Cerebrospinal Fluid Profile of Lipid Mediators In Alzheimer's Disease

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Research

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Abstract

Background: Alzheimer's disease (AD) develops into dementia over a period of several years, during which subjective cognitive impairment (SCI) and mild cognitive impairment (MCI) are used as intermediary diagnoses of increasing severity. Chronic neuroinflammation resulting from insufficient resolution is involved in the pathogenesis of AD and is associated with cognitive impairment. Specialized pro-resolving lipid mediators (LMs) that promote the resolution of inflammation may be valuable markers in AD diagnosis and as therapeutic targets.

Methods: Liquid chromatography-tandem mass spectrometry was used to analyze pro-resolving and pro-inflammatory LMs in cerebrospinal fluid (CSF) from patients with cognitive impairment ranging from subjective impairment to a diagnosis of AD, and correlated to cognition, CSF tau and β-amyloid (Aβ), and an inflammation biomarker (YKL-40).

Results: RvD4, neuroprotectin D1, MaR1, and RvE4 were lower in AD and/or MCI compared to SCI. The pro-inflammatory LTB_4 and 15-HETE were higher in AD and MCI, respectively, while PGD_2 and PGE_2 were decreased in AD, compared to SCI. RvD4 was also negatively correlated to AD tangle biomarkers. Many differences were dependent on gender.

Conclusion: In this exploratory study of the lipidome in CSF of AD, MCI and SCI, the results indicate a gender-dependent shift in the LM profile from pro-resolving to pro-inflammatory in progression to AD, suggesting that it may be of use as a biomarker when followed by confirmation by replication studies.

Background

Alzheimer's disease (AD) is the most common dementia in the aged population [1]. AD brain pathology includes neuronal and synapse loss as well as widespread deposits of senile plaques mainly consisting of β-amyloid (Aβ) peptide, neurofibrillary tangles of phosphorylated (p)-tau protein, and activated microglia [2–4]. As AD dementia is an insidious process and usually takes decades, diagnostic categories reflecting disease progression have been developed. A commonly used nomenclature of increasing severity starts with subjective cognitive impairment (SCI) [5], then mild cognitive impairment (MCI) [6], and finally, dementia due to AD [7]. In SCI, the individual experiences memory problems, but clinical assessments are within the normal range [5]. In MCI, symptoms are characterized by decreased cognitive function in clinical assessment but minimal or no functional decline [6]. In the dementia stage, the ability of patients to function is significantly impaired [8].

Molecular biomarkers in cerebrospinal fluid (CSF) of Ab and tau pathology are used to facilitate AD diagnosis [9]. However, AD is a heterogeneous and multifactorial disease and should be regarded from a broader perspective than from only Ab and tau [10]. It is important to expand the range of molecular factors used as biomarkers reflecting other mechanisms of the pathogenesis. Inflammatory responses in AD are well-known [4,11], and there is accumulating evidence of its occurrence early in the disease process [12]. Aside from being regulated by cytokines and chemokines, inflammation engages a

prominent network of lipid mediators (LMs) with well-known bioactivities, such as the fever response [13,14] and pain [15]. However, LMs also play an essential role in the resolution of inflammation and the initiation of tissue restoration, an active process regulated by specialized pro-resolving LMs. These include the lipoxins (LX), protectins (PD), resolvins (Rv), and maresins (MaR), which are derived from the omega-3 and -6 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA), arachidonic acid (AA), and eicosapentaenoic acid (EPA) [16,17]. Although a relatively new field of research, studies on various pathologies revealed LM involvement [18–22].

AD brains demonstrate lower levels of pro-resolving LMs than healthy controls [23–25], while the expression of their receptors is increased [24,26]. *In vitro* studies demonstrate that pro-resolving LMs improve cell survival, reduce Ab production in neuronal models [23,25,27–30], and down-regulate inflammation and increase Ab phagocytosis in glia [23,25,27–30]. Reduction of AD pathologies and attenuation of cognitive impairment [27–29,31,32] have been shown in *in vivo* models. To pave the way for future treatments and biomarkers based on the resolution of inflammation, we aimed at analyzing the pro-inflammatory and pro-resolving lipidome in CSF in cohorts of AD, MCI, or SCI patients and how the lipidome is associated with cognitive dysfunction and biomarkers of plaques and tangles, as well as with the inflammatory marker YKL-40. In view of the known higher incidence of AD in women, we also included gender comparisons in the analyses.

Methods

Recruitment of study subjects

The study population consisted of 136 participants with SCI (n = 53), MCI (n = 43), or AD (n = 40) from the Memory Clinic at Karolinska University Hospital, Huddinge, Sweden. **Table 1** lists the demographics of the study population. The recruitment procedure details are outlined in **Fig. 1**. Data on age, gender, cognition, CSF AD biomarkers (Ab_{42}, total (t)-tau and phosphorylated (p)-tau), the mini-mental state examination (MMSE) test [33], and clinical diagnosis were retrieved from the biobank database at the clinic. The CSF biomarker levels were determined by ELISAs (INNOTEST®, Innogenetics, Ghent, Belgium) with the following cut-off values indicating pathology: $A\beta_{42}$ < 550 pg/ml, t-tau > 400 pg/ml, and p-tau > 80 pg/ml. The ICD-10 criteria were used for AD diagnosis [34], and the Winblad criteria were used for the diagnosis of MCI [8]. A diagnosis of SCI was established when clinical tests did not indicate pathology [5,35].

The clinical data and CSF samples of the study subjects were collected from 2015 to 2017. All participants gave informed consent and agreed to donate their CSF to the Gedoc biobank for scientific research. The study was approved by the Regional Human Ethics Committee of Stockholm (2011/680-31, 2014/1921-32, and 2020-02023).

