Plasma phosphatidylcholines and vitamin B12/folate levels are possible prognostic biomarkers for progression of Alzheimer’s disease

Imrich Blasko a,*, Michaela Defrancesco a, Herbert Oberacher b, Lorin Loacker c, Georg Kemmler a, Josef Marksteiner d, Christian Humpel a

a Department of Psychiatry, Psychotherapy and Psychosomatics, Division of Psychiatry I, Medical University of Innsbruck, Innsbruck, Austria
b Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Innsbruck, Innsbruck, Austria
c Central Institute of Medicinal and Chemical Laboratory Diagnostics, University Hospital, Innsbruck, Austria
d State Psychiatric Hospital, Hall in Tirol, Austria

ARTICLE INFO

Section Editor: Stephane Baudry

Keywords:
Mild cognitive impairment
Alzheimer’s disease
Plasma levels
Phosphatidylcholines
Lysophosphatidylcholines
Folate
Vitamin B12

ABSTRACT

Objectives: In clinical practice it is important to identify patients suffering from mild cognitive impairment (MCI) who will progress to Alzheimer’s disease (AD). The purpose of this study is to investigate whether lipid metabolites and vitamin B12 and folate levels are effective biomarker for an accurate prediction of MCI-to-AD conversion.

Methods: During the standard diagnostic assessment at our memory clinic 48 cognitively healthy subjects and MCI patients were recruited. These participants were followed up after 7–9 years. Blood was collected, various biochemical markers (including vitamin B12 and folate) analysed and plasma lipids were measured using the AbsoluteIDQ p150 Kit.

Results: There was no significant change in lipid levels in controls converting to MCI. However, we found significant changes in five lipids in converters from controls to AD. Interestingly, also two lipids were altered when MCI re-converted to controls. Vitamin B12 levels were not affected by conversion but folate levels significantly decreased in MCI-AD conversion.

Conclusions: Taken together, our study provides evidence that some plasma lipids are significantly altered in subjects converting to AD. Future studies will investigate whether the peripheral lipid changes correspond with changes in the brain during the course of the disease. Although this is a small study, there are indications that lipids may be suitable as prognostic markers.

1. Introduction

1.1. Biomarkers in Alzheimer’s disease

Alzheimer’s disease is a severe neurodegenerative disorder of the brain, characterized by extracellular beta-amyloid plaques, intraneuronal tau fibrillary tangles, cell death of cholinergic neurons, vascular pathology and inflammation. Within the last years the concept of Alzheimer’s disease has changed significantly. There is broad consensus that sporadic AD may be a slowly progressing disease with a long preclinical phase which fluently merge to Mild Cognitive Impairment (MCI) and finally exacerbates in clinically manifest Alzheimer dementia (AD) in old age (Sperling et al., 2011). MCI is thought to represent a transitional state between clinically silent and early dementia stages. In individuals 65 years of age and older, the incidence of AD is 1–2% per year in the general population, and approximately 4–20% in those with MCI depending on applied diagnostic criteria and study setting. However, the correct classification of MCI corresponding to preclinical AD remains challenging due to mild symptoms of cognitive impairment, various aetiologies and pathologies. Approximately 50% of patients with MCI reconvert back to normal cognitive state or show no conversion to dementia. As AD is a very heterogeneous disease, the diagnosis is complex and mainly based on clinical examination, neuroimaging and neuropsychological assessment methods.

The analysis of biomarkers is a challenge in research. As we are exploring brain diseases, it would be optimal to look directly into the
brain. Unfortunately, brain imaging methods such as cerebral magnetic resonance imaging (MRI) or positron emission tomography (PET) are not suitable to measure lipid metabolites or vitamins. Further, brain biopsy is not possible. Thus, the use of cerebrospinal fluid (CSF) is state-of-the art to test biomarkers (beta-amyloid-40 and -42, tau and pTau181), as the brain is directly connected to CSF (Blasko et al., 2006; Blennow, 2005; Blennow et al., 2010; Humpel, 2011). However, CSF collection is limited as it is an invasive procedure. Thus as we expect a high number of AD patients within the next 50 years there is a need to search for biomarkers in other human fluids, such as blood, urine or saliva. Recent results showed that blood-based tests measuring plasma phosphorylated tau-181 highly correlate with brain Tau load measured by 18F-Flortaucipir PET (Thijssen et al., 2020). Further, changes of neurofilament light (NFL) chain - another promising biomarker for the diagnosis of AD - was found to be elevated in serum and CSF (Preichte et al., 2019).

Definitely peripheral blood biomarkers may become important for AD and MCI diagnosis and also as markers for MCI conversion to AD. However, so far no blood specific AD-biomarkers have been established in routine diagnosis, although there are indications that the ratio of beta-amyloid-42/40 (Tang and Kumar, 2008) or some phospho-tau species (Thijssen et al., 2020) could become effective markers for predicting the risk of MCI/AD development. Thus, these examples provide evidence for a good comparability of some blood and brain markers. However, peripheral fluids can be influenced by many exogenous factors and e.g. inflammations in the periphery affect blood biomarkers, and may have nothing to do with the brain. Future studies will further investigate whether the peripheral lipid changes correspond with changes in the brain during the course of the disease.

