that neurodegenerative processes and AD symptoms are more frequent in females. Moreover progression of the disease was observed to be more rapid among elderly women (Laws et al. 2016). Women comprise two-thirds of all AD patients. Mechanisms underlying sex differences are not yet well understood. However, it was confirmed that men affected with AD outperform women with AD across a range of cognitive domains. It may be related with men having greater cognitive reserve interpreted as a sum of brain injuries that person can suffer before reaching a clinical threshold for impairment (Laws et al. 2016). Moreover, it is suggested that disease progression might be related to the different inflammation state in males and females (Hanamsagar and Bilbo 2016).

Previously, we have shown that patients with Alzheimer's have a reduced level or deficiency in innate immunity of peripheral blood leukocytes (PBLs) that correlated with the clinical severity of the disease (Sochocka et al. 2019). We have hypothesized that in AD patients, innate immune mechanisms are impaired because of strong pro-inflammatory features of PBLs. Cellular resistance to viral infections is one of the innate immune reactions. To estimate the degree of resistance, a test based on vesicular stomatitis virus (VSV) replication in freshly isolated PBLs ex vivo was used. VSV

was selected as an indicatory virus among other viruses, to the test of PBLs resistance since it is an animal virus and do not cause natural infections among human population. Therefore, there is no association between VSV infection and AD. Here, we investigated whether there are differences in PBLs response between AD men and AD women knowing that AD is more common in women. The aim of this study was to investigate the correlation between sex and innate immune response of PBLs ex vivo in people with AD vs. age-matched controls.

Materials and Methods

Study Design

This study evaluated the possible correlation between peripheral innate immune response and sex among patients diagnosed with AD and controls in relevant age. Blood samples were obtained from 39 participants: 22 AD patients (15 females, 7 males) and 17 healthy adult volunteers (10 females, 7 males) aged 37–90 years. This study has been reviewed, approved, and conducted in accordance with the guidelines of the Ethics Committee of Wroclaw Medical University (No. KB-349/2016). Signed consent was obtained from all participants of the study or their legal representative. AD patients were recruited into the study successively (newly diagnosed cases) during the period of investigation. Thus, the sample of AD patients was a representative sample of the general population of AD sufferers. All participants were late-onset AD except one, a 37-year-old men with early-onset AD. Patients did not receive any immunomodulators and had no infectious diseases (with antibiotic treatment) in the 3 months period before inclusion in the study. Patients underwent psychiatric and neurological examinations, as well as laboratory tests, electroencephalographic examinations and computed tomography (CT) or magnetic resonance imaging (MRI) structural studies at the Department of Psychiatry of the Medical University in Wroclaw, Poland. All patients met DSM-V and NINCDS-ADRDA criteria for probable AD dementia. The mini-mental state examination (MMSE) scale was used for the screening of dementia. A diagnosis of AD was made when specific symptoms were present and CT and MRI of the brain were performed to exclude other causes of dementia such: anemia, brain tumor, chronic infection, intoxication from medication, severe depression, stroke, thyroid disease, and vitamin deficiencies. A semistructured interview with the patient and informant, a physical exam, evaluation of neurological status, and a psychiatric exam were obtained. Vital signs and blood samples (for hematology, chemistry panel, urinalysis, B12, thyroid-stimulating hormone: TSH) were collected. Exclusion criteria: patients older than 90 years, any significant neurological disease such as Parkinson's disease, multi-infarct dementia, Huntington disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, or history of significant head trauma followed by persistent neurologic defaults or known structural brain abnormalities. MRI scan with evidence of infection, infarction or other focal lesions, subjects with multiple lacunes or lacunes in a critical memory structure, which may indicate the existence of mixed dementia (AD + vascular). Major depression, bipolar disorder, agitation or behavioral problems within the last 3 month, history of schizophrenia, alcohol abuse, history of alcohol or substance abuse or dependence within the past 2 years; unstable medical condition, clinically significant abnormalities in B12, rapid plasma reagin or TSH, current use of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics or sedative hypnotics, etc.). Patients were excluded if they did not agree to respond to the test questions and/or if they had life-threatening diseases other than AD. Controls were collected as a representative for an age-matched population of people without AD. Controls as well as AD patients did not receive any immunomodulators before and during the study. Exclusion criteria: diabetes, cancer, serious infectious diseases (with antibiotic treatment) that occurred in the 3 months period before the inclusion in the study, autoimmune diseases, smokers.

Virus and Cell Line

A wild-type Indiana VSV (*vesicular stomatitis virus, Rhabdoviridae*) serotype was used. VSV was obtained from Dr. C. Buckler (National Institutes of Health, Bethesda, MD, USA). The virus was grown and titrated in L_{929} cells. Viral titer was expressed with reference to the $TCID_{50}$ (tissue culture infectious dose) value, based on the cytopathic effect caused by this virus in approximately 50% of infected cells. L_{929} (ATCC CCL1), a murine fibroblast-like cell line, was maintained in complete RPMI 1640 medium (HIIET, Wroclaw, Poland) with antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin), 2 mM L-glutamine, and 2% fetal bovine serum (FBS; all from Sigma-Aldrich, USA).

Isolation of peripheral blood leukocytes (PBLs)

Peripheral blood samples from AD patients and healthy controls were taken into heparin tubes. PBLs were isolated within 2 h of collection according to a standard protocol from 10 ml of peripheral blood by gradient centrifugation in Gradisol G (Aqua-Med, Łódź, Poland) and maintained in RPMI 1640 medium (HIIET, Wroclaw, Poland) with antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin),

2 mM L-glutamine, and 2% FBS (all from Sigma-Aldrich, USA).

Determination of Resistance/Level of Innate Immunity of PBLs

Resistance/innate immunity was determined by infection of leukocytes (1×10^6 cells/ml) ex vivo with a VSV dose of 100 $TCID_{50}$. After 40 min of adsorption at room temperature, the virus was washed out three times with RPMI medium with 2% FBS and the cells were suspended in 1 ml of RPMI 2% FBS for investigations of PBLs resistance/innate immunity and cytokine production. A sample of the infected cells was kept at 4 °C and served as a control of the starting level of the virus. Next cells were incubated at 37 °C for 24 h. After that time samples of medium above the cells were collected and titrated in L_{929} cells. Viral titer was expressed in $TCID_{50}$. Resistance of PBLs to VSV infection was assessed as follows: a VSV titer ≥ 4 log $TCID_{50}$ was considered as a lack of resistance (deficiency in innate immunity), a titer of > 1 log $TCID_{50}$ indicated partial resistance, and a titer of 0–1 log $TCID_{50}$ indicated complete resistance to VSV infection (high level of innate immunity).

Statistical Analysis

Due to the highly skewed and heavy right tailed distributions of the data, all cytokine measurements x were transformed with the log function, i.e., $x' = \ln x$. Means and standard deviations (SD) as well as confidence intervals, CI 95%, for the mean, were estimated with bootstrap sampling due to small sample sizes. PBLs were control level, i.e., before infection with VSV in men (in tables marked as $\boldsymbol{a_m}$) and women($\boldsymbol{a_w}$). [PBLs + VSV] was VSV-infected PBLs for men ($\boldsymbol{b_m}$) and women ($\boldsymbol{b_w}$). Difference [PBLs + VSV]− PBLs was the relative effect of VSV for men ($\boldsymbol{c_m}$) and for women ($\boldsymbol{c_w}$). The difference between average levels of cytokine in men and women, produced by VSV-infected PBLs, was estimated as $\boldsymbol{b_m - b_w} = [\text{PBLs + VSV}]_{\text{men}} - [\text{PBLs + VSV}]_{\text{women}}$. The difference between men and women according to the relative effect of VSV was estimated as $\boldsymbol{c_m - c_w}$**.** In the case of AD patients, a priori formulated hypothesis, according to the aim of this investigation, was that average level of cytokine, produced by VSV-infected PBLs, in women is lower than in men, i.e., $H0_1 : \boldsymbol{b_m - b_w} \leq 0$**;** opposite to the alternative $H1_1 : \boldsymbol{b_m - b_w} > 0$. Similarly, the hypothesis regarding the different magnitude of relative reaction to VSV stated: $H0_2 : \boldsymbol{c_m - c_w} \leq 0$, opposite to the alternative $H1_2 : \boldsymbol{c_m - c_w} > 0$, i.e., relative reaction on VSV stimulation is also lower in women (one-side tests). In case of healthy controls, there were no a priori reasonable assumptions that average level of investigated cytokine, produced by VSV-infected PBLs, in men and women is different. In this case, the hypothesis stated: $H0_1 : \boldsymbol{b_m - b_w} = 0$ (average levels are equal) and $H0_2 : \boldsymbol{c_m - c_w} = 0$ (relative reactions to VSV stimulation are the same) opposite to alternatives which stated: $H1_1 : \boldsymbol{b_m - b_w} \neq 0$; $H1_2 : \boldsymbol{c_m - c_w} \neq 0$ (two-side test). t − Student statistic for two independent samples (men and women) was used to test the null hypotheses. Due to the small sample sizes, the distributions of t-statistics were estimated numerically with bootstrap sampling. All confidence intervals are bootstrapped percentile two-side intervals. To better describe the observed differences with taking into account the levels of witching group variability, effect size was estimated with Cohen's d statistic according to Sawilowsky (2009)

$$|d| \leq 0.2, \text{verysmall}; 0.2 < |d| \leq 0.5, \text{small};$$
$$0.5 < |d| \leq 0.8, \text{moderate}; 0.8 < |d| \leq 1.2, \text{large};$$
$$1.2 < |d| \leq 2, \text{verylarge}; |d| > 2, \text{huge}.$$