Analysis of lipid mediators

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to assess a total of 22 lipids in the CSF samples, including pro-resolving LMs (LXA_4, MaR1, MaR2, neuroprotectin D1 (NPD1), RvD1, RvD3, RvD4, RvE1, and RvE4), pro-inflammatory LMs (leukotriene B_4, LTB_4), prostaglandins (PGD2, PGE2, and PGF2a), their precursors (EPA, AA, and DHA) and the intermediate products in the metabolic pathways (14-hydroxy-docosahexaenoic acid (14-HDHA), 17-HDHA, 20-HDHA, 12-hydroxyeicosatetraenoic (12-HETE), 14-HETE and 15-HETE) (**Fig. 2**). Fatty acids were extracted from CSF samples using a liquid-liquid lipid extraction method based on $CHCl_3$/MeOH extraction [36]. Briefly, since the volume of CSF samples was small (< 700ul), extraction was done by adding 9 ml of $CHCl_3$/MeOH = 2:1. Internal standard mix (PGD2-d4, LTB4-d4, 15-HETE-d8, EPA-d5, and AA-d8) was added. Then 2 ml of pH3.5 H2O was added, the resulting upper aqueous phase discarded, and the bottom organic phase was dried down under a gentle N2 gas stream. The lipid extract was re-constituted in 50ul of MeOH/H2O = 1:1 solvent and samples loaded onto a Xevo TQ-S equipped with Acquity I Class UPLC (Waters, Milford, MA, USA). We used the CORTECS C18 2.7um column (4.6 x 100 mm; Waters, Milford, MA, USA) for chromatographic separation. Initially, 56.2% of the mobile phase A (MeOH/H2O = 2:8, 0.01% AcA) gradually decreased to 25% for the first 8 min, then 3 min of isocratic run, followed by 100% B (MeOH, 0.01% AcA) at 18.1 min. The isocratic run of 100% B till 25 min is followed. Finally, it comes back to the initial condition for 5 min. The capillary voltage was -2.5kV, desolvation temperature at 600C, desolvation gas flow at 1100L/Hr, cone gas at 150 L/Hr, and nebulizer pressure at 7.0 Bar with the source temperature at 150^{o}C.

Analysis of the inflammatory factor YKL-40

The concentration of chitinase-3-like protein 1 (YKL-40) was determined by ELISA according to the distributor instructions (R&D Systems, Abingdon, United Kingdom).

Statistical analysis

To investigate differences between diagnostic groups, Kruskal-Wallis was performed in Statistica v13 (Tibco, Palo Alto, USA), followed by Dunn's *post-hoc* test corrected for multiple comparisons. The association of LMs to cognition, AD biomarkers, and inflammatory factors was tested with Spearman Rank Order Correlation in Statistica v13. A *P*-value of < 0.05 was considered statistically significant.

Results

CSF samples from cases with different degrees of memory dysfunction according to objective tests (AD or MCI) or subjective memory complaints (SCI) were analyzed by LC-MS/MS with regard to bioactive LMs, their fatty acid precursors, and intermediate derivatives. The median detected level and interquartile range for each LM within the diagnostic groups are presented in **Table 2**.

Differences in lipids between diagnostic groups and genders

Diagnostic groups

The analysis of LMs in CSF samples showed that levels of the pro-resolving LMs RvD4 and NPD1 were lower in the AD ($P < 0.00005$ and $P < 0.05$, respectively) and MCI ($P < 0.0005$ and $P < 0.05$, respectively) group compared to SCI (**Fig. 3A**), whereas levels of the pro-inflammatory LM LTB_4 were increased in AD ($P < 0.001$) and MCI ($P < 0.05$) compared to SCI (**Fig. 3C**). The pro-resolving MaR1 ($P < 0.005$) (**Fig. 3A**) and RvE4 ($P < 0.005$) (**Fig. 3B**) were lower in CSF samples from MCI patients compared to SCI cases.

The levels of PGD_2 ($P < 0.0005$) and PGE_2 ($P < 0.0001$) were lower in MCI patients compared to SCI; PGD_2 levels were also lower in AD compared to SCI ($P < 0.005$) (**Fig. 3C**).

There were no significant differences between the three diagnostic groups regarding the n-3 and n-6 PUFA precursors AA, EPA, or DHA.

Gender

Analysis according to gender revealed additional differences between the diagnostic groups, demonstrating a pattern regarding PUFAs where the median levels were almost half in women in the non-impaired SCI group compared to men with the same diagnosis (DHA ($P < 0.0001$), EPA ($P < 0.0005$), and AA ($P < 0.0001$)) (**Fig. 3**). In addition, within the MCI group, AA levels were about 30% lower in women than in men (**Fig. 3C**). The levels of DHA in men were slightly lower but statistically significant in AD than in SCI ($P < 0.05$) (**Fig. 3A**).

A gender difference in the SCI group was also found for the DHA derivative 20-HDHA, where women showed almost half the median levels of this LM compared to men ($P < 0.005$) (**Fig. 3A**), and for 12-HETE, which also had lower levels for women ($P < 0.005$) (**Fig. 3C**). The pro-resolving LM RvE1 showed higher levels in women than in men within the AD group ($P < 0.05$), and men had more than 30% lower levels of this LM in the AD group compared to men with a diagnosis of SCI ($P < 0.005$) (**Fig. 3B**).

In the MCI group, the LXA_4 levels were higher in women than in men ($P < 0.005$), and the levels in men in this group were lower than in men in the SCI group ($P < 0.05$) (**Fig. 3C**).

As described above, the levels of RvD4 were reduced in both AD and MCI compared to SCI, and the difference between AD and SCI cases was also seen in both women ($P < 0.005$) and men ($P < 0.05$) (**Fig. 3A**), whereas the difference between MCI and SCI was attributed only by a difference in women ($P < 0.005$). The lower levels of MaR1 seen in MCI compared to SCI were due to differences between men in those groups ($P < 0.005$) (**Fig. 3A**).

The reduction in PGD_2 and PGE_2 abundance in MCI cases compared to SCI was also found upon analysis of women ($P < 0.01$ for women) and men ($P < 0.05$ for men) separately (**Fig. 3C**). Comparisons of PGE_2 levels between AD and SCI showed a difference only for women ($P < 0.01$) (**Fig. 3C**). The significantly higher levels of LTB_4 in AD cases compared with SCI cases can be attributed to differences between women ($P < 0.01$), while the higher levels seen in LTB_4 in MCI compared to SCI cases can be attributed to the difference in men ($P < 0.05$).