1.2. Plasma lipid metabolites in Alzheimer’s disease

The importance of lipid measures in blood, including brain-derived cholesterol species and lipid peroxidation products, is in their potential to produce detectable signatures in the blood of MCI and AD patients. These signatures may be indicators of AD-associated neurodegeneration, producing candidate biomarkers of disease progression. There are some few studies demonstrating that distinct lipid sets in the blood are associated with AD diagnosis, with CSF Tau or with CSF beta-amyloid (Barupal et al., 2019). Phosphatidylcholines (PC) are also linked to decline of cognitive functioning in AD, to memory loss in healthy aging and some of these molecules correlated also with hippocampal atrophy occurring in different stages of dementia progression (Kim et al., 2017; Simpson et al., 2016). A ground-breaking work has been published some years ago, where it has been postulated that a set of ten lipids (C3, lypoPcA4C18:2, PCaAc36:6, C16:1-OH, PCaAc38:0, PCaAc38:6, PCaAc40:1, PCaAc40:2, PCaAc40:6, and PCaAc40:6) from peripheral blood predicted the conversion to MCI or AD within a 2–3 year timeframe with >90% accuracy (Mapistone et al., 2014). However, this study was very enthusiastic and so far the pattern of these 10 lipids could not be reproduced from other laboratories and also this set did not go into routine analysis. We ourselves could not find the same pattern of lipids but found that the ratio of phosphatidylcholines to lysophosphatidylcholines (PCaAc34:4 and lypoPcA4C18:2) in plasma differentiated healthy controls from patients with AD and MCI (Klavins et al., 2015). Very recently a study showed that 3 serum lipids (SM(OH)C24:1, SM C24:0 and PCaC44:3) differentiated MCI and early stage AD patients (Weng et al., 2019). Thus there is a clear need to further explore lipid metabolic changes in blood in order to find a common strategy to distinguish novel biomarkers.

1.3. Plasma vitamin B12, folate and Alzheimer’s disease

Low levels of vitamin B12 and folate have been reported to affect cognitive functions such as memory in healthy aging and dementia (de Wilde et al., 2017; Li et al., 2008). Both of them are used in standard clinical assessment of cognitive deterioration in old age. Vitamin B12 and folate are nutrient-dependent determinants of homocysteine and represent risk factors of conversion to dementia (Smith et al., 2018). It has been hypothesized that a lower CSF/brian availability for these nutrients may correlate with cognitive impairment (de Wilde et al., 2017). This could be due to a reduced uptake via the blood-brain barrier, a reduced uptake via the gut or an enhanced metabolism in the blood. Interestingly, persons with vitamin B12 deficiency showed a close connection between vitamin B12, markers of mitochondrial function and oxidative stress parameters such as acylcarnitines and plasmalogens and the metabolome (Brito et al., 2017).

We hypothesize that blood lipids are altered in the course of AD and are linked to vitamin B12/folate. Thus, the aim of the present longitudinal follow-up study was to investigate the metabolism of different plasma lipids together with folate and vitamin B12 in elderly patients with and without mild cognitive impairment. We present data on changes of plasma lipids of healthy controls and MCI patients who either remain stable or convert to AD over a very long period of 7–9 years. Our data will show that some phosphatidylcholines are altered during the progression of dementia and linked to vitamin B12.

2. Methods

2.1. Study design and patients

For this retrospective, observational study participants were recruited at the Memory clinic, Department Psychiatry and Psychotherapy, at the Medical University of Innsbruck, Austria. All participants completed a clinical examination, a neuropsychological assessment, and a blood sample was taken at baseline and at least at one follow-up visit after 7–9 years. Clinical data including information on somatic comorbidities and the APO-E genotype, were obtained from medical records, patients and caregivers. At follow-up patients were re-evaluated and classified into 6 groups: (1) cognitively healthy subjects not converting to MCI or AD (n = 13), (2) control subjects converting to MCI (n = 6), (3) control - AD conversion (n = 6), (4) MCI - AD conversion (n = 8), (5) MCI patients stable on MCI (n = 7), and (6) MCI - control re-conversion (n = 8). Inclusion criteria were a diagnosis of MCI (single domain or multi-domain type), German language proficiency and age of ≥60 years. Subjects were only excluded if major haematological, infectious, neurologic, oncologic illness or any other psychiatric disorder (including delirium, alcohol or drug dependency, and schizophrenia) was present. The study was approved by the Local Ethics Committee of Innsbruck Medical University and was performed in accordance with the Helsinki Declaration. All participants provided informed consent for study inclusion.