Cohen's d effect size is the most common measure of magnitude of an effects obtained in empirical studies (Lakens 2013). It is the standardized mean difference of the effect, in which the difference between two means is divided by pooled standard deviation. So, effect size presents the difference measured in number of standard deviations and Cohen's d range between 0 (no effect) and infinity. Cohen's d was also translated into point-biserial correlation coefficient $r = \frac{d}{\sqrt{d^2+4}}$ according to Rosenthal et al. (2000) to easier interpret magnitude of the effect size. Coefficient r is the correlation between an individual's measurement (i.e., cytokine level etc.) and belongings of the individual to the one of the two groups (e.g., AD or control, men or women, etc.). Additionally, we translated Cohen's d statistic into the two other commonly understood measures: (a) Ovl—percent of overlapping of two populations to each other; $Ovl = 2\Phi(-|d|/2)$ ranges from 0 to 100%, where $Ovl = 100\%$ when Cohen's $d = 0$ (no effect, no differences between two groups); (b) AUC—area under ROC curve—the probability that a randomly chosen person from a population with a higher average level will have a higher value of their score than a randomly chosen person from a population with a lower average level; $AUC = \Phi\left(\frac{d}{\sqrt{2}}\right)$ ranges from 50% when Cohen's $d = 0$ (no effect) to 100% when two populations are completely separated. In both equations, Φ is the cumulative distribution function of the standard normal distribution $N(0, 1)$.

As all data presented in the tables were log-transformed, one can re-transform the values using formula $x = e^{x'}$, where $e = 2.7183$ is the base for natural logarithms. The difference between two logarithms, e.g., between two

log-means is $D = \ln A - \ln B = \ln\frac{A}{B}$ so if D is the difference between two log-means, and the value e^D is the ratio of two geometric means on their original (not transformed) scales. Data presented in the figures were log-transformed but labels on the y-axis are re-transformed, i.e., labels are in an original scales as pg/ml, not log(pg/ml). The S_n statistic (Rousseeuw and Croux 1993) was computed as the measure of variability: $S_n = \text{med}\left\{\text{med}\left|x_i - x_j\right| i, j = 1, ..n\right\}$. S_n is the typical distance between two randomly selected individuals and can be used as a measure of variability instead of standard deviation when the median is used instead of the arithmetic mean.

Results

Characterization of Study Groups

Table 1 includes basic characteristics of AD patients and healthy age-matched controls. There were 15 females and 7 males among AD patients and 10 females and 7 males among healthy controls. The median age for AD patients was 67.5 years (67 years for females/71 years for males) and for controls median age was 59 years (59 years for females/59 years for males). The median MMSE score for AD females was 18.75 and 18.5 for AD males. Patients were collected randomly to estimate DGN and DSMV differences between sex. There were 26.7% females and 42.9% males with mild AD, 40% females and 28.6% males with moderate AD and 33% females and 28.6% males with serious AD. A chi-square test $\chi^2_{\text{df}=2} = 0.598, p = 0.856$ showed no difference in DGN and DSMV between AD women and AD men.

Table 1 Characteristics of participants

Variable	Group	Median	Sn	Min–Max
Age	AD	67.5	11	37–80
	Control	59	9.5	42–90
Sex	Group	Women	Men	% Men
	AD	15	7	31.8
	Control	10	7	41.2
MMSE	AD Group	Median	Sn	Min–Max
	Women	18.75	3	12–24
	Men	18.5	3.5	12–23
DGN	AD Group	Mild	Moderate	Serious
	Women	4	6	5
	%	26.7	40.0	33.3
	Men	3	2	2
	%	42.9	28.6	28.6
DSMV	AD Group	Mild	Moderate	Serious
	Women	4	6	5
	%	26.7	40.0	33.3
	Men	3	2	2
	%	42.9	28.6	28.6

Sn, measure of variability; MMSE, mini-mental state examination; DGN, the diagnostic according to the guidelines of the German Society of Neurology; DSMV, fifth edition of diagnostic and statistical manual of mental disorders classification (criteria for major neurocognitive disorder)

Sex-Dependent Differences in Innate Immunity/PBLs Resistance of AD Patients

First, we investigated the sex differences in the level of innate immunity of PBLs ex vivo in AD patients and healthy age-matched controls. Table 2 presents the average level of innate immunity measured with a test based on PBLs resistance to VSV infection among AD patients and healthy, age-matched controls. A higher VSV titer (marked on a logarithmic scale) means a lower level of innate immunity as follows: a VSV titer ≥ 4 log $TCID_{50}$ was considered as a lack of resistance (deficiency in innate immunity), a titer of > 1 log $TCID_{50}$ indicated partial resistance, and a titer of 0–1 log $TCID_{50}$ indicated complete resistance to VSV infection (high level of innate immunity). We have observed that the average level of VSV titer/level of innate immunity in women with AD was lower 2.99 log $TCID_{50}$ than in men with AD 1.70 log $TCID_{50}$. The difference was $d_{\text{AD}} = -1.27$ with a confidence interval CI95%(−2.61;0.17). A one-sided test for this difference gave the result $p = 0.013$. Interestingly, sex differences were not observed in the control group. The

Table 2 Mean level of innate immunity/PBLs resistance in AD patients and control group, divided into the men and women as well as differences between men and women

Group	Statistics	Men	Women	Men − Women
AD	Mean	1.70	2.99	−1.27
	SD	1.80	1.15	0.63
	CI 95%*	0.50; 3.02	2.39; 3.54	−2.61; 0.17
	p value**			0.013
	$H0$: Men − Women ≥ 0;$H1$: Men − Women < 0			
Control	Mean	3.36	3.75	−0.39
	SD	1.14	0.95	0.51
	CI 95%*	2.49; 4.18	3.14; 4.31	−1.40; 0.60
	p value***			0.422
	$H0$: Men − Women = 0;$H1$: Men − Women ≠ 0			

All values in log scale

SD, standard deviation (in case of difference between two means SD is standard error of the difference)

*Two-side confidence interval

**One-side test

***Two-side test

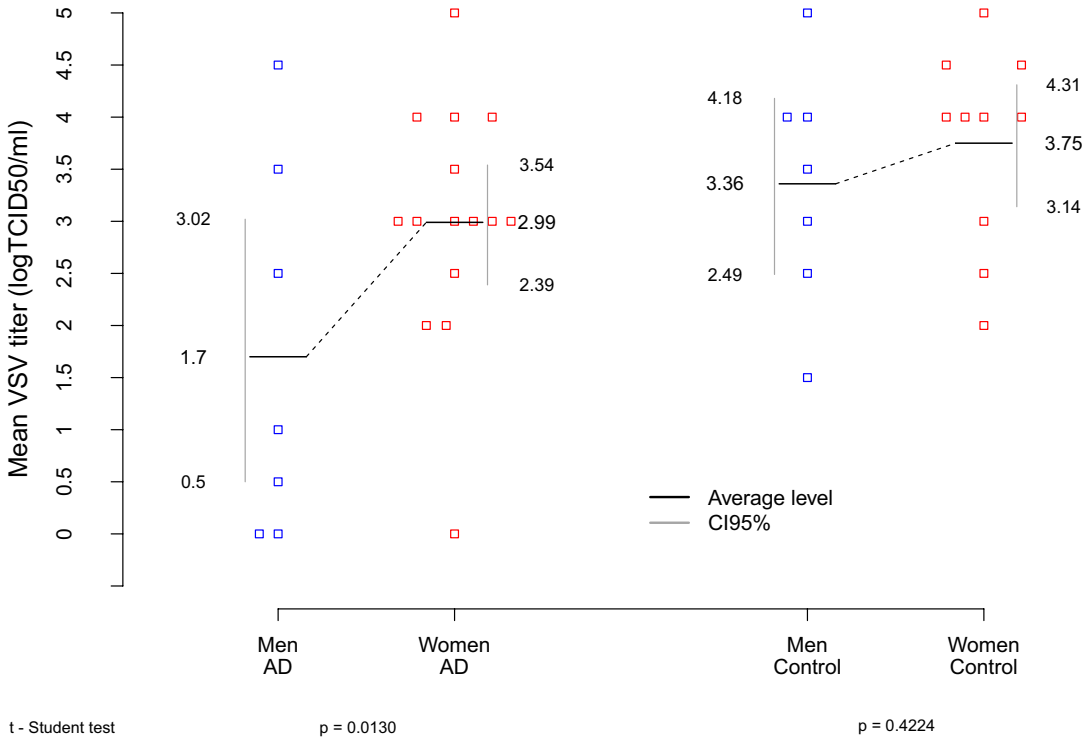


Fig. 1 Innate immunity/PBLs resistance among men and women. Average level with confidence interval, CI 95%, according to AD patients and controls. See also Table 2