Female SCI cases showed slightly higher but statistically significant CSF levels of RvD3 compared to those diagnosed with MCI ($P < 0.05$) (**Fig. 3A**), while the comparison of the 15-HETE levels between the same groups showed that the levels were slightly lower but statistically significant in female SCI than in female AD cases ($P < 0.05$) (**Fig. 3C**).

Correlations to cognitive function, CSF biomarkers of plaque and tangle pathology, and the CSF inflammation marker YKL-40

Correlative relationships were investigated using the Spearman rank-order test. The LMs where significant correlations could be found are presented in **Table 3**. The complete results from the analysis of correlations, including all LMs and PUFAs, can be seen in **Supplementary Table 1**.

MMSE

Our analyses of correlations suggest that for several lipids, high levels are associated with are protection against the deterioration of cognition seen in AD as assessed by the MMSE test. Analysis of the entire cohort showed that the levels of RvD4 displayed the strongest correlation to cognition as evaluated by the MMSE test (R = 0.29, $P < 0.001$). Other lipids showing a positive correlation to MMSE when including all three diagnostic groups were DHA (R = 0.21), EPA and PGE_2 (R = 0.18), and RvD1 (R = 0.17), all with a significance level of $P < 0.05$. Separating the cases according to diagnosis provided stronger correlative relationships. In the group of AD cases, the strongest correlations to MMSE were by DHA and 14-HDHA (R = 0.53, $P < 0.0005$ for both), EPA (R = 0.51, $P < 0.001$), AA (R = 0.42, $P < 0.01$), and 20-HDHA (R = 0.33, $P < 0.05$). Among the cases diagnosed with MCI, only one negative correlation was found with MMSE, *i.e.*, MaR1 (R = -0.32, $P < 0.05$). Cases diagnosed with SCI showed positive correlations between MMSE and RvD4 (R = 0.42, $P < 0.005$), RvD1 (R = 0.36, $P < 0.01$), RvE4 (R = 0.34, $P < 0.05$), and LTB_4 (R = 0.29, $P < 0.05$).

Ab_{42}

Analysis of all diagnostic groups together showed that the CSF levels of Ab_{42} were positively correlated to the levels of RvD4 (R = 0.29, $P < 0.001$), RvE1 (R = 0.23, $P < 0.01$), RvD1 (R = 0.18, $P < 0.05$), and NPD1 (R = 0.18, $P < 0.05$). The analysis of correlations according to diagnostic group showed a positive correlation between Ab_{42} and 12-HETE (R = 0.42, $P < 0.01$), LXA_4 (R = 0.35, $P < 0.05$), LTB_4 (R = 0.33, $P < 0.05$), and RvE4 (R = 0.32, $P < 0.05$) among the AD cases.

t-tau and p-tau

The CSF levels of the tangle biomarkers t-tau and p-tau showed weak correlative relationships to the lipids analyzed. Analysis of the entire cohort showed a negative correlation between RvD4 and t-tau (R = -0.17, $P < 0.05$) while there was no correlation to p-tau. The analysis according to the diagnostic group showed that for AD cases, MaR1 was negatively correlated to t-tau (-0.35, $P < 0.05$), and PGD_2 was positively correlated to p-tau (R = 0.32, $P < 0.05$). In cases diagnosed with MCI, there was a negative

correlation between the levels t-tau and those of LXA_4 and 12-HETE (R = -0.33, $P < 0.05$ and R = -0.32, $P < 0.05$, respectively). There was no correlation to the CSF levels of t- or p-tau within the SCI group.

YKL-40

To probe for the relationship to inflammation, correlations between the CSF levels of lipids to YKL-40 were investigated. In the entire cohort, the levels of AA and EPA were positively correlated to those of YKL-40 (R = 0.21, $P < 0.05$ and R = 0.2, $P < 0.05$, respectively). Analysis of the diagnostic groups separately showed a positive correlation in the SCI group between PGE_2 and PGD_2 levels and YKL-40 (R = 0.37, $P < 0.01$ and R = 0.29, $P < 0.05$, respectively).

Discussion

The inflammatory lipidome consists of pro-inflammatory LMs and also of LMs that end and resolve inflammation while promoting restoration and regeneration of the tissue, *i.e.*, healing [37]. We have previously shown decreased levels of pro-resolving LMs in the human AD brain [23–25]. Two of these LM, LXA_4 and RvD1, were present in lower levels in the CSF of AD patients compared to those without clinical evidence of memory deficits, *i.e.*, diagnosed with SCI, and were positively correlated to the scores from the MMSE test.

A decrease in RvD1 in the CSF of AD patients was not seen in the present study, possibly due to the greater specificity of LC-MS compared to immunochemical assays in which signals may consist of several compounds with molecular similarities. Compared to SCI patients, the levels of RvD4 and NPD1 were lower in both AD and MCI patients, while RvE4 and MaR1 were lower in MCI patients only. The differences between the diagnostic groups in RvD4 and MaR1 are also seen in a gender-separated comparison. However, this was not evident for NPD1.

The pool size of the LM precursors DHA, EPA, and AA in CSF was substantially lower in women than in men in the SCI group and, in the case of AA, also in the MCI group. However, except for slightly reduced but statistically significant levels of DHA in men with AD compared to SCI, there were no differences in the levels of DHA, EPA, or AA between the diagnostic groups. Lohner *et al*. [38] describe in a review that the plasma lipid and plasma phospholipid compartments of women contained higher levels of both DHA and AA, whereas in erythrocyte membranes, only DHA levels were higher in women. Several factors may influence the levels of lipids and give rise to differences between men and women, including diet, age, sex hormones, and the ability to synthesize lipids. Indeed, the ability to synthesize long-chain fatty acids was shown to be higher for women than men, as suggested by a higher conversion rate of a-linoleic acid (ALA) to DHA and EPA [39]. In a study on mice [40], females had higher brain levels of PUFAs than males, both after a Western-style high fat diet and regular chow diet, while plasma levels were similar. Extier *et al.* showed that female rats could replenish cortical DHA more efficiently than male rats, a result the authors attribute to higher expression in the desaturases needed for conversion of ALA to DHA [41]. Our

results, which contradict these previous findings, may be due to differences in cohort composition, species differences, or in the tissue analyzed.