2.2. Clinical and neuropsychological assessment

All patients completed a neuropsychological assessment and a clinical interview at baseline and at least one follow-up visit within a period of seven years. Only data from those patients who completed at least one follow-up were included into this retrospective analysis. MCI was diagnosed according to the criteria of Petersen (Petersen et al., 2001) if patients reported subjective memory complaints over the previous 6 months, and showed an impaired memory function (verbal or figural) and/or an impairment of another cognitive domain in the neuropsychological assessment of >1.5 standard deviation (SD) related to age and education. Healthy controls were defined as presenting with no clinical and neuropsychological measureable cognitive impairment. At follow-up, progression from MCI to AD was defined as follows: presence of subjective memory complaints over the previous 6 months, impaired neuropsychological function of >2 SD or more corrected for age and education in one memory function (verbal or figural memory) and at least one other cognitive domain, deficits in activities of daily living assessed with a clinical interview. Diagnosis of dementia due to Alzheimer’s disease was done accordingly to the revised National Institute
on Aging-Alzheimer’s Association (NIA-AA) workgroups criteria (McKhann et al., 2011). All patients had undergone a neuropsychological assessment including Mini Mental State Examination (MMSE) (Folstein et al., 1975) and subtests of the “Consortium to Establish a Registry for Alzheimer’s Disease” (CERAD) battery (Rosen et al., 1984) at baseline and follow-up visits. Depressive symptoms were assessed the 30-items version of the geriatric depression scale (GDS (Yesavage et al., 1982)).

2.3. Blood collection

Blood samples were taken between 9:30 and 11:00 AM. Participants had a fasting time ranging from 1 to 3 h. From each patient 10 mL of EDTA blood was collected and samples were processed within 3 h at the same day. The samples were centrifuged (400 g, 30 min), and the upper plasma phase was collected, general blood biomarkers analysed within the same day and plasma immediately frozen at −80 °C. The samples remained frozen until lipid analysis.

2.4. Analysis of folate, vitamin B12 and other plasma markers

Total cholesterol, HDL- and LDL-cholesterol, triglycerides and creatinine plasma concentrations were measured using enzymatic photometric assays (“HDL3C”, “LDL-C”, “TRIGL”, “CREP2” and “UA2”, Roche, Mannheim, Germany.) Folate and Vitamin B12 serum concentrations were measured using a competitive immunonassay (“Folate III” and “Vitamin B12”, Roche, Mannheim, Germany). All procedures were done on a Roche Cobas c701/702 or e601 analyzer, respectively (Roche Diagnostics). Hba1c plasma concentrations were measured using ion exchange chromatography on an ADAMS A1c HA-8160 analyzer (Menarini, Florence, Italy). ApoE genotyping was performed by using an allele-specific multiplex PCR assay (“GenoType ApoE”, HAIN Life science, Nehren, Germany) on a Verity Thermal PCR Cycler (Thermo Fisher, Waltham, USA) following the manufacturer’s instructions.

2.5. Targeted metabolomic analysis of plasma lipids

In total, approx. 150 different metabolites were analysed using the well established AbsoluteIDQ p150 Kit (Biocrates Life Science AG) but we focused only on phosphatidylcholines (PCs), lysophosphatidylcholines (lysoPCs) and sphingomyelins (SMs). The lipid metabolites were analysed with a targeted quantitative and quality controlled metabolomics approach using the AbsoluteIDQ p150 Kit (Biocrates Life Science AG) as described previously by us (Klavins et al., 2015; Koal et al., 2015; Oberacher et al., 2017). Ten microliter plasma was pipetted onto filter spots suspended in the wells of a 96-well filter plate. The filter plate was fixed on top of a deep-well plate serving as a receiving plate for the extract later on, i.e. a combi-plate structure. After drying under a nitrogen stream, 50 μL of a 5% phenylisothiocyanate solution was added to enable derivatization of amino acids. After 20 min of shaking and nitrogen drying, 300 mL of 5 mM ammonium acetate in methanol was added to the wells. After 30 min of incubation, the combi-plate was centrifuged to move the extracts into the lower receiving deep-well plate, which was then detached from the upper filter plate. After adding another 300 μL of 5 mM ammonium acetate in methanol to the extracts and briefly shaking, the plate was placed in the autosampler of the FIA-MS/MS system for analysis. The FIA-MS/MS system consisted of a Krüner K-1001 LC pump (Krüner, Berlin, Germany), a CTC-PAL HTS9 autosampler (CTC Analysis AG, Zwingen, Switzerland), and a QTrap 3200 mass spectrometer (Sciex, Toronto, Canada). The injection volume was 30 μL. The flow rate was set to 30 μL/min. Metabolite concentrations (μM) were automatically calculated by the MetIDQ software package part of the AbsoluteIDQ p150 Kit.