average level of VSV titer/level of innate immunity among control women was 3.75 log $TCID_{50}$ and in control men it was 3.36 log $TCID_{50}$. The difference was $d_{\text{Control}} = -0.39$. There were no a priori reasonable assumptions that such a difference in controls will manifest in specified direction, so it was two-side tested with null hypothesis formulated as $H0 : \text{Men} - \text{Women} = 0$ opposite to alternative $H1 : \text{Men} - \text{Women} \neq 0$ was performed. The observed difference was not significant ($p = 0.422$). Results are presented in Fig. 1.

PBLs of AD Men and AD Women Differ in Spontaneous and VSV-Induced Cytokine Production

The next step was to examine sex differences in cytokine production by uninfected and VSV-infected PBLs from AD patients and healthy age-matched controls.

AD Patients

Table 3 presents the results of measuring the level of cytokines: TNF-α, IFN-γ, IL-10, IL-1β in AD group. Data were log-transformed.

The mean level of **TNF-α** produced by unstimulated/uninfected PBLs from AD men was $e^{4.20} = 66.9$pg/ml. In contrast, in AD women, it was $e^{2.73} = 15.4$pg/ml. Data are presented in Fig. 2. After VSV infection the mean level of TNF-α in AD men increased to $e^{4.91} = 135.6$pg/ml (the mean level was $e^{0.71} = 2$ fold higher compared to unstimulated PBLs) and in women it was $e^{3.43} = 31$pg/ml (the mean level of TNF-α also increased $e^{0.7} = 2$ fold compared to unstimulated PBLs). Data are presented in Fig. 3. Comparing the quantity of TNF-α produced by VSV-infected PBLs between men and women, we can see that the mean level in men was $e^{1.467} = 4.3$-fold higher than in women. This difference was significant ($p = 0.061$; Table 3). In summary, uninfected and VSV-infected PBLs from AD women secreted lower levels of TNF-α than PBLs from AD men. Nevertheless, TNF-α level increased as much after PBLs infection with VSV in both men and women (Table 3, last column).

In the case of **IFN-γ** production by uninfected and VSV-infected PBLs, there was no difference between AD men and AD women. The mean level of IFN-γ secreted by uninfected PBLs of AD men was $e^{2.33} = 10.2$pg/ml whereas in AD women it was $e^{2.34} = 10.4$pg/ml ($p = 0.981$; Table 3). Data are presented in Fig. 2. There was also no difference between AD men $e^{2.20} = 9$pg/ml and AD women $e^{2.46} = 12$pg/ml in IFN-γ production by VSV-infected PBLs ($p = 0.674$; Table 3). Data are presented in Fig. 3. The level of IFN-γ

Table 3 Mean level of TNF-α, IFN-γ, IL-10 and IL1β in AD group divided into the men and women, and differences between men and women

Cytokine	Statistics	Men (m)			Women (w)			Difference Men – Women		
		PBLs (a_m)	PBLs + VSV (b_m)	(PBLs + VSV) – PBLs (c_m)	PBLs (a_w)	PBLs + VSV (b_w)	(PBLs + VSV) – PBLs (c_w)	$a_m - a_w$	$b_m - b_w$	$c_m - c_w$
TNF-α	Mean	4.20	4.91	0.71	2.73	3.43	0.70	1.47	1.47	0.01
	SD*	2.34	2.32	0.70	2.24	2.11	1.20	1.01	0.98**	0.41**
	CI 95%	2.46	3.19	0.17	1.59	2.32	0.10	–0.57	–0.51	–0.81
		5.74	6.35	1.28	3.78	4.39	1.31	3.35	3.27	0.84
	$H0_1 : b_m - b_w \le 0; H0_2 : c_m - c_w \le 0; H0_3 : a_m - a_w \le 0$; p values***							0.071	0.061	0.488
IFN-γ	Mean	2.33	2.20	–0.13	2.34	2.46	0.13	–0.02	–0.27	–0.26
	SD	0.59	0.61	0.43	1.45	1.69	0.45	0.45	0.50	0.25
	CI 95%	1.82	1.65	–0.53	1.66	1.69	–0.13	–0.93	–1.28	–0.73
		2.80	2.64	0.28	3.09	3.33	0.39	0.83	0.65	0.23
	$H0_1 : b_m - b_w \le 0; H0_2 : c_m - c_w \le 0; H0_3 : a_m - a_w = 0$; p values							0.981	0.674	0.958
IL-10	Mean	5.32	4.62	–0.70	3.89	3.13	–0.76	1.43	1.48	0.06
	SD	1.03	1.00	0.69	2.20	2.21	1.31	0.68	0.67	0.44
	CI 95%	4.54	3.81	–1.18	2.79	2.02	–1.39	0.10	0.15	–0.79
		6.04	5.27	–0.10	4.94	4.18	–0.09	2.77	2.81	0.93
	$H0_1 : b_m - b_w \le 0; H0_2 : c_m - c_w \le 0; H0_3 : a_m - a_w \le 0$; p values							0.046	0.040	0.448
IL-1β	Mean	6.31	6.33	0.02	5.56	5.68	0.12	0.75	0.65	–0.10
	SD	0.16	0.14	0.17	0.79	0.78	0.48	0.26	0.26	0.21
	CI 95%	6.01	6.04	–0.28	5.14	5.23	–0.14	0.26	0.17	–0.51
		6.61	6.63	0.33	5.94	6.04	0.39	1.28	1.18	0.31
	$H0_1 : b_m - b_w \le 0; H0_2 : c_m - c_w \le 0; H0_3 : a_m - a_w \le 0$; p values							0.002	0.010	0.800

Cytokine' levels (x) are log-transformed as $x' = \log_e x$

To get results in original scale re-transformation must be done as $x = e^{x'}$

CI 95%, confidence interval at 95% level; PBLs, unstimulated/uninfected PBLs; PBLs + VSV PBLs, infected with VSV

*SD standard deviation

**In the case of difference between two means SD is standard error of the difference (SE)

***One-side t-Student for difference for two independent means with H0s defined in the table and $H1_1 : b_m - b_w > 0; H1_2 : c_m - c_w > 0$, i.e., mean effects in the men are higher than in the women. Nulls and alternatives for difference $a_m - a_w$ depend on both results of testing $H0_1$ and $H0_2$ because $H0_1, H0_2$ and $H0_3$ logically and statistically are not independent (only two of them are independent to each other)

increased to the same amount after PBLs infection with VSV in both men and women (Table 3, last column).

The mean level of **IL-10** produced by uninfected PBLs from AD men was $e^{5.32} = 203.4$pg/ml; while in AD women, it was much lower $e^{3.89} = 48.8$pg/ml ($p = 0.046$; Table 3). Data are presented in Fig. 2. After infection with VSV the level of IL-10 in AD men decreased to $e^{4.62} = 101.7$pg/ml and in AD women it also decreased to $e^{3.13} = 23$pg/ml. Thus, it was $e^{1.48} = 4.4$ times lower than in men ($p = 0.040$; Table 3). Data are presented in Fig. 3. The magnitude of PBL response to VSV was the same in men and women (Table 3, last column).

In the case of **IL-1β**, the mean level of this cytokine produced by uninfected PBLs from AD men was $e^{6.31} = 551.1$pg/ml; whereas in AD women, it was over two times lower $e^{5.56} = 259.7$pg/ml and this difference was significant ($p = 0.002$; Table 3). Data are presented in Fig. 2. After VSV infection the level of IL-1β in AD men scarcely increased to $e^{6.33} = 561.7$pg/ml whereas in AD women it was $e^{5.68} = 292.9$pg/ml. However in AD women it was 1.9 times lower than in AD men ($p = 0.007$, Table 3). Data are presented in Fig. 3. The magnitude of PBLs response to VSV was the same in men and women (Table 3, last column).