Production of pro-inflammatory or resolving LMs from PUFAs is dependent on a series of enzymatic reactions. Phospholipases catalyze the liberation of PUFAs from membrane phospholipids, while cyclooxygenases and lipoxygenases act on the free PUFAs together with hydrolases and epoxidases and, in some cases, enzymes specific for producing a certain LM (*e.g.,* prostaglandin synthases) [42,43]. Gender-specific patterns of expression and activity of these enzymes are suggested by a study showing that the lower incidence of asthma in males is attributed to androgenic steroids acting on neutrophils leading to decreased trafficking and activity of 5-LOX, and thus less leukotriene production [44]. Although knowledge is not yet sufficient to explain the gender differences seen in our study, the literature so far indicates the possibility for different profiles according to gender.

Although analyses of LTB_4 in CSF have been performed since the eighties [45], the significance of its presence in CSF in the context of AD is not known. We show that LTB_4 in CSF of both AD and MCI patients was slightly higher but statistically significant than in SCI and negatively correlated to levels of Ab_{42}. In studies on multiple sclerosis (MS) [46,47], higher levels of LTB_4 were found in the CSF of MS patients compared to controls, suggesting LTB_4 as an indicator of inflammation in the brain, which is supported by our finding of a positive correlation of LTB_4 to YKL-40, suggested to be a marker of astrocytosis [48] and prognostic biomarker for AD [49]. LTB_4 increased the production of Ab in neurons in culture [50], providing a direct link to the molecular pathology in AD. In addition, we found that the CSF levels of 15-HETE, an intermediary in the synthesis of LTB_4, were slightly higher but statistically significant in women with AD compared to women with SCI diagnosis and negatively correlated to MMSE scores. Yao *et al*. previously detected 15-HETE in CSF [51] and increased levels in AD patients.

In contrast, the pro-inflammatory LMs PGD_2 and PGE_2 were lower in the CSF of MCI patients compared to SCI, and in the case of PGE_2, also reduced in AD patients compared to SCI. Neither PGD_2 nor PGE_2 has been studied in the CSF in the context of AD, but analysis of human *post mortem* entorhinal cortex showed higher levels of PGD_2 in AD compared to non-demented controls [25]. PGD_2 synthetase and the PGD_2 receptor DP1 were upregulated in plaque-associated glia in *post mortem* AD brains and an AD mouse model [52]. PGD_2 mediated neuronal cell death in *in vitro* cocultures of neurons and microglia exposed to Ab_{42} [53]. PGE_2 is increased in the CSF of patients with severe MS [18]. In a mouse model of AD, PGE_2 increased production of Aβ production [54]. The literature thus suggests that PGD_2 and PGE_2 play harmful roles in AD, which makes our findings on lower levels of these factors in the CSF of AD patients hard to explain without further studies.

Our novel finding of the presence of RvD4, NPD1, and RvE4 in human CSF highlight the abundance of pro-resolving LMs, and the present data on decreased levels in patients with cognitive dysfunction are in line with our previous research showing impaired resolution in AD. Of these, NPD1 is the most well-studied, and beneficial effects in the brain have been shown [23,55,56], as well as direct protection on

human neuronal cells [25]. NPD1 here showed a positive correlation to the CSF levels of Ab_{42}, known to be decreased in AD patients.

The decreased levels of MaR1 in CSF of MCI cases can mainly be attributed to reduced levels in women with MCI. We previously found decreased levels of MaR1 in the hippocampus [24] and entorhinal cortex [25] of AD patients and beneficial effects of MaR1 in several cellular models [25,30]. In the only previous study on MaR1 in human CSF [57], Tang *et al.* showed that the levels did not differ pre- and post-operatively, suggesting that a deficiency in resolution is a feature of chronic brain diseases. Surprisingly, there was a negative correlation of MaR1 to the MMSE scores in MCI patients, indicating a more complex nature of immune regulation in this heterogeneous group of patients than previously thought. However, in general, the correlative relationships between the lipids and cognition and AD CSF biomarkers indicated a positive role, where the levels of AA, DHA, and EPA all showed a comparatively strong positive correlation to cognition in AD cases, while within the group of SCI cases the LMs derived from DHA and EPA showed such a relationship. Of note, we, along with other researchers, consistently detect the presence of pro-resolving LMs in pathological as well as healthy tissues, which adds credibility to an evolving concept of the resolution pathway as an ever-present “care-taker-guardian” of the tissue rather than a response that is elicited only on demand. Studies in animal models of cancer [58–60] provide a fascinating perspective on resolution as a defender of the tissue, adding further support to this concept in which future therapies for disorders that today are hard to treat may be found.

Limitations

This is an explorative study, original in that it uses LC-mass-spectrometry to analyze the pro-inflammatory and pro-resolving lipidome in samples from cases of AD and MCI as well as subjective cognitive impairment (SCI) in a reasonably large cohort. Although we suggest the use of the CSF lipidomic profile as a biomarker of cognitive decline, its usefulness as a novel biomarker must be determined in replication studies, including longitudinal observations of cognitive decline. We are in the process of performing such studies and hope that the results from the present study motivate other researchers to explore and hopefully confirm the association of alteration in CSF LMs that we believe can be seen in our data. Age and gender were included in our analyses, and the influence of gender on the abundance of LMs is a major finding in the study.

The majority of the differences observed reach the threshold of 0.005 or 0.001, and in some cases even 0.0001. Considering the explorative nature and novel findings in our study, the analyses resulting in a p-value of > 0.005 should be interpreted with caution and as an impetus for further investigation rather than hard evidence. Although we did not perform a sensitivity power analysis prior to our investigation, we believe that the sample size in our cohort is large enough for an explorative study.