2.6. Statistical methods

Statistical analysis was performed by using the IBM SPSS statistics program (version 24.0). Variables were expressed as means ± SEMs (unless otherwise stated). The baseline characteristics of cognitively healthy persons and MCI-patients were compared by means of the appropriate two-sample tests. The Mann-Whitney U test was used for non-normally distributed metric variables and Pearson Chi-square test for categorical variables. The blood biochemical parameters were initially compared by means of Kruskal-Wallis test. In those parameters with $p \leq 0.2$ difference, the Mann-Whitney U test was used for calculating of differences between cognitive groups. The lipid metabolites were calculated as percentage changes. Kruskal-Wallis test was used for assessing the levels of significance in each plasma lipid metabolite between the six cognitive groups. In second step, the change over the time in each plasma lipid metabolite was assessed by means of non-parametrical Wilcoxon Signed Ranks Test. In such calculations, raw phosphatidylcholines and lysophosphatidylcholines plasma values as obtained in metabolomics analysis were utilised. The level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patients demographic and clinical characteristics

After a follow-up period of 7–9 years, 13 (52%) out of 25 remained cognitively healthy, six participants (24%) developed MCI and six (24%) developed AD. From 23 MCI-cases at baseline visit, eight (35%) patients recovered from MCI to a cognitively healthy state, seven (30%) remained stable MCI and eight (35%) developed AD. In total, 14 (29%) out of 48 patients converted to AD until follow-up (Table 1). At follow-up visit cognitively healthy persons, MCI and AD patients were comparable in age, in time of follow up, in years of education and frequency of other medical and vascular risk indices such as diabetes, stroke or transitory ischemic attacks, heart disease and...
3.2. Vitamin B12, folate and other biochemical markers

Neither total cholesterol, HDL-cholesterol, LDL-cholesterol or triglycerides, Hba1C, vitamin B12 and creatinine showed any significance group differences at baseline (Table 2). Plasma folate showed higher levels in stable controls but a significant decreased in MCI patients converting to AD (Table 2).

3.3. Plasma lipid metabolites

Out of more than 100 lipids only 11 were affected in this conversion study. None of the lipids were affected in controls converting to MCI (Table 3), but 5 lipids were significantly increased when controls converted to AD: PcaC32:1, PcaC34:1, PcaC42:1, PcaCae34:1 and lysoPCaC18:0 (Table 3). Interestingly, also 2 lipids were altered when MCI re-converted to controls: PcaC40:3 and lysoPcaC18:0 (Table 3).

3.4. Vitamin B12 and folate correlation with lipids

Associations between folate, vitamin B12 and phosphatidylcholines in healthy controls are displayed in Table 4. Folate did not correlate significantly with any of the investigated lipids at baseline or follow-up. However, folate showed a positive correlation with vitamin B12 at baseline. In AD patients vitamin B12 correlated positively again with PCaeC34:1 (r = 0.541, p = 0.046).

Table 3

| Plasma lipid metabolites and conversion. |
|-----------------------------------------|
| Baseline cognition:                     |
| Controls converted to:                  |
| MCI converted to:                       |
| Follow up cognition:                    |
| raw values (μM) | Co | MCI | AD | AD | MCI | Co |
| N | 13 | 13 | 6  | 6  | 8   | 7  | 8  |
| PcaC32:1 | 12.5 ± 108 | 130 | 177 | 80 ± 10 | 86 ± 38 | 100 |
| PcaC34:1 | 172 ± 109 | 118 | 148 | 118 ± 139 | 104 | 14 ± 31 | 8 |
| PcaC38:0 | 2.5 ± 10 | 128 | 110 | 127 ± 115 | 103 |
| PcaC40:6 | 19.4 ± 108 | 105 | 109 | 124 ± 133 | 106 |
| PCaeC32:1 | 2.6 ± 6 | 11 | 16 | 10 ± 25 | 10 |
| PCaeC34:1 | 0.23 ± 129 | 231 | 129 | 131 ± 168 | 117 |
| PCaeC38:0 | 0.03 ± 26 | ± 24 | ± 82 | ± 25 |
| PCaeC39:1 | 7.4 ± 109 | 112 | 140 | 122 ± 140 | 115 |
| PCaeC40:6 | 0.5 ± 6 | ± 8 | ± 9 |
| PCaeC36:1 | 7.0 ± 104 | 117 | 146 | 119 ± 152 | 116 |
| PCaeC40:3 | 1.0 ± 113 | 105 | 122 | 134 ± 132 | 134 |
| Folate | 0.1 ± 9 | ± 16 | ± 11 | ± 16 |
| lysoPcaC16:1 | 3.9 ± 88 | 116 | 113 | 109 ± 92 | 83 ± 83 |
| lysoPCaC18:0 | 4.3 ± 60 | 10 | 11 | 11 ± 4 * |
| SMC24:0 | 32.4 ± 106 | 86 | 88 | 91 ± 98 | 97 ± 97 |
| Serum creatinine | 2.1 ± 7 | 10 | 13 | 4 * |

The second column gives raw values obtained from metabolomics analysis in all investigated subjects. Abbreviations: Co – cognitively healthy controls, MCI - mild cognitive impairment; AD – Alzheimer’s dementia.

Table 4

| Associations of folate and vitamin B12 with phosphatidylcholines - findings of Spearman rank correlation analysis. |
|-----------------------------------------------------------|
| Vitamin B12 | Folate | Vitamin B12 |
|---------------|-------------|---------------|
| r = -0.073, n.s. | - | r = -0.384, p = 0.008 |
| r = -0.311, p = 0.035 | - | r = -0.394, p = 0.007 |

r = Spearman rank correlation coefficient n.s. – not significant, p > 0.05.