We can conclude, that among AD patients, sex differences in cytokine production by uninfected and VSV-infected PBLs ex vivo were observed. The mean level of TNF-α, IL-10 and IL-1β was much higher in AD men than in AD women. There was no difference between AD men and AD women in IFN-γ production.

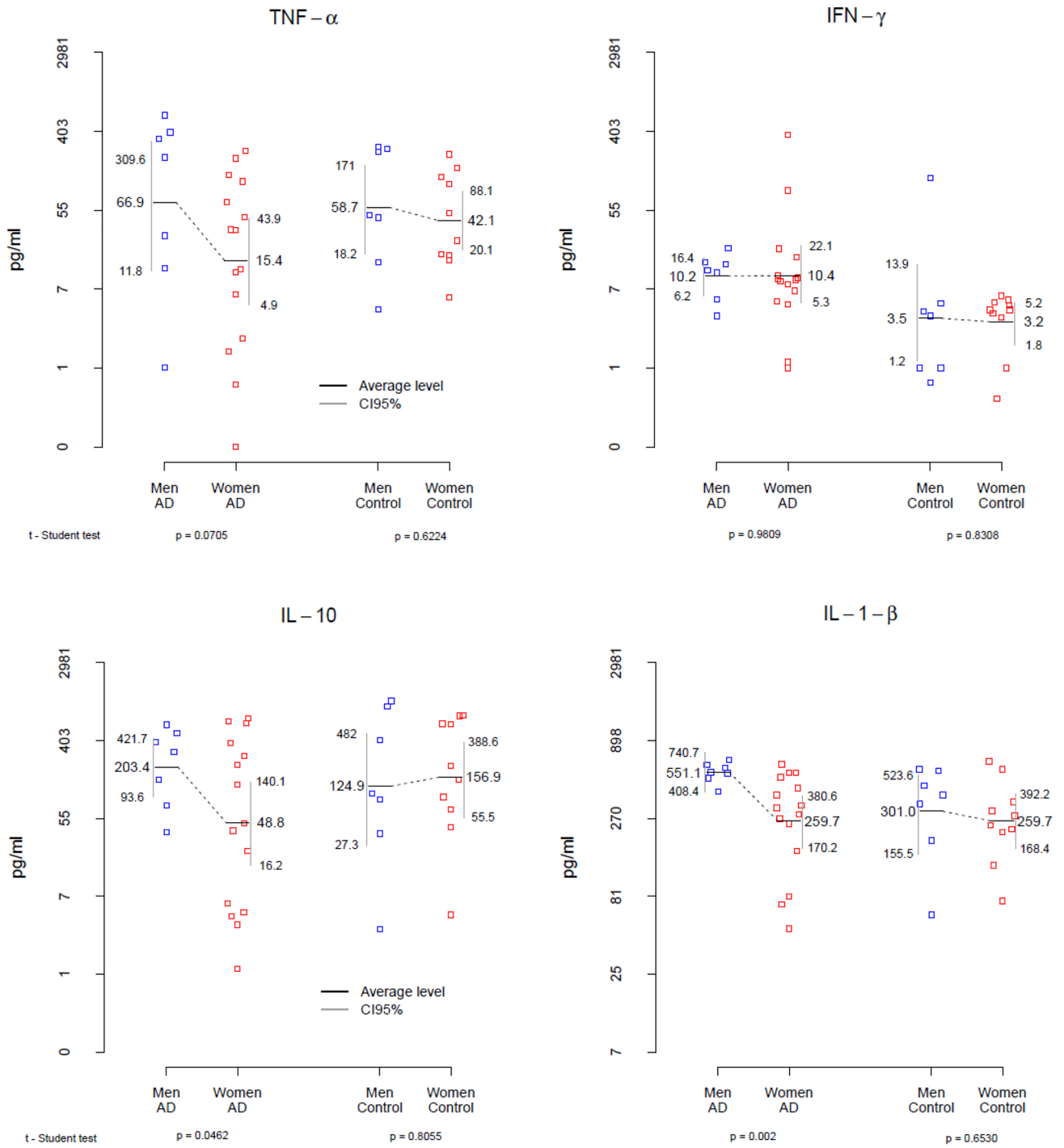


Fig. 2 Mean level of TNF-α, IFN-γ, IL-10 and IL-1β produced by unstimulated/uninfected PBLs (spontaneous production) from AD patients and controls (men vs. women). Average level with confidence interval, CI 95%, according to AD patients and controls. See also Tables 3 and 4

Healthy, Age-Matched Controls

Importantly, sex differences in cytokine release by PBLs were not observed among healthy controls of relevant age. Table 4 presents the results of TNF-α, IFN-γ, IL-10, IL-1β in healthy controls. Data were log-transformed.

In the case of **TNF-α,** spontaneous (uninfected PBLs) release of this cytokine was similar for men and women ($p = 0.622$; Table 4). The mean level of TNF-α for control men was $e^{4.07} = 58.7$pg/ml and for control women it was $e^{3.74} = 42.1$pg/ml. Data are presented in Fig. 2. TNF-α production increased in all healthy controls after infection

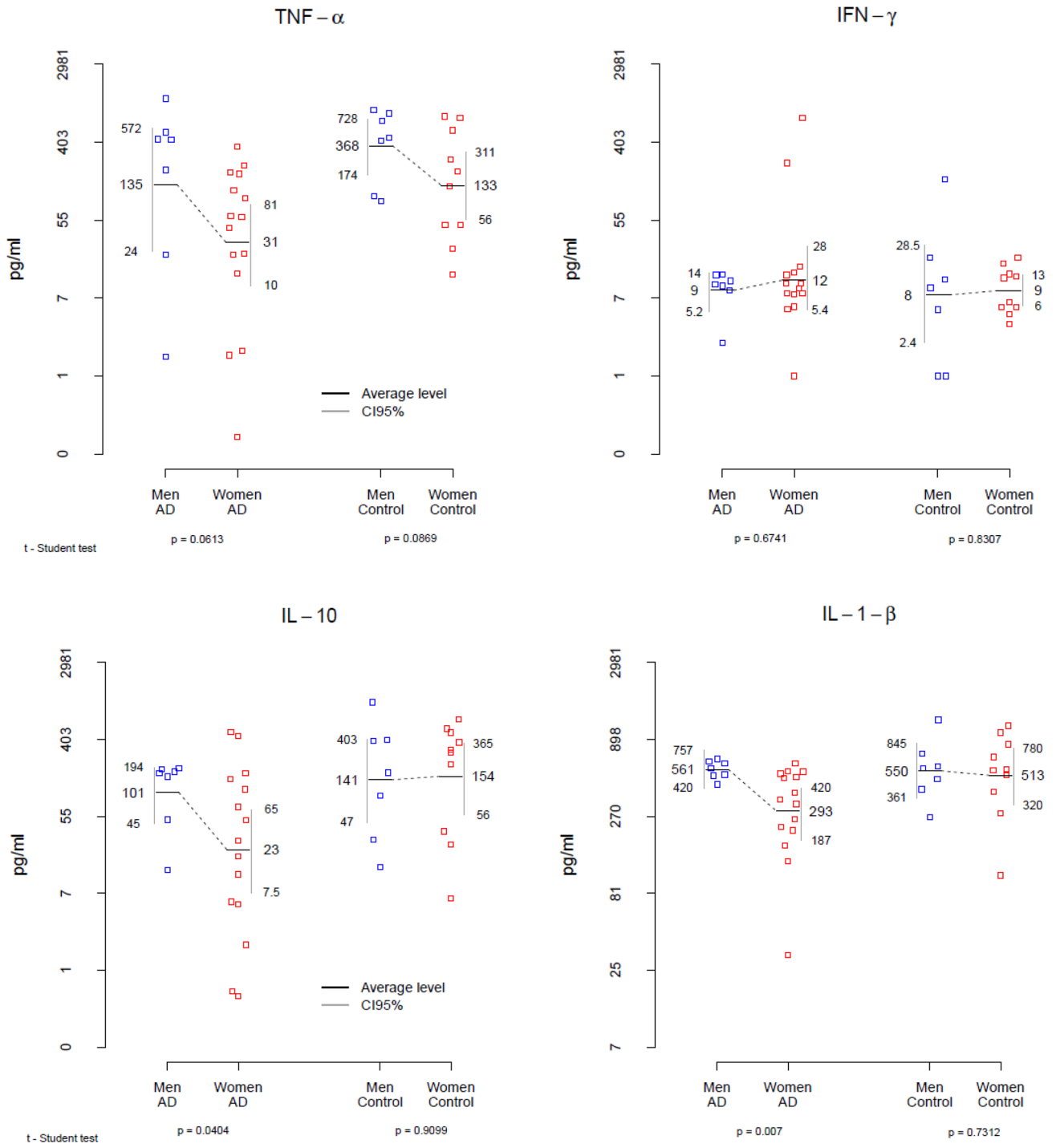


Fig. 3 Mean level of TNF-α, IFN-γ IL-10 and IL-1β produced by VSV-infected PBLs from AD patients and controls (men vs. women). Average level with confidence interval, CI 95%, according to AD patients and controls. See also Tables 3 and 4

with VSV. The mean level of TNF-α was $e^{5.91} = 368$pg/ml for men and for women it was $e^{4.89} = 133$pg/ml. Results were not statistically significant ($p = 0.087$; Table 4). Data are presented in Fig. 3.