Conclusions

Our results uncovered alterations in the pro-resolving CSF lipidome during the dysfunctions of inflammatory resolution in AD. Our data demonstrate that gender has an important influence on the activities of pathways of LM synthesis and metabolism that are key onset events. This is an area of research where studies may improve the design of new AD treatments by including our LMs as biomarkers tracking dysfunctional resolution associated with increased AD risk. Overall we hope that our findings open inroads for innovative AD therapeutic approaches.

List Of Abbreviations

AA: arachidonic acid; Aβ: β-amyloid; AD: Alzheimer's disease; ALA: a-linoleic acid; CSF: cerebrospinal fluid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; HDHA: hydroxy-docosahexaenoic acids; HETE: hydroxyeicosatetraenoic; LC-MS/MS: liquid chromatography with tandem mass spectrometry; LMs: lipid mediators; LTB4: leukotriene B4; LX: lipoxin; MaR: maresin; MCI: mild cognitive impairment; MMSE: mini-mental state examination; MS: multiple sclerosis; NPD1: neuroprotectin D1; PD: protectins; PG: prostaglandin; p-tau: phosphorylated tau; PUFAs: polyunsaturated fatty acids; Rv: resolvin; SCI: subjective cognitive impairment; t-tau: total tau; YKL-40: chitinase-3-like protein 1

Declarations

Ethics approval and consent to participate

All participants signed an informed consent. The study was approved by the Regional Human Ethics Committee of Stockholm (2011/680-31).

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author’s contributions

The study was designed by EH, MS, and NB. YW took part in identifying the lipids to be analyzed, organized and handled the samples, and wrote a first draft of the manuscript. KVD, BJ, and MAIK extracted the lipids and analyzed the samples by LC-MS. YW, EH, and MS performed the statistical analysis. EH, MS, and NB edited and finalized the manuscript.

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Tables

Table 1. Cohort characteristics

	SCI n = 53 (F = 33, M = 20)			**MCI** n = 43 (F = 23, M = 20)			**AD** n = 40 (F = 24, M = 16)		
	Median	Lower Q	Upper Q	Median	Lower Q	Upper Q	Median	Lower Q	Upper Q
Age (y)	64	59	67	66	60	69	79	72	81
MMSE	29	28	30	28	27	29	24	22	26
Ab_{42}	909	769	987	851	645	1000	434	367	529
t-tau	260	214	320	269	185	377	523	388	809
p-tau	37	31	46	40	29	52	59	46	84

Data are described as median with interquartile (Q) range in pg/mL. Aβ = β-amyloid; AD = Alzheimer's disease; F = female, M = male, CSF = cerebrospinal fluid; MCI = mild cognitive impairment; MMSE = mini-mental state examination; p-tau = phosphorylated tau; SCI = subjective cognitive impairment; t-tau = total tau; y = years

Table 2. LMs in CSF from patients diagnosed with SCI, MCI or AD

	SCI			MCI			AD		
	Median	Lower Q	Upper Q	Median	Lower Q	Upper Q	Median	Lower Q	Upper Q
RvE1	0,95	0,73	1,27	0,95	0,73	1,27	1,18	0,93	1,53
RvE4	1,36	0,94	1,54	1,36	0,94	1,54	1,39	1,28	1,74
RvD1	7,85	3,72	8,06	7,85	3,72	8,06	7,95	7,82	8,38
RvD3	0,76	0,61	0,91	0,76	0,61	0,91	0,78	0,67	1,17
RvD4	0,93	0,78	1,01	0,93	0,78	1,01	1,10	0,94	1,35
MaR1	0,24	0,16	0,34	0,24	0,16	0,34	0,26	0,18	0,33
MaR2	0,20	0,17	0,26	0,20	0,17	0,26	0,20	0,18	0,26
NPD1	0,30	0,25	0,37	0,30	0,25	0,37	0,38	0,29	0,50
LXA_4	0,42	0,35	0,56	0,42	0,35	0,56	0,48	0,41	0,64
PGD_2	22,06	20,37	23,91	22,06	20,37	23,91	29,74	20,46	38,53
PGE_2	28,86	25,04	32,10	28,86	25,04	32,10	35,43	27,56	57,11
PGF_{2a}	10,50	4,95	11,73	10,50	4,95	11,73	9,35	8,04	13,34
LTB_4	0,45	0,32	0,64	0,45	0,32	0,64	0,32	0,20	0,40
12-HETE	1,32	0,28	1,76	1,32	0,28	1,76	1,08	0,00	1,55
15-HETE	0,99	0,43	1,22	0,99	0,43	1,22	0,40	0,20	0,74
14-HDHA	0,20	0,14	0,25	0,20	0,14	0,25	0,17	0,13	0,24
17-HDHA	0,22	0,17	0,31	0,22	0,17	0,31	0,20	0,14	0,25
20-HDHA	0,76	0,61	0,96	0,76	0,61	0,96	0,67	0,54	1,01
EPA	54,89	38,05	84,34	54,89	38,05	84,34	58,65	42,92	84,08
AA	138,57	112,77	161,06	138,57	112,77	161,06	116,21	95,79	186,70
DHA	503,75	381,83	582,39	503,75	381,83	582,39	498,17	421,98	702,67

The levels of lipid mediators (LMs) and polyunsaturated fatty acids (PUFAs) in cerebrospinal fluid (CSF) samples from individuals with subjective cognitive impairment (SCI), mild cognitive impairment (MCI) or Alzheimer's disease (AD), are presented as median and interquartile (Q) range in pg/ml. AA = arachidonic

acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, HDHA = hydroxy-docosahexaenoic acids, HETE = hydroxyeicosatetraenoic, LTB4 = leukotriene B4, LX = lipoxin, MaR = maresin, NPD1 = neuroprotectin D1, PG = prostaglandin, Rv = resolvin