4. Discussion

In this study, we found an increase in levels of some phosphatidylcholines and lysophosphatidylcholines in healthy controls who developed AD within a period to 7 to 9 years. The long follow-up period allowed us the evaluation of changes in plasma lipids in very early stages within the continuum of Alzheimer’s dementia.

4.1. Characterization of demented patients

All our patients were clinically characterized by well experienced psychiatrists with state-of-the-art methods. In fact, we have extensive experience in diagnosing MCI and AD patients and performed several studies either with lipids in CSF (Koal et al., 2015), lipids in plasma (Klavins et al., 2015), lipids in platelets (Oberacher et al., 2017), platelets (Defrancesco et al., 2018) or bile acid metabolites in plasma
4.2. Phosphatidylcholines as early markers of preclinical AD (CO→AD)

Phospholipids and sphingomyelins are substantial parts of the membrane in cells of the neuronal and peripheral nervous system, but also in all organs and blood cells, where they stabilize their function and sustain energy metabolism (Moreira et al., 2006; Perry et al., 2003; Smith et al., 2018). It is well known that perturbations in the lipid metabolism are consistently associated with preclinical and prodromal AD (Varma et al., 2018). Our data provide evidence that several PCs (PCaaC32:1, PCaaC34:1, PCaaC42:1, PCaeC34:1, PCaeC36:1) were significantly enhanced in healthy controls who converted to AD within 7–9 years.

4.3. Phosphatidylcholines as markers of MCI stage (MCI→MCI)

We found specific changes in lipid metabolism in stable MCI patients. Our data show that 4 lipids were increased at the MCI stage: PCaaC34:1, PCaaC40:6, PCaeC34:1 and PCaeC40:3. We did not detect changes in triglycerides as reported by Yin et al. (2012), who reported that triglycerides were negatively associated with MCI, and could preserve cognitive decline in the elderly. In 2016, He et al. (2016) analysed the total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels in patients with MCI compared with healthy individuals. This study found higher TC levels in MCI individuals compared with controls. In our study, we did not find similar changes for cholesterol. Characterizing individuals using blood markers to identify MCI patients who converted to AD is still a very high challenge. Our pilot study suggests that changes in lipid levels may not further contribute to better characterize MCI patients at risk to convert to AD.

4.4. Phosphatidylcholines in conversion and re-conversion (MCI→AD and MCI→Co)

At MCI stage baseline levels of some phosphatidylcholines were already high. In course of progression to AD they can further increase, but the relative value remains low. This might be the reason, why we could not reproduce the results by conversion from Co→AD. Interestingly also PCaeC40:3 increased and lysoPCaeC18:0 decreased when MCI re-converted to controls. These changes of lipids may reflect a complex metabolic situation in elderly persons with different co-morbidities and with different concomitant medications. This bidirectional influence on lipid metabolites is supported by a recent metabolomics study where the lipid metabolites is supported by a recent metabolomics study where changes of blood lipids are not causal linked to clinical disease progression but represent a reliable marker of ongoing disease process due to other hospitals. Definitely, such a broad conversion study is a huge challenge and complex but makes this study exceptional. On the other hand, our data provide clear evidence that the changes in plasma lipid biomarkers is only moderate, which can also be followed up with only a low number of subjects per group. Definitely a consecutive study with a higher n number would be necessary.

4.6. Limits of the study

This study has several limitations:

1. The major limitation of this study is the low number of included persons. However, our sample size is comparable with other longitudinal studies measuring blood lipids as biomarkers for AD or healthy aging (Simpson et al., 2016). Our study was performed during 12 years (2007–2019) and we started with a high number of approx. 300 persons, but the number of subjects which could be included till the final follow-up of this study decreased finally to 48. The reason was that some patients died, some patients refused to be further involved or some patients were transferred to other hospitals. Definitely, such a broad conversion study is a huge challenge and complex but makes this study exceptional. On the other hand, our data provide clear evidence that the changes in plasma lipid biomarkers is only moderate, which can also be followed up with only a low number of subjects per group. Definitely a consecutive study with a higher n number would be necessary.

2. Another limitation of this study might be, that diagnostic assessments slightly have changed over the long time course of the study. Further, changes of responsible psychiatrist could lead to an interrer-variability in clinical diagnosis of the patients. Along with these changes also the diagnostic criteria slightly changed, while some psychiatrists more focused on psychological-based criteria others also included more imaging techniques. Thus we cannot exclude that a small number of subjects had a more heterogeneous diagnosis as it would be if only the same psychiatrist diagnoses the patients. Thus, we also learned and suggest that a consensus on diagnostic criteria (not only local but also international) is an important feature in performing such longitudinal studies (see also O’Bryant et al. (2015)). The detailed and standardized clinical and neuropsychological examination ensure reliable data on changes of cognitive function and clinical state. Even though, we have no neuropathological confirmation of AD in cerebrospinal fluid or by amyloid/tau-PET, AD and MCI can be diagnosed clinically with high accuracy (Schmand et al., 2010). Thus, diagnostic misclassification may play a minor role. Further, we assume that the changes of blood lipids are not causal linked to clinical disease progression but represent a reliable marker of ongoing disease progression on clinical and neuropathological level. Therefore, we termed the analyses lipid markers as prognostic markers for AD but not as target for primary disease prevention strategies.