Likewise, sex differences were not observed in **IFN-γ** production. The mean spontaneous level of IFN-γ in control men was $e^{1.26} = 3.5$pg/ml whereas in control women it was $e^{1.17} = 3.2$pg/ml ($p = 0.831$; Table 4). Data are presented in Fig. 2. An increased level of IFN-γ in control men $e^{2.08} = 8$pg/ml and in women $e^{2.19} = 9$pg/ml

Table 4 Mean levels of cytokines in the control group divided into the men and women group and differences between men and women

Cytokine	Statistics	Men (m)			Women (w)			Difference Men – Women		
		PBLs (a_m)	PBLs + VSV (b_m)	(PBLs + VSV) − PBLs (c_m)	PBLs (a_w)	PBLs + VSV (b_w)	(PBLs + VSV) − PBLs (c_w)	$a_m - a_w$	$b_m - b_w$	$c_m - c_w$
TNF-α	Mean	4.07	5.91	1.84	3.74	4.89	1.15	0.34	1.02	0.69
	SD*	1.59	0.98	1.76	1.21	1.44	0.92	0.69**	0.58**	0.70**
	CI 95%	2.90	5.16	0.76	3.00	4.02	0.60	–1.03	–0.11	–0.55
		5.14	6.59	3.19	4.48	5.74	1.74	1.67	2.13	2.16
	$H0_1 : b_m - b_w = 0$; $H0_2 : c_m - c_w = 0$; $H0_3 : a_m - a_w = 0$ p values***							0.622	0.087	0.276
IFN-γ	Mean	1.26	2.08	0.82	1.17	2.19	1.03	0.09	–0.13	–0.22
	SD	1.76	1.76	1.23	0.86	0.59	0.99	0.69	0.67	0.55
	CI 95%	0.17	0.87	0.06	0.58	1.80	0.46	–1.13	–1.43	–1.25
		2.63	3.35	1.80	1.64	2.59	1.68	1.53	1.20	0.93
	$H0_1 : b_m - b_w = 0$; $H0_2 : c_m - c_w = 0$;$H0_3 : a_m - a_w = 0$ p values							0.831	0.831	0.646
IL-10	Mean	4.83	4.95	0.12	5.06	5.04	–0.02	–0.22	–0.09	0.14
	SD	2.06	1.52	1.66	1.63	1.57	0.73	0.89	0.73	0.64
	CI 95%	3.31	3.85	–0.93	4.02	4.03	–0.46	–2.00	–1.47	–1.02
		6.18	6.00	1.38	5.96	5.90	0.48	1.49	1.36	1.49
	$H0_1 : b_m - b_w = 0$; $H0_2 : c_m - c_w = 0$; $H0_3 : a_m - a_w = 0$ p values							0.806	0.910	0.776
IL-1β	Mean	5.71	6.31	0.61	5.56	6.24	0.68	0.15	0.08	–0.09
	SD	0.80	0.47	0.89	0.64	0.69	0.75	0.38	0.31	0.42
	CI 95%	5.05	5.89	–0.01	5.13	5.77	0.19	–0.63	–0.52	–0.88
		6.26	6.74	1.31	5.97	6.66	1.15	0.85	0.71	0.74
	$H0_1 : b_m - b_w = 0$; $H0_2 : c_m - c_w = 0$;$H0_3 : a_m - a_w = 0$ p values							0.653	0.731	0.844

Measured cytokines levels (x) are log-transformed as $x' = \log_e x$

To get results in original scale re-transformation must be done as $x = e^{x'}$

CI 95%, confidence interval at 95% level; PBLs, unstimulated/uninfected PBLs; PBLs + VSV PBLs, infected with VSV

*SD standard deviation

**In the case of difference between two means SD is standard error of the difference (SE)

***Two-side t-Student test for difference for two independent means with H0s defined in the table and $H1_1 : b_m - b_w \neq 0$; $H1_2 : c_m - c_w \neq 0$ and $H0_3 : a_m - a_w \neq 0$ i.e., mean effects in the men could be higher or lower than in the women

was noticed after PBLs infection with VSV ($p = 0.831$; Table 4). Data are presented in Fig. 3.

The mean level of **IL-10** produced by uninfected PBLs from control men was $e^{4.83} = 125$pg/ml whereas in control women it was $e^{5.06} = 157$pg/ml ($p = 0.806$; Table 4). Data are presented in Fig. 2. VSV-infected PBLs from healthy men released $e^{4.95} = 141$pg/ml IL-10 and $e^{5.04} = 154$pg/ml ($p = 0.910$; Table 4) from control women. Data are presented in Fig. 3.

Similar results were obtained for **IL-1β**. The average level of spontaneous IL-1β production in control men was $e^{5.71} = 301$pg/ml and in control women it was $e^{5.56} = 259.7$pg/ml. The difference between sexes was not statistically significant ($p = 0.653$; Table 4). Data are presented in Fig. 2. The mean level of IL-1β produced by VSV-infected PBLs from healthy men was $e^{6.31} = 550$pg/ml and from healthy women it was $e^{6.24} = 513$pg/ml ($p = 0.731$; Table 4). Data are presented in Fig. 3.

Effect Size Calculation

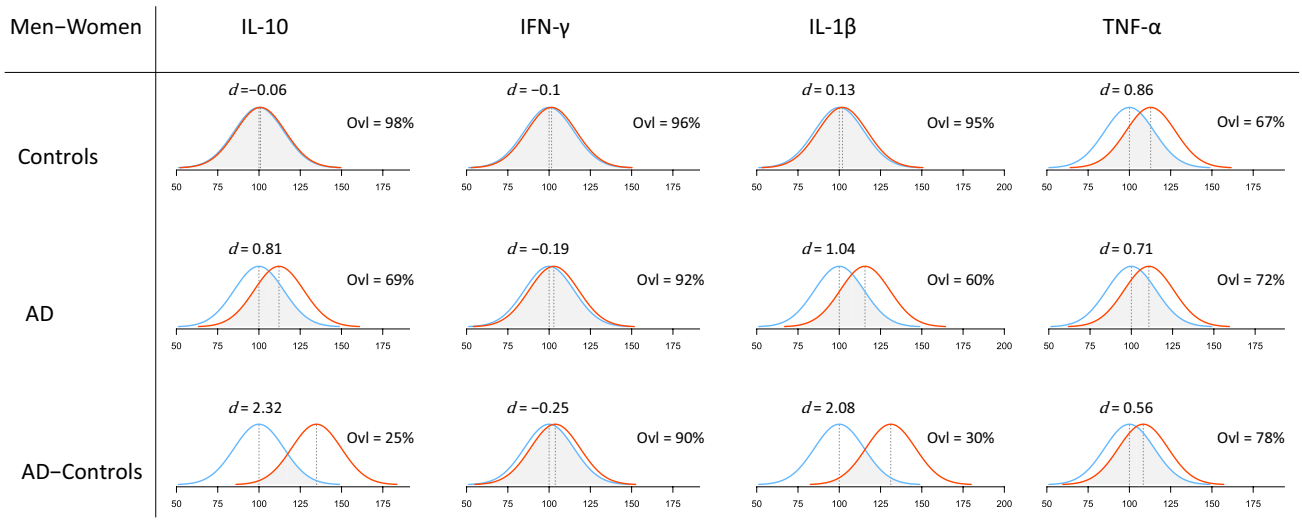
An effect sizes are the most important outcomes of empirical studies. Table 5 presents the magnitude of the observed difference between men and women among AD patients and healthy age-matched controls. Effect size, measured here with Cohen's d measure, takes into consideration the observed differences between men and women and also within groups variability expressed as standard deviation (SD). For every value of the Cohen's d statistic three other effect size measures: correlation coefficient r, Ovl and AUC have been computed (Statistical analysis; Table 5—legend) and presented in Table 5. Based on Table 5 and Fig. 4 we can see, that for IL-10 ($d = -0.06$) and for IL-1β