Table 3. Correlation of LMs to cognition, CSF plaque and tangle biomarkers, and CSF inflammation marker YKL-40

		All		AD		MCI		SCI	
		R	p-value	R	p-value	R	p-value	R	p-value
MMSE	RvE4	0,11	0,2025	-0,03	0,8303	-0,21	0,1699	**0,34**	**0,0125**
	RvD1	**0,17**	**0,0434**	-0,24	0,1434	-0,12	0,4496	**0,36**	**0,0090**
	RvD4	**0,29**	**0,0007**	-0,13	0,4264	-0,22	0,1657	**0,42**	**0,0018**
	MaR1	0,02	0,8306	0,09	0,5931	**-0,32**	**0,0377**	0,14	0,3206
	PGE2	**0,18**	**0,0395**	0,02	0,8808	-0,10	0,5435	0,01	0,9501
	LTB4	-0,09	0,2987	0,02	0,8844	0,19	0,2235	**0,29**	**0,0351**
	14-HDHA	0,13	0,1366	**0,53**	**0,0004**	0,19	0,2153	0,18	0,1980
	20-HDHA	0,04	0,6121	**0,33**	**0,0355**	0,30	0,0539	-0,06	0,6632
	EPA	**0,18**	**0,0373**	**0,51**	**0,0008**	0,05	0,7398	0,16	0,2433
	AA	0,04	0,6732	**0,42**	**0,0066**	0,04	0,8158	0,01	0,9356
	DHA	**0,21**	**0,0143**	**0,53**	**0,0005**	0,09	0,5557	0,10	0,4847
$A\beta_{42}$	RvE1	**0,23**	**0,006**	-0,08	0,6158	0,19	0,2117	**0,27**	**0,0474**
	RvE4	0,1	0,2698	**0,32**	**0,0437**	0,03	0,8573	-0,07	0,5965
	RvD1	**0,18**	**0,0319**	0,14	0,3728	0,23	0,1461	-0,14	0,3008
	RvD4	**0,29**	**0,0007**	0,15	0,3664	0,14	0,37	0,11	0,4434
	NPD1	**0,18**	**0,0398**	-0,09	0,5884	0,03	0,8456	0,12	0,3993
	LXA4	0,15	0,0891	**0,35**	**0,0279**	-0,08	0,6197	0,05	0,7091
	PGF2a	0,01	0,9201	0,32	0,044	-0,05	0,7538	-0,08	0,5872
	LTB4	-0,15	0,0779	**0,33**	**0,0375**	-0,05	0,7555	0	0,9938
	12-HETE	-0,05	0,6017	**0,42**	**0,0064**	-0,05	0,7263	-0,08	0,5466
t-tau	RvD4	**-0,17**	**0,0464**	-0,03	0,8369	0,1	0,535	-0,01	0,9226
	MaR1	-0,08	0,328	**-0,35**	**0,0262**	-0,06	0,6805	0,05	0,7274
	LXA4	-0,09	0,3167	0,19	0,2507	**-0,33**	**0,0304**	0,2	0,157
	12-HETE	-0,07	0,3905	-0,24	0,1393	**-0,32**	**0,034**	0,08	0,5471
p-tau	LXA4	-0,05	0,5703	0,22	0,1689	-0,33	0,0306	0,23	0,1047
	PGD2	0,07	0,4107	0,32	0,0408	0,07	0,6785	0,15	0,2799
YKL-40	PGD2	0,15	0,0899	0,08	0,6158	0,21	0,1848	**0,29**	**0,038**

PGE2	0,16	0,0576	0,02	0,8949	0,28	0,0735	**0,37**	**0,007**
EPA	**0,2**	**0,0176**	0,28	0,0791	0,05	0,773	0,25	0,0745
AA	**0,21**	**0,0126**	0,19	0,2364	0,2	0,1878	0,19	0,1764

The correlations of lipid mediator (LM) levels to the mini-mental state examination (MMSE) test scores, and to the levels of Ab_{42}, total (t)-tau, phosphorylated (p)-tau, and chitinase-3-like protein 1 (YKL-40) are presented by the R-value according to Spearman rank-order test. Significant correlations are presented with their R- and p-values in bold digits. AA = arachidonic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, HDHA = hydroxy-docosahexaenoic acids, HETE = hydroxyeicosatetraenoic, LTB4 = leukotriene B4, LX = lipoxin, MaR = maresin, NPD1 = neuroprotectin D1, PG = prostaglandin, Rv = resolvin