3. A correct statistical analysis is the state-of-the-art of such studies. In fact, the statistical analysis has been done by a professional statistician in order to exclude as many as possible of random factors. Our study presents some slight statistical alteration, with p-values of <0.05. Based on many previous studies in cerebrospinal fluid and blood, it could be argued that biomarkers with such a low p-value may never become a biomarker in routine diagnosis. In fact, we also think, that the lipids may not enter clinical routine, as the changes are very small and influenced by exogenous factors. On the other hand, the changes reflected in the lipids, may tell us more on the progression of the disease and not on the causes of the disease.
(4) Changes of lipids reflect a complex metabolic situation in elderly persons with different co-morbidities and with different concomitant medications. The distribution of common diseases such as diabetes, hypertension, stroke, heart disease or depression did not differ between diagnostic groups and is also included in the present study. Similarly, the use of cholesterol lowering drugs was similar in controls, mildly impaired and persons with AD. Definitely, such studies are a challenge in AD research, as many exogenous factors can influence the outcome of the study.

(5) Finally, we cannot exclude that the stability of the samples was affected. Although we processed the samples within 3 h at room temperature and stored the samples immediately at −80°C, it may happen that some specific lipids may degrade over a period of 12 years. This could be reflected by a high variance already in the controls groups. On the other hand we measured similar raw values as we did in a previous plasma lipid study (Klavins et al., 2015). A strength of this study is the long follow-up period compared to numerous other published studies which assessed risk markers for the conversion of MCI to AD. Especially the long follow-up period of healthy controls and MCI patients allow a reliable differentiation between MCI due to AD and MCI due to other etiology. Further, our study sample is small but homogeneous regarding ethnicity and socio-cultural background.

Taken together, this study aimed to demonstrate what kind of changes occurs in metabolism of plasma lipids together with folate and vitamin B12 in the course of cognitive deterioration leading to AD. The changes observed are caused by the development of the AD and associated neurodegeneration. Our conversion study supports our initial hypothesis and provides evidence that some plasma phosphatidylcholines are moderately affected during progression of dementia. This goes in lines with changes of folate and a correlation to vitamin B12. Some of these phosphatidylcholines could identify the degree of clinical differences, goes the clinical information or add an additional value to therapies, which target to stabilize the membrane function. Definitely, the changes and heterogeneity is too high that these biomarkers could currently become reliable and stable biomarkers for dementia.

CRediT authorship contribution statement

Imrich Blasko: patients recruitment, writing - original draft, methodology, formal analysis, writing - review and editing; Michaela Defrancesco: conceptualization, reviewing and editing; Herbert Oberacher: performed the experiments and analysed the data; Lorin Loacker: performed the experiments; Georg Kemmler: statistical analysis and visualization; Josef Marksteiner: supervision, conceptualization; Christian Humpel: conceptualization, methodology, data analysis, writing – final version, writing - review and editing.

Declaration of competing interest

The authors have declared that no competing interests exist.

Acknowledgement

The authors thank Mr. Wolfgang Egger and nursing staff of outpatient ward of Psychiatry Department for continuous support by blood sample collection.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Barupal, D.K., Baillie, R., Fan, S., Saykin, A.J., Meikle, P.J., Arnold, M., Nho, K., Fiebel, K., Kaddurah-Daouk, R., Alzheimer Disease Metabolomics Center, 2018. Sets of coregulated serum lipids are associated with Alzheimer’s disease pathophysiology. Alzheimers Dement (Amst) 11, 619–627.

Blasko, I., Lederrer, W., Oberbauer, H., Walch, T., Kemmler, G., Hinterhuber, H., Marksteiner, J., Humpel, C., 2006. Measurement of thiomolecular biomarkers in CSF of patients with Alzheimer’s disease and other dementias. Dement. Geriatr. Cogn. Disord. 21, 9–15.

Blenkow, K., 2005. CSF biomarkers for Alzheimer’s disease: use in early diagnosis and evaluation of drug treatment. Expert. Rev. Mol. Diagn. 5, 661–672.

Blenkow, K., Humpel, H., Weiner, M., Zetterberg, H., 2010. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat. Rev. Neurol. 6, 131–144.

Brito, A., Grapov, D., Fahrmann, J., Harvey, D., Green, R., Miller, J.W., Fedosov, S.N., Shahab Verfows, S., Humpel, D., Pedersen, T.L., Fiehn, O., Newman, J.W., Li, Q., Huang, G., Ma, F., 2016. Relationship between plasma lipids and mild cognitive impairment in the elderly Chinese: a case-control study. Lipids Health Dis. 15, 146.