Table 5 Effect size as Cohen's *d* for differences between two means

n - Women	Effect size	IL - 10	IFN-γ	IL-1β	TNFF-α
Controls	Cohen's *d*	−0.06 (very small)*	−0.1 (very small)	0.13 (very small)	0.86 (large)
	r	− 0.03	− 0.05	0.06	0.39
	Ovl (%)	97.6	96	94.8	66.7
	AUC (%)	51.7	52.8	53.7	72.8
AD	Cohen's *d*	0.81 (large)	− 0.19 (very small)	1.04 (large)	0.71
	r	0.35	−0.09	0.44	0.31
	Ovl (%)	68.5	92.4	60.3	72.3
	AUC (%)	71.7	55.3	76.9	69.2
AD - Controls	Cohen's *d*	2.32 (huge)	− 0.25 (small)	2.08 (huge)	0.56 (moderate)
	r	0.75	−0.12	0.72	0.27
	Ovl (%)	24.6	90.1	29.8	77.9
	AUC (%)	95	57	92.9	65.4

Mean level of selected cytokine produced by VSV-infected PB of men was compared to the mean level of this cytokine in women, for both AD and controls. Additionally, presented is effect size for difference between AD and controls, i.e., (men–women) difference in AD minus (men–women) difference in controls. An interpretation* of the magnitude of the effect size was shown in the parentheses, according to Sawilowsky (2009). Cohen's *d* was also translated into: *r*—point-biserial correlation coefficient between individual's value and membership to the one of the compared groups; Ovl—percent of overlapping of two populations to each other; AUC—probability that person sampled at random from the group with higher average value will have a higher score then a person sampled at random from the group with lower average value (see "Statistical analysis" and Fig. 4). Cohen's *d* interpretation $|d| \le 0.2$, very small; $0.2 < |d| \le 0.5$, small; $0.5 < |d| \le 0.8$, moderate; $0.8 < |d| \le 1.2$, large; $1.2 \langle |d| \le 2$, very large; $|d| \rangle 2$, huge;

r—point-biserial correlation coefficient, $r \in [-1, 1]$, where $r = 0$ if $d = 0$. Ovl—overlapping of two distributions, Ovl ∈ [0, 100%], where Ovl = 100% if $d = 0$; AUC—area under the receiver operating characteristics, AUC ∈ [50%, 100%], where AUC = 50% if $d = 0$



Graphical interpretation of Cohen's *d* effect size. Smaller average (blue) is set to 100. Larger average based on the effect size *d* (orange). In both groups, the standard deviation is set to 15. Shaded area is percent of overlapping (Ovl) of two populations to each other; for $d = 0$ (no differences, no effect) overlapping is Ovl = 100%.

Abbreviations:

Controls, AD – difference between averages in men and women (men-women) in controls and AD patients, respectively.

AD – Controls – difference between (men-women) in AD patients and (men-women) in controls.

Fig.4 Graphical interpretation of two measures of the effect size: standardized differences between two means as Cohen's *d* statistic and percent of overlapping of two distributions to each other. For four cytokines differences between men and women average levels were measured among Controls an AD patients. Additionally, these differences were compared between AD patients and Controls. See also Table 5

($d = 0.13$) small differences were observed between men and women in the control group. In contrast, among AD men and women large differences were observed for these two interleukins with $d = 0.81$ and $d = 1.04$, respectively. There were substantial differences between AD patients and controls, i.e., sex-dependent level of interleukin IL-10 ($d = 2.32$) and IL-1β ($d = 2.08$). In the case of TNF-α, the mean level for men was higher than for women both in AD patients ($d = 0.71$) and controls ($d = 0.86$). Table 5 and Fig. 4 showed that AD group and controls were similar in this regard ($d = 0.56$). In the case of IFN-γ, no differences between men and women were observed in AD patients and controls.

In conclusion, the presented data showed a difference in innate immune response of PBLs between AD men and AD women. Such changes were not observed among healthy age-matched controls. IFN-γ, however, was the only cytokine that did not express sex differences in AD or controls. Nevertheless we have noticed that the level of spontaneous IFN-γ differed between AD patients and controls, $e^{2.336} = 10.34$pg/ml and $e^{1.205} = 3.37$pg/ml, respectively. The average level of spontaneous IFN-γ in AD patients was three times higher than in controls, with CI 95% (1.42; 6.72 times), $p = 0.001$. Data are presented in Fig. 2. Moreover, after VSV infection an increased in IFN-γ production was noticed only in control group. Data are presented in Fig. 3.

Discussion

The key risk factor of AD—advanced age—as well as lifestyle-related factors such as poor diet, excessive overweight, smoking, abuse of alcohol, or physical inactivity modulate susceptibility to dementia in males and females. Consequently, sex differences display in the frequency, prevalence, clinical manifestation and progression of the disease (Podcasy and Epperson 2016) with women being more afflicted by AD (Ferretti et al. 2018). Moreover, the rate of cognitive decline, neurodegeneration and clinical symptoms occur more rapidly in females (Podcasy and Epperson 2016). Thus, considerable heterogeneity in AD pathology is observed. This could be related to the activation of multiple pathways that differ across individuals (Nebel et al. 2018). Notwithstanding the fact that our knowledge about AD presentation and pathology has increased over the past decade, sex differences have not been studied systematically or with limited attention. Meanwhile, to accelerate the development of near and future treatment of AD, sex differences in AD, especially in relation to immune system reactions, must be better explored, understood and measured. The underlying cause of AD still remains unclear; however, several theories have suggested looking the role of neuroinflammation and immune dysregulation (Armstrong 2013; Bettcher et al. 2021; Jevtic et al. 2017). Generally, males and females differ in innate and adaptive immune responses to foreign (infectious) and self-antigens which manifests, e.g., in a different cytokine and chemokine production (Klein and Flanagan 2016). This is directly linked with the immune system functioning which slowly deteriorates during aging (Pawelec 2018). In fact, the importance of the immune system and the inflammatory pathway has been highlighted in many neurodegenerative diseases where dysregulation of immune system was also connected with sex biases (Lopez-Lee et al. 2021). The importance of the inflammatory pathway in neurodegeneration has been illustrated by many studies (Bektas et al. 2018). Anti-inflammatory therapies, however, have not been sufficient for AD treatment (Imbimbo et al. 2020). Therefore, there is a need to identify the disease-specific immune mechanisms of microglia and peripheral immune cells to better target therapeutic purposes. An interesting aspect that may underline of differences in disease prevalence and progression, may be heterogeneity in peripheral immune cells in males and females that determine immune system response to foreign stimuli.

Our previous results and those presented in this manuscript allow for the drawing of interesting conclusions towards the immunological characteristics of patients with AD. First, men and women with AD differ in their peripheral immune system condition. Second, altered pattern of the immune response of PBLs was noticed, in contrast to healthy individuals in AD men and women. We have shown earlier that the level of innate immunity, measured with a test based on ex vivo PBLs resistance to VSV infection, was reduced among AD patients and positively correlated with disease severity (Sochocka et al. 2019). Complete resistance of PBLs to VSV infection was noticed among patients with mild AD. PBLs sensitivity to viral infection deteriorated with disease progression from moderate to serious AD (Sochocka et al. 2019). Cellular resistance to viral infection is one of the innate immune reactions which was found to have unspecific character. The selection of VSV to the test of PBLs resistance was not incidental. Other viruses, such as: human herpesvirus 1 (*Herpesviridae*) and encephalomyocarditis virus (*Picornaviridae*), have been tested. VSV as the indicatory virus does not cause natural infection among human population, thus no specific anti-VSV antibodies are presented in human sera (Jatczak et al. 2012; Rybka et al 2003). There are also no implications of VSV in AD development. In this study, sex differences in the level of innate immunity were observed in AD but not among healthy controls. Interpretation of the results was based on the assumption that the lack of virus replication (defined as 0–1 log $TCID_{50}$/ml) indicated complete immunity. Deficiencies emerged when the virus replicated over 1 log (about 2–3 log) which indicates partial immunity. When the virus replicated

over 4 log it evidenced high deficiency in innate immunity. Analysis of the results let us conclude that the mean level of innate immunity of all participants showed deficiency in innate immunity. It was consistent with previous studies that this mechanism progressively drops with advanced age due to severe deterioration of both adaptive and innate immunity in the elderly (Jatczak et al. 2012; Rybka et al 2003). Interestingly, although all of our participants were immunodeficient, sex differences were observed only in AD. This disparity indicated on importance of stratifying sex in AD. Therefore, it was expected that the innate immunity of AD females will be reduced compare to males. We suggest that it could be related to the dementia state of the patients. In our study, most of recruited men were with mild AD, while most of women were with moderate or serious AD, and as mentioned earlier, lower innate immunity was noted among patients with moderate to severe AD (Sochocka et al. 2019). Moreover, frequency of AD is often reported to be higher for women than men, and women suffer more severely from AD having longer disease duration (Zhu et al. 2021). Importantly, women first-time diagnosed are usually in worse clinical condition, which might be also the reason that among our AD patients women were more severe than AD men. However, the better immune condition of men with AD deserves much attention. More detailed research on a larger sample size are required to confirm our assumptions. The study of the level of pro- and anti-inflammatory cytokines released by peripheral immune cells was to explain the observed differences.

