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REVIEW

A Review on MS-Based Blood Biomarkers for Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia and there is no cure to date. Biomarkers in cerebrospinal fluid (CSF) are already included in the diagnostic work-up of symptomatic patients but markers for preclinical diagnosis and disease progression are not available. Furthermore, blood biomarkers are highly appreciated because they are minimally invasive and more accessible in primary care and in clinical studies. Mass spectrometry (MS) is an established tool for the measurement of various analytes in biological fluids such as blood. Its major strength is the high selectivity which is why it is also preferred as a reference method for immunoassays. MS has been used in several studies in the past for blood biomarker discovery and validation in AD using targeted MS such as multiple/selected reaction monitoring (MRM/SRM) or unbiased approaches (proteomics, metabolomics). In this short review, we give an overview on the status of current MS-based biomarker candidates for AD in blood plasma and serum.
Plain Language Summary: Plain language summary available for this article.

Keywords: Alzheimer's disease; Biomarker; Blood; Mass spectrometry; MRM; Plasma; Serum

Key Summary Points

Mass spectrometry (MS) is an established tool to measure analytes in blood.

Reliable MS-based biomarkers in blood for Alzheimer´s disease (AD) are still rare.

Most promising MS-based biomarkers for AD at the moment seem to be the determination of Aβ peptides by IP-MS and the "genotyping" of ApoE by MRM.

MS will be essential in the definition of reference methods for the measurement of AD biomarkers in blood.

PLAIN LANGUAGE SUMMARY

Alzheimer´s disease (AD) is the most common form of neurodegenerative dementia and there

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is no cure to date. A biomarker is a naturally occurring molecule, gene, or characteristic by which a particular disease can be identified or its progression monitored. Biomarkers in cerebrospinal fluid are used in clinical diagnosis of AD, but markers for pre-clinical diagnosis and disease progression are not available. Blood biomarkers are accessible in primary care in a minimally invasive way, convenient to patients, with little cost and suitable for repeated sampling for longitudinal assessments. Mass spectrometry (MS) is an established analytical tool for measuring markers in biological fluids such as the blood, with the benefit of higher selectivity than alternative methods. MS has been used in several studies in the past for blood biomarker discovery and validation in AD and a review of the available research identified several biomarker candidates, of which the most promising are Aβ peptides, proteins released by AD pathology, and ApoE genotyping, although both markers require targeted, specific methods of MS and require further research for validation. Mass spectrometry is expected to be an essential tool in the definition of reference methods for the measurement of AD biomarkers in blood.

INTRODUCTION

Alzheimer´s disease (AD) is a neurodegenerative disease and the most common form of dementia. The characteristic feature of AD is the formation of amyloid plaques and neurofibrillary tangles in the brain accompanied with the degeneration of synapses. To date, the cause of AD is still unclear and there is no cure available making it a serious health problem in the aging societies of industrial countries [1]. Diagnosis is mainly based on clinical symptoms, although the measurement of tau protein and the amyloid β peptide (Aβ42) in cerebrospinal fluid (CSF) was included into the diagnostic criteria in 2011 to support AD neuropathology [2]. Biomarkers for presymptomatic diagnosis and disease progression are not yet available but are essential for early treatment of patients and drug development.

In contrast to CSF, the measurement of biomarkers in blood has several advantages: it is more convenient for patients, it is less costly and it is more suitable for repeated sample collection in longitudinal assessments. Mass spectrometry (MS) is a valuable tool for biomarker measurement in blood (reviewed in [3]). Its major strength is the high selectivity. This is an important advantage compared with immunoassays which often struggle with matrix effects and unspecific signals in blood samples. In MS, the target analyte is measured by its mass-to-charge ratio (m/z). In modern hybrid MS instruments more than one mass analyzer is included. This allows additional fragmentation of the target analyte and quantification of the specific fragments. This approach is called multiple/selected reaction monitoring (MRM/SRM) or parallel reaction monitoring (PRM) depending on the mass analyzer used. It significantly increases specificity compared with single m/z measurement. MS can also be used for unbiased discovery of new biomarker candidates (e.g. proteomics, metabolomics). Here, a successive acquisition of full-scan and fragment mass spectra is performed followed by matching with a database to identify the measured analytes. Very often, MS is coupled to gas or liquid chromatography to perform an additional separation of molecules before MS analysis.

In this short review we give an overview on biomarker (candidates) for AD in blood plasma and serum which were measured using MS techniques.

METHODS

We searched the PubMed database in June 2019 for articles matching the following search terms: Alzheimer's disease AND blood or plasma or serum AND mass spectrometry or MRM or SRM or proteomics or proteomic or proteome or metabolomics or metabolomic or metabolome or lipidomics or lipidomic or lipidome or glycomics or glycomic or glycome. A total of 483 articles were found. After exclusion of review articles, the titles and abstracts of 422 articles were screened for studies using MS-based

techniques for measuring endogenous molecules in serum/plasma (not blood cells) from human AD patients. From the remaining 151 articles, we further excluded articles which used MS for protein identification only (not quantification). In order to focus on the most reliable biomarker candidates, we included unbiased discovery approaches only if the identified candidates were verified using MRM/PRM (proteomics) or using synthetic compounds (metabolomics). With the exception of the Mapstone panel (examined in several studies), we also did not include biomarker panels because they were unique to the respective study populations. Finally, 66 articles fulfilled our criteria.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