Humpel, C., 2011. Identifying and validating biomarkers for Alzheimer’s disease. Trends Biotechnol. 29, 26–32.

Kim, M., Nevada-Holgado, A., Whiley, L., Snowden, G., Soininen, H., Klouwer, J., Meccoli, P., Tsoaki, M., Vellas, B., Thambisetty, M., Dobson, R.J.B., Powell, J.F., Lupton, M.K., Simmons, A., Velayudhan, L., Lovestone, S., Proitsi, P., Legido-Quigley, C., 2017. Association between plasma ceramides and phosphorylatedolines and hippocampal brain volume in late onset Alzheimer’s disease. J. Alzheimers Dis. 60, 809–817.

Klavins, K., Koal, T., Hallmalm, G., Marksteiner, J., Kemmler, G., Humpel, C., 2015. The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer’s disease and mild cognitive impairment. Alzheimers Dement (Amst) 1, 295–302.

Koal, T., Klavins, K., Seppi, D., Kemmler, G., Humpel, C., 2015. Sphingomyelin SM(18:1/18:0) is significantly enhanced in cerebrospinal fluid samples dichotomized by pathological amyloid-beta42, tau, and phospho-tau-181 levels. J. Alzheimers Dis. 44, 1193–1201.

Lee, H., Choi, J.M., Cho, J.Y., Kim, T.E., Lee, H.J., Jung, B.H., 2018. Regulation of endogenic metabolites by rosuvastatin in hyperlipidemia patients: an integration of metabolomics and lipidomics. Chem. Phys. Lipids 214, 69–83.

Li, L., Cao, D., Desmond, R., Rahman, A., Lah, J.J., Levey, A.J., Zamrini, E., 2008. Cognitive performance and plasma levels of homocysteine, vitamin B12, folate and lipids in patients with Alzheimer disease. Dement. Geriatr. Cogn. Disord. 26, 384–396.

Mapstone, M., Cheema, A.K., Fiandaca, M.S., Zhong, X., Mhyre, T.R., MacArthur, L.H., Hall, W.J., Fisher, S.G., Peterson, D.R., Haley, J.M., Nazar, M.D., Rich, S.A., Berlau, D.J., Peltz, C.B., Tao, M.T., Kawasaki, C.H., Fedoroff, H.J., 2014. Plasma phospholipids identify antecedent memory impairment in older adults. Nat. Med. 20, 415–418.

Marksteiner, J., Blasko, I., Kemmler, G., Koal, T., Humpel, C., 2018. Bile acid identification of 20 plasma metabolites identifies hyodeoxycholic acid as a putative biomarker in Alzheimer’s disease. Metabolomics, 14, 1.

McGuinness, B., O’Hare, J., Craig, D., Bullock, R., Passmore, P., 2010. Statins for the treatment of dementia. Cochrane Database Syst. Rev. 8, CD007514.

McGuinness, B., Craig, D., Bullock, R., Passmore, P., 2016. Statins for the prevention of dementia. Cochrane Database Syst. Rev. 1, CD003160.

McKernan, G.M., Knopman, D.S., Cherkin, J.W., Hyman, B.T., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rosen, M.N., Schelins, P., Carrillo, M.C., Thies, B., Weinshu, S., Phelps, C.H., 2011. The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 7, 263–269.

Morris, P.L., Zhu, X., Liu, Q., Honda, K., Siedlak, S.L., Harris, P.L., Smith, M.A., Perry, G., 2006. Compensatory responses induced by oxidative stress in Alzheimer disease. Biol. Res. 39, 7–13.

O’Barr, H., Arnhard, K., Lindhart, C., Divo, A., Marksteiner, J., Humpel, C., 2017. Targeted metabolomic analysis of soluble lysates from platelets of patients with mild cognitive impairment and Alzheimer’s disease compared to healthy controls: is PC acylco6:4 a promising diagnostic tool? J. Alzheimers Dis. 57, 493–504.

O’Bryant, S.E., Gupta, V., Henriksen, K., Edwards, M., Jerome, A., Lister, S., Benzeit, C., Soares, H., O’Ke, F., Fie, S., Humpel, H., Montine, T., Blennow, K., Carrillo, M., Graff-Radford, N., Laskie, C., Breetler, M., Shaw, L., Trojanowski, J.O., Schupf, N., Rissman, R.A., Fagan, A.M., O’boeri, P., Uemek, R., Weiner, M.W., Grammas, P., Posner, H., Martin, R., Star, b., groups, R.W., 2015. Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer’s disease research. Alzheimers Dement. 11, 549–560.

Perry, G., Nunomura, A., Raina, A.K., Aliev, G., Siedlak, S.L., Harris, P.L., Casadesus, G., Petersen, R.B., Bligh-Glover, V., Balraj, E., Poter, G.J., Smith, M.A., 2003. A metabolic basis for Alzheimer disease. Neurochem. Res. 28, 1549–1552.
Petersen, R.C., Doody, R., Kurz, A., Mohs, R.C., Morris, J.C., Rabins, P.V., Ritchie, K., Rossor, M., Thal, L., Winblad, B., 2001. Current concepts in mild cognitive impairment. Arch. Neurol. 58, 1985-1992.