The next part of the study was to investigate sex differences in PBLs response to VSV infection ex vivo by quantifying several cytokine production. Natural immunity measured in the whole PBLs has been found to be the most informative and repeatable and dependent on endogenous cytokines such as interferons and TNF-α (Orzechowska et al. 2003; Zaczyńska et al. 2008). We chose to investigate cytokines: TNF-α, IFN-γ, IL-1β, IL-10 and additionally IFN-α. As we mentioned above, although in all our participants innate immune response was decreased, altered pattern of immune response/cytokine production by PBLs was recognized in AD patients. In contrast to controls, AD patients were characterized with increased investigated inflammatory markers as shown by cytokine measurements produced by uninfected PBLs. Yet, PBLs of AD women released much less cytokines compared to men what may indicated sex differences in immune response of leukocytes. This effect was not observed among healthy controls. Perhaps in AD patients, an enhancement of spontaneous inflammatory cytokine release by PBLs ex vivo has a reflection in immune condition of whole organism what expressed in cytokine level in peripheral circulation. We believe that this trend of immune research in AD should be continued. Significantly higher levels of IL-10 and IL-1β, in plasma samples of AD patients were noticed by King et al. (2018). Elevated levels of TNF-α, IL-1β, IFN-γ and other peripheral inflammatory markers in AD were also confirmed by Lai et al. (2017) and Su et al. (2019), however, in those studies sex differences were not explored. It should be noted that immune response measured by cytokine production by PBLs in response to stimuli better reflect inflammatory mechanisms that has been going in the body in comparison to cytokine levels detected in the serum/plasma. Whole amount of inflammatory markers in serum/plasma is a result of immune cells activity but also daily leakage of CNS metabolites into the peripheral system (Arosio et al. 2014; Esteras et al. 2016). Interestingly, in our study, the presence of viral stimuli changed the profile of investigated cytokines produced by these cells. Immune response in AD, however, was also altered compared to healthy age-matched controls. Leukocytes of healthy controls reacted with several-time increase in TNF-α, IFN-γ, IL-10 and IL-1β production in response to VSV infection while PBLs from AD patients responded with low increase in TNF-α and IL-1β production and decrease in IL-10 compared to unstimulated PBLs. It was presented that VSV replicates in monocytes and monocyte-derived dendritic cells leading to an increase of several cytokine and chemokine production such as TNF-α, IFN-α and IFN-γ inducible protein-10 (Tomczyk et al. 2018). Different response of PBLs from AD patients to VSV infection could result from the activation of different intracellular signaling pathways or changes in the peripheral cell population. For instance, GSK3 has been identified as promoting the production of IL-6, IL-1β, and TNF-α, and decreasing the level of IL-10 in human monocytes (Martin et al. 2005). In AD patients, an exacerbation of the age-related change in immune system cell distribution and function was noted. Studies of blood count changes in people with AD found a decrease in lymphocyte number and increase in neutrophil number (Lutshumba et al. 2021). Sex differences were not investigated.

One of the most surprising but interesting results was the observation of IFN-γ production by PBLs from AD and controls. First, in AD patients, sex bias in the level of IFN-γ secreted by uninfected and VSV-infected leukocytes were not observed. However, even though there were no sex differences, significant disparity were noticed between AD and controls in spontaneous IFN-γ production. Uninfected PBLs from AD patients released three times more interferon compared to controls. However, no stimulation of IFN-γ pathway was noticed after PBLs infection with VSV, while leukocytes of healthy controls reacted properly producing elevated level of this cytokine. Possibly PBLs of AD patients overexpressed of IFN-γ and were not able to boost this cytokine after viral infection. This may indicate altered pattern of immune response to viral infection. The question was why an over-exuberance of IFN-γ by PBLs of AD patients was noticed? Mastrangelo et al. (2009) induced chronic of IFN-γ

production as a response to serotype-1 recombinant adeno-associated virus vector in triple-transgenic mouse model of AD. They exhibited protective role of IFN-γ related to maintenance of virus latency after infection, diminished phospho-tau pathology and evidence of increased neurogenesis. On the other hand, they pointed on that elevated level of IFN-γ was deleterious what resulted in an increase in microglial activation, pro-inflammatory cytokine production, and severity of amyloid-related pathology. In contrast to our results, Jabbari Azad et al. (2014) showed that the level of IFN-γ in the T lymphocytes of AD patients stimulated with PMA was increased compared to control individuals. Different stimulants, however, were used in our study (virus) thus different intracellular pathways were activated. In addition, it was noticed the steady state production of IFN-γ in AD which ensures a constant low level of cell trafficking between the brain and the periphery. Upregulating of IFN-γ in the peripheral immune system was observed in AD after the insult/injury, such as infections, trauma, relative ischemia or metabolic alterations, and increased of BBB permeability. It is believed that this might be beneficial for dampening development/progression of AD and postulated cross-talk communication between the AD brain and the periphery (Schwartz and Deczkowska 2016). Perhaps, our observations of sustained increase of IFN-γ in AD patients, mean that continuous danger signal coming from the brain or from periphery, e.g., due to chronic infection, has occurred. The role of chronic infection in AD pathogenesis was postulated from several years and characterized by at least three different frameworks as currently presented by Butler and Walker (2021). Additionally, the level of IFN-α was also investigated. Results showed that uninfected PBLs from AD and controls did not produce IFN-α what was expected. Infection with VSV resulted in IFN-α production by PBLs in both groups. However, PBLs from controls produced 88.47 pg/ml of IFN-α, over eight times more than leukocytes from AD – 10.49 pg/ml. We did not present these findings in the results due to IFN-α was not assessed for all study participants (only for a half of them) thus we were unable to perform sex difference analysis. However this observation, showing a significant disparity between AD and controls was interesting. Our research indicated that, as well as with IFN-γ, PBLs from AD patients were unable to release higher amounts of IFN-α in response to viral infection.

The main limitation of this study was a sample size according to precision of the estimation of the interesting effects. Nevertheless, the observed differences were statistically significant and effects size were strong. In the case of participants' age, there was no serious age gap between AD patients and controls, and the difference between medians age was 8.5 years. Typical difference between two randomly selected AD patients was 11 years and 9.5 years between controls. Thus, AD patients differed each other more than was the difference in median age between both groups. Another limitation was the number of AD women and AD men. In this study, the female/male ratio was not 1:1 what resulted from the sampling method. Although the ratio also corresponded to the natural ratio in the AD population (more females affected with AD), the total number of patients was limited. In the case of small study groups (sexes) from a statistical point of view, they should contain the same (or very similar) numbers of patients to avoid mis-interpretation of the obtained results. To confirm these results and to draw prominent conclusions, future studies are needed. The challenges ahead are research involving larger groups of patients and controls in an appropriate age. The effect size of the future obtained results should also be estimated. In addition, to avoid the misleading factors (e.g., serious, chronic inflammatory or infectious diseases) influencing on immune background, the control group should be choose carefully. Investigations of a wider panel of cytokines both externally and intracellularly. Inflammatory-genes expression that might be responsible for, e.g., IFN-γ overproduction should also be investigated.

Concluding Remarks

In this study, measured innate immune system mechanisms did not differ among sexes in controls; whereas in AD, this disparity was strongly visible. In healthy individuals, the pattern of leukocyte response to viral infection still remained proper with an increase of pro- and anti-inflammatory molecules. Among AD patients, a derangement in investigated reactions of innate immune system was noted in relation to sex. AD sufferers were probably sustained over-stimulated with constant increase in inflammatory markers. In AD, the persistent activation of the immune system is detrimental (Sochocka et al. 2017), and different susceptibility, and vulnerability to chronic inflammation will vary among males and females (Hall et al. 2013). On the other hand, our observations may result at least partially from hormonal alteration observed in postmenopausal women in which hormone decline occur more rapidly than in males, with immune system disability (Klein and Flanagan 2016). A stronger immune responses is characterized for pre-menopausal adult women in contrast to children, men, or post-menopause women. Sex hormones have a great impact on immune response. Estrogen has immune-stimulatory activity, while progesterone and androgens play an immune-suppressive role. It was showed that estrogen can regulate IL-1β, IL-10 or IFN-γ (Ciarambino et al. 2021). In addition, an aging of the immune system is related with the development of a sub-clinical chronic low-grade systemic inflammation with elevated level of serum levels of pro-inflammatory mediators

such as TNF-α, IL-6, and IL-8 or CRP (Ciarambino et al. 2021). In this context, immunosenescence in females is characterized with greater extent in aberrant chronic low-grade pro-inflammatory state (Fuentes et al. 2017; Klein and Flanagan 2016). It is important to consider sex as a biological variable in AD due to it promises to advance our understanding of mechanisms of AD pathology and may be the basis for future treatment of AD. Nevertheless, much more research are still need to understand innate immune dysregulation and the role of peripheral immune cell crosstalk in AD pathophysiology.