Figures

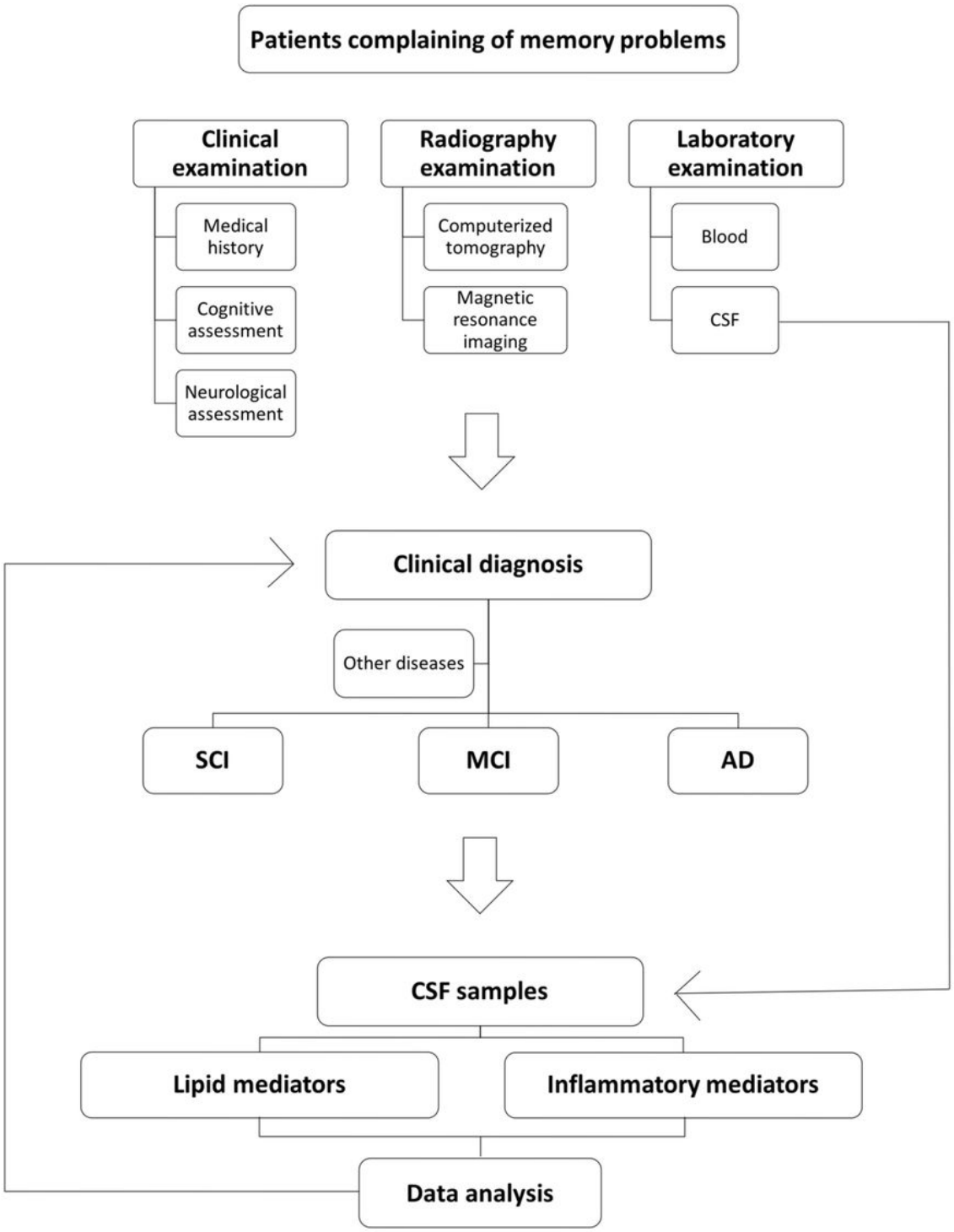

Figure 1

Study flow-chart. Cerebrospinal fluid (CSF) samples were obtained from a cohort of 136 patients subjected to clinical, radiography and laboratory examinations for the diagnosis of subjective cognitive impairment (SCI) (n = 53), mild cognitive impairment (MCI) (n = 43), and Alzheimer's disease (AD) (n = 40).

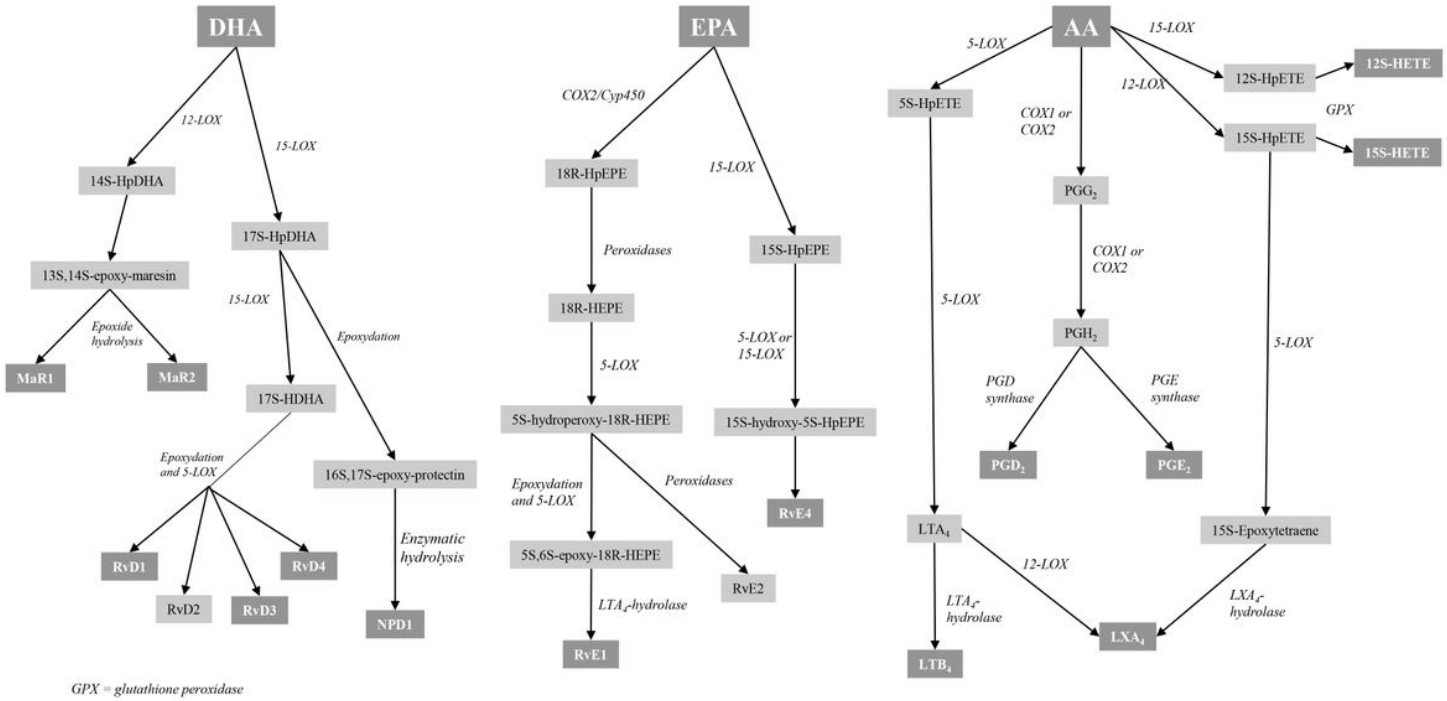

Figure 2

Metabolic pathways of lipid mediators. Pro-resolving and pro-inflammatory lipid mediators (LMs) are derived from the omega-3 and -6 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA). The figure shows the LMs analyzed in the present study and the biosynthetic enzymes involved in their synthesis. COX = cyclooxygenase, GPX = glutathione peroxidase, HDHA = hydroxydocosahexaenoic acid, HEPE = hydroxyeicosapentaenoic acid, HETE = hydroxyeicosatetraenoic acid, LOX = lipoxygenase, LT = leukotriene, LX = lipoxin, MaR = maresin, NPD = neuroprotectin D1, PG = prostaglandin, Rv = resolvin.