Preische, O., Schultz, S.A., Apel, A., Kuhle, J., Kaeser, S.A., Barro, C., Graber, S., Kuder-Buletta, E., LaFougere, C., Lomke, A., Vogelein, J., Levin, J., Masters, C.L., Martins, R., Schofield, P.R., Rossor, M.N., Graff-Radford, N.R., Salloway, S., Ghebreme, R., Ringman, J.M., Noble, J.M., Ghitiz, J., Goate, A.M., Benzing, T.L.S., Morris, J. C., Bateman, R.J., Wang, G., Fagan, A.M., McDade, E.M., Gordon, B.A., Jucker, M., Dominantly Inherited Alzheimer, N., 2019. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer’s disease. Nat. Med. 25, 277–283.

Rosen, W.G., Mohs, R.C., Davis, K.L., 1984. A new rating scale for Alzheimer’s disease. Am. J. Psychiatry 141, 1356–1364.

Schmand, B., Huizenga, H.M., van Gool, W.A., 2010. Meta-analysis of CSF and MRI biomarkers for detecting preclinical Alzheimer’s disease. Psychol. Med. 40, 135–145.

Simpson, B.N., Kim, M., Chuang, Y.F., Beason-Held, L., Kinter-Triolo, M., Kraut, M., Lirette, S.T., Windham, B.G., Griswold, M.E., Legido-Quigley, C., Thambisetty, M., 2016. Blood metabolite markers of cognitive performance and brain function in aging. J. Cereb. Blood Flow Metab. 36, 1212–1223.

Smith, A.D., Refsum, H., Bottiglieri, T., Fenech, M., Hooshmand, B., McCaddon, A., Miller, J.W., Rosenberg, I.H., Obeid, R., 2018. Homocysteine and dementia: an international consensus statement. J. Alzheimers Dis. 62, 561–570.

Sperling, R.A., Aisen, P.S., Beckett, L.A., Bennett, D.A., Craft, S., Fagan, A.M., Iwatsubo, T., Jack Jr., C.R., Kaye, J., Montine, T.J., Park, D.C., Reiman, E.M., Rowe, C.C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M.C., Thies, B., Morrison-Bogorad, M., Wagster, M.V., Phelps, C.H., 2011. Toward defining the preclinical stages of Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 7, 280–292.

Tang, B.L., Kumar, R., 2008. Biomarkers of mild cognitive impairment and Alzheimer’s disease. Ann. Acad. Med. Singap. 37, 406–410.

Thijssen, E.H., La Joie, B., Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., VandeVrede, L., Chai, X., Proctor, N.K., Airey, D.C., Scherbinin, S., Duggan Evans, C., Sims, J.R., Zetterberg, H., Bremow, K., Karydas, A.M., Tsunij, C.E., Kramer, J.H., Grinberg, L.T., Seely, W.W., Rosen, H., Boeve, B.F., Miller, B.L., Rabinovici, G.D., Dage, J.E., Rojas, J.C., Boxer, A.L., Advancing, R., Treatment for Frontotemporal Lobar Degeneration, i, 2020. Diagnostic value of plasma phosphorylated tau181 in Alzheimer’s disease and frontotemporal lobar degeneration. Nat. Med. 26, 367–367.

Varma, V.R., Oommen, A.M., Varma, S., Casanov, R., An, Y., Andrews, R.M., O’Brien, R., Pletnikova, O., Troncoso, J.C., Toledo, J., Baillie, R., Arnold, M., Kastenmueller, G., Nho, K., Dorsaiswamy, P.M., Saykin, A.J., Kudurah-Daouk, R., Legido-Quigley, C., Thambisetty, M., 2018. Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: a targeted metabolomics study. PLoS Med. 15, e1002482.

Weng, W.C., Huang, W.Y., Tang, H.Y., Cheng, M.L., Chen, K.H., 2019. The differences of serum metabolites between patients with early-stage Alzheimer’s disease and mild cognitive impairment. Front. Neurol. 10, 1223.

de Wilde, M.C., Vellas, B., Girault, E., Yavuz, A.C., Siibben, J.W., 2017. Lower brain and blood nutrient status in Alzheimer’s disease: results from meta-analyses. Alzheimers Dement (N Y). 3, 416–431.

Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., Leier, V.O., 1982. Development and validation of a geriatric depression screening scale: a preliminary report. J. Psychiatr. Res. 17, 37–49.

Yin, Z.X., Shi, X.M., Kraus, V.B., Fitzgerald, S.M., Qian, H.Z., Xu, J.W., Zhai, Y., Sereny, M.D., Zeng, Y., 2012. High normal plasma triglycerides are associated with preserved cognitive function in Chinese oldest-old. Age Ageing 41, 600–606.