Acknowledgements The authors wish to acknowledge the nursing staff of the Wroclaw Medical University for the support in recruiting participants and blood samples collection.

Author Contributions JL, MS: contributed to the study conception and design. MO, MS: performed laboratory experiments. JL: was responsible for clinical examination of the patients. Data collection and analysis were performed by MS, MO and MS. The first draft of the manuscript was written by MS and MS. MS, JL, BO revised the manuscript critically for important intellectual content and editing language. All authors read and approved the final manuscript.

Funding This work was supported by internal research funds of the Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences and Wroclaw Medical University.

Data Availability Data available on request from the authors.

Declarations

Conflict of Interest The authors report no conflicts of interest, financial or otherwise.

Ethical Approval This study has been reviewed, approved, and conducted in accordance with the guidelines of the Ethics Committee of the Wroclaw Medical University (No. KB-349/2016).

Consent to Participate Informed consent was obtained from all individual participants or their legal guardians included in the study.

Consent for Publication Not applicable.

References

Armstrong RA (2013) What causes Alzheimer's disease? Folia Neuropathol 51:169–188

Arosio B, D'Addario C, Gussago C et al (2014) Peripheral blood mononuclear cells as a laboratory to study dementia in the elderly. BioMed Res Int 2014:169203

Bektas A, Schurman SH, Sen R et al (2018) Aging, inflammation and the environment. Exp Gerontol 105:10–18

Bettcher BM, Tansey MG, Dorothée G et al (2021) Peripheral and central immune system crosstalk in Alzheimer disease—a research prospectus. Nat Rev Neurol 17:689–701

Butler L, Walker AK (2021) The role of chronic infection in alzheimer's disease: instigators, co-conspirators, or bystanders? Curr Clin Microbiol Rep 8:199–212

Ciarambino T, Para O, Giordano M (2021) Immune system and COVID-19 by sex differences and age. Womens Health 17:17455065211022262

Esteras N, Alquézar C, de la Encarnación A et al (2016) Lymphocytes in Alzheimer's disease pathology: altered signaling pathways. Curr Alzheimer Res 13:439–449

Ferretti MT, Iulita MF, Cavedo E et al (2018) Sex differences in Alzheimer disease—the gateway to precision medicine. Nat Rev Neurol 14:457–469

Fuentes E, Fuentes M, Alarcón M et al (2017) Immune system dysfunction in the elderly. An Acad Bras Cienc 89:285–299

Giri M, Zhang M, Lü Y (2016) Genes associated with Alzheimer's disease: an overview and current status. Clin Interv Aging 11:665–681

Hall JR, Wiechmann AR, Johnson LA et al (2013) Biomarkers of vascular risk, systemic inflammation, and microvascular pathology and neuropsychiatric symptoms in Alzheimer's disease. J Alzheimers Dis 35:363–371

Hanamsagar R, Bilbo SD (2016) Sex differences in neurodevelopmental and neurodegenerative disorders: focus on microglial function and neuroinflammation during development. J Steroid Biochem Mol Biol 160:127–133

Heneka MT, Carson MJ, Khoury JE et al (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388–405

Imbimbo BP, Lozupone M, Watling M et al (2020) Discontinued disease-modifying therapies for Alzheimer's disease: status and future perspectives. Expert Opin Investig Drugs 29:919–933

Jabbari Azad F, Talaei A, Rafatpanah H et al (2014) Association between cytokine production and disease severity in Alzheimer's disease. Iran J Allergy Asthma Immunol 13:433–439

Jatczak B, Leszek J, Siemieniec I et al (2012) Age- and disease-related innate immunity of human leukocytes ex vivo. Exp Gerontol 47:8–13

Jevtic S, Sengar AS, Salter MW et al (2017) The role of the immune system in Alzheimer disease: etiology and treatment. Ageing Res Rev 40:84–94

King E, O'Brien JT, Donaghy P et al (2018) Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. J Neurol Neurosurg Psychiatry 89:339–345

Kinney JW, Bemiller SM, Murtishaw AS et al (2018) Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement 4:575–590

Klein SL, Flanagan KL (2016) Sex differences in immune responses. Nat Rev Immunol 16:626–638

Lai KSP, Liu CS, Rau A et al (2017) Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. J Neurol Neurosurg Psychiatry 88:876–882

Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol 4:863

Laws KR, Irvine K, Gale TM (2016) Sex differences in cognitive impairment in Alzheimer's disease. World J Psychiatry 6:54–65

Le Page A, Dupuis G, Frost EH et al (2018) Role of the peripheral innate immune system in the development of Alzheimer's disease. Exp Gerontol 107:59–66

Lim A, Krajina K, Marsland AL (2013) Peripheral inflammation and cognitive aging. Mod Trends Pharmacopsychiatry 28:175–187

Lopez-Lee C, Kodama L, Gan L (2021) Sex differences in neurodegeneration: the role of the immune system in humans. Biol Psychiatry 91:72–80

Lutshumba J, Nikolajczyk BS, Bachstetter AD (2021) Dysregulation of systemic immunity in aging and dementia. Front Cell Neurosci 15:652111

Martin M, Rehani K, Jope RS et al (2005) Toll-like receptor—mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat Immunol 6:777–784

Mastrangelo MA, Sudol KL, Narrow WC et al (2009) Interferon-{gamma} differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. Am J Pathol 175:2076–2088

Morris JK, Honea RA, Vidoni ED et al (2014) Is Alzheimer's disease a systemic disease? Biochim Biophys Acta 1842:1340–1349

Nebel RA, Aggarwal NT, Barnes LL et al (2018) Understanding the impact of sex and gender in Alzheimer's disease: a call to action. Alzheimers Dement 14:1171–1183

Orzechowska B, Antoszków Z, Błach-Olszewska Z (2003) Individual differentiation of innate antiviral immunity in humans; the role of endogenous interferons and tumor necrosis factor in the immunity of leukocytes. Arch Immunol Ther Exp 51:51–60

Pawelec G (2018) Age and immunity: what is "immunosenescence"? Exp Gerontol 105:4–9

Podcasy JL, Epperson CN (2016) Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin Neurosci 18:437–446

Rosenthal R, Rosnow RL, Rubin DB (2000) Contrasts and effect sizes in behavioral research—a correlational approach. Cambridge University Press

Rousseeuw PJ, Croux C (1993) Alternatives to the median absolute deviation. J Am Stat Assoc 88:1273–1283

Rybka K, Orzechowska B, Siemieniec I et al (2003) Age related natural antiviral non-specific immunity of human leukocytes. Med Sci Monit 9:BR413-417

Sawilowsky SS (2009) New effect size rules of thumb. J Mod Appl Stat Methods 8(2): 597–599

Schwartz M, Deczkowska A (2016) Neurological disease as a failure of brain-immune crosstalk: the multiple faces of neuroinflammation. Trends Immunol 37:668–679

Sochocka M, Diniz BS, Leszek J (2017) Inflammatory response in the CNS: friend or foe? Mol Neurobiol 54:8071–8089

Sochocka M, Ochnik M, Sobczyński M et al (2019) New therapeutic targeting of Alzheimer's disease with the potential use of proline-rich polypeptide complex to modulate an innate immune response—preliminary study. J Neuroinflammation 16:137

Su C, Zhao K, Xia H et al (2019) Peripheral inflammatory biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. Psychogeriatrics 19:300–309

Tomczyk T, Wróbel G, Chaber R et al (2018) Immune consequences of in vitro infection of human peripheral blood leukocytes with vesicular stomatitis virus. J Innate Immun 10:131–144

Wang MM, Miao D, Cao XP et al (2018) Innate immune activation in Alzheimer's disease. Ann Transl Med 6:177

Zaczynska E, Duś D, Paprocka M et al (2008) Resistance of human leukocytes to vesicular stomatitis virus infection as one of the innate antiviral immune activities; participation of cell subpopulations. Folia Histochem Cytobiol 46:39–43

Zhu D, Montagne A, Zhao Z (2021) Alzheimer's pathogenic mechanisms and underlying sex difference. Cell Mol Life Sci 78:4907–4920