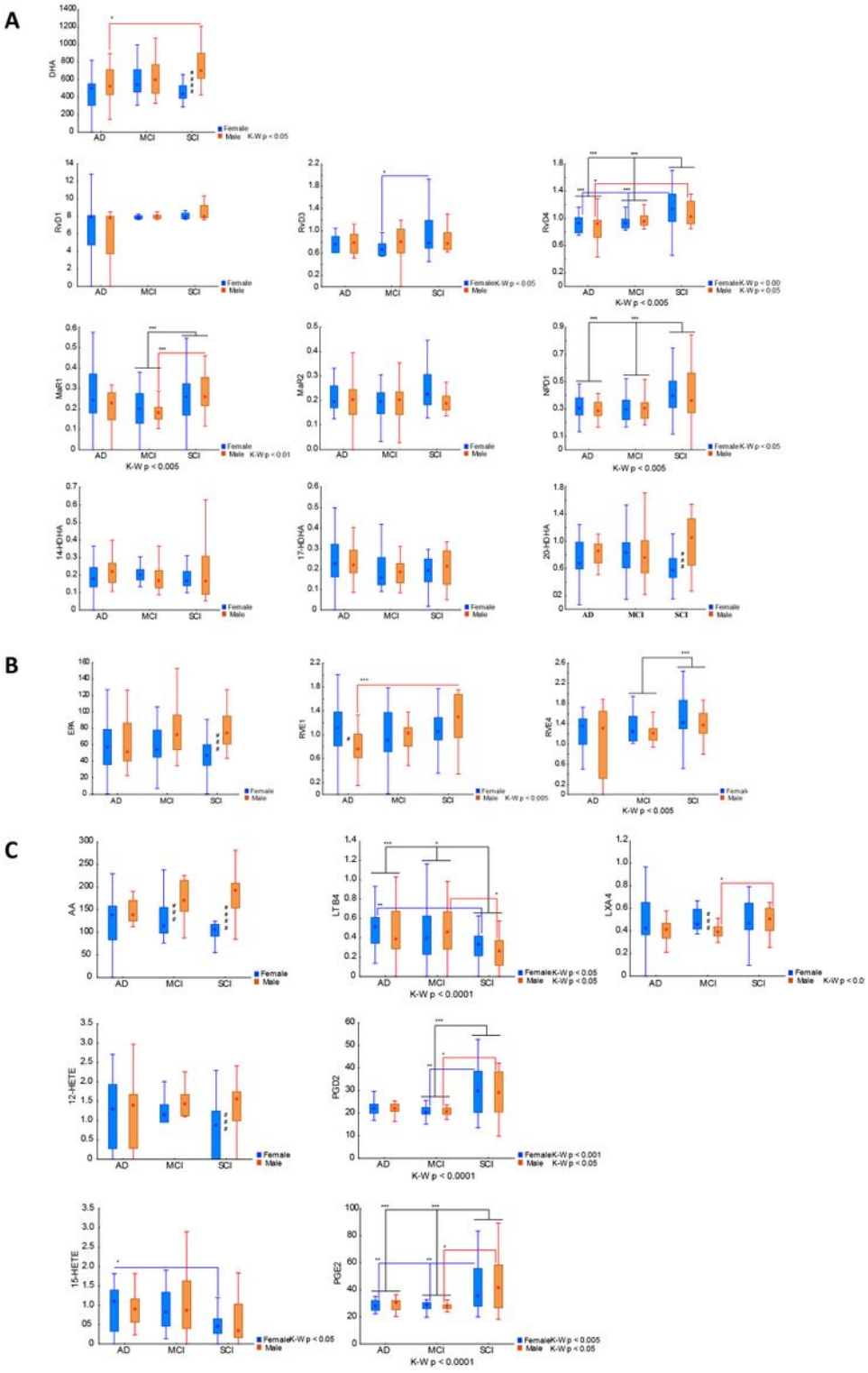

Figure 3

Gender differences and reduced pro-resolving LMs in CSF samples from MCI and AD patients. LMs were assessed in the cerebrospinal fluid (CSF) samples from patients with Alzheimer's disease (AD) (n = 40), mild cognitive impairment (MCI) (n = 43) or subjective cognitive impairment (SCI) (n = 53), using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results are presented according to the polyunsaturated fatty acid (PUFA) and the respective derivatives, i.e., (A) docosahexaenoic acid (DHA),

(B) eicosapentaenoic acid (EPA), and (C) arachidonic acid (AA). Note that the levels of all PUFAs were significantly lower in women than in men in the SCI group and for AA also in the MCI group. Comparisons between groups were performed by Kruskal-Wallis ANOVA with Dunn's multiple comparisons post hoc test (*P < 0.05, **P < 0.005, ***P < 0.001, ****P < 0.0001). Black lines indicate differences between diagnostic groups, and blue or red lines indicate differences between diagnostic groups when comparing women (blue) or men (red) separately. Comparisons between women and men within one diagnostic group were performed using Mann-Whitney test (# P < 0.05, ## P < 0.01, ### P < 0.005, #### P < 0.001). HDHA = hydroxydocosahexaenoic acid, HETE = hydroxyeicosatetraenoic acid, LTB4 = leukotriene B4, LXA4 = lipoxin A4, MaR1 = maresin 1, NPD1 = neuroprotectin D1, PG = prostaglandin, Rv = resolvin.

Supplementary Files

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