RESULTS

Aβ Peptides

The accumulation of Aβ in amyloid plaques in the brain is a major hallmark of AD. Alterations of the proteolytic APP (amyloid precursor protein) processing and release of the Aβ peptides is a key event in AD pathophysiology [1]. The measurement of Aβ42 in CSF is an established AD biomarker of amyloid pathology and included in the diagnostic criteria [2]. MRM is used as a reference method for Aβ measurement in CSF [4, 5]. Pannee and colleagues used their antibody-free CSF reference MRM also to investigate blood levels of Aβ38, Aβ40 and Aβ42. In their small cohort of 9 AD and 10 control patients, they did not observe significant differences between the two groups [6]. There was also no correlation of Aβ42 levels in plasma and CSF but CSF was analyzed by ELISA. They also characterized additional Aβ variants in plasma by immunoprecipitation (IP) and MALDI-ToF MS (Aβ15, Aβ17, Aβ19, Aβ20, Aβ33, Aβ34, Aβ5-40, Aβ37, Aβ3-40 and Aβ39) but observed no differences between AD and control samples ($n = 2$).

Another group also reported an antibody-free MRM method with the aim to measure total Aβ in plasma [7]. However, they performed tryptic digestion of all plasma proteins as an initial step of sample preparation. The tryptic peptide (Aβ17-28) selected for Aβ quantification in this study is not specific for Aβ and is also released from full-length APP during tryptic digestion. Thus, measurement results did not represent Aβ in plasma. This is also reflected in the reported concentration which is more than tenfold higher compared with the more reliable method from Pannee et al. [6].

A Japanese group published a first study in 2014 using IP-MALDI ToF MS to measure Aβ peptide levels in plasma. They investigated subjects with and without cerebral amyloid deposition which was determined by positron emission tomography (PET) with Pittsburgh Compound B (PiB) [8]. The IP-MALDI ToF MS method used was already validated in terms of precision and dilution linearity previously [9]. The authors chose this pathology-driven approach because AD-like amyloid deposition is also found in a substantial number of non-demented aged individuals [10]. In this context, a syndromal comparison (i.e. AD patients vs controls) might underestimate the diagnostic performance of amyloid-related plasma biomarkers. Thereby, they also followed the recent suggestion of a biological definition of AD by the ATN classification of biomarkers [11]. Kaneko et al. measured plasma levels of Aβ40, Aβ42 and APP669-711 in 22 PiB-negative and 40 PiB-positive (including AD, MCI and healthy controls) subjects. They observed significantly reduced Aβ42 and Aβ42/Aβ40 and increased APP669-711/Aβ42 in PiB-positive vs PiB negative subjects [8]. Subgroup comparison showed, that the APP669-711/Aβ42 ratio was also increased in PiB-positive AD compared with PiB-positive healthy controls. The ratio APP669-711/Aβ42 yielded a high sensitivity and specificity (93% and 96%) for the detection of cerebral amyloid pathology. The authors confirmed their results recently with a slightly modified method in two additional patient cohorts from the Japanese National Center for Geriatrics and

Gerontology (NCGG, $n = 121$) and Australian Imaging, Biomarker and Lifestyle Study of Ageing (AIBL, $n = 252$) [12]. APP669-711/Aβ42 and Aβ40/Aβ42 showed good discriminatory power for amyloid positive and negative subjects in the NCGG cohort (sensitivity 68% and 96%, specificity 92% and 87%) and AIBL cohort (sensitivity 83% and 85%, specificity 70% and 68%). In addition, they created a composite biomarker from both ratios with even better performance in the NCGG and AIBL cohort (sensitivity 86% and 86%, specificity 89% and 78%). They used stable isotope-labeled (SIL)-Aβ38 as internal standard (IS) for all Aβ peptides, which has the advantage that the Aβ ratios are independent from the spiked amount of IS. Therefore, the established cut-off values are transferable. A drawback could be that the different properties of the Aβ peptides are not optimally reflected by Aβ38. This might contribute to the center differences of the Aβ ratios between the NCGG and AIBL cohort due to different matrix effects.

Most recently, the Bateman group published a study confirming the good predictive power of Aβ40/Aβ42 in plasma for amyloid PET status [13]. They used tryptic digestion and MRM instead of MALDI-ToF but observed a similar sensitivity (88%) and specificity (76%) for the discrimination of amyloid positive and negative subjects compared with Nakamura et al. [12].The high performance for detection of cerebral amyloid pathology and the consistent findings with IP-MS methods in multiple cohorts and by different research groups makes it a very promising MS-based biomarker.

Phospholipids/Sphingolipids

Phospholipids are an integral part of the plasma membrane and their composition substantially influences the function of membrane proteins, receptors, enzymes and ion channels. The reduced phospholipid amount in AD brains gives a first link of phospholipids to AD pathophysiology. Furthermore, the cleavage of APP, releasing the Aβ peptides, takes place at the cell membrane and thus in direct contact with phospholipids [14].

In 2014, Mapstone and colleagues [15] reported a panel of phospholipids (Table 1) with significant changes in plasma already in the preclinical phase of AD. The phospholipids and other metabolites were measured using a commercially available MRM kit. They compared AD/amnestic MCI (mild cognitive impairment) patients with cognitively normal controls from a longitudinal observational study. They especially looked at subjects converting from cognitively normal to AD/aMCI during the observational period. Here, they included plasma samples from the time before and after conversion. Combining the panel of eight phospholipids with two acylcarnitines (C3 and C16:1-OH, Table 1), they were able to identify AD/aMCI patients in their cohort of 106 patients at a time, when they were cognitively normal (on average 2.1 years before conversion). The sensitivity and specificity was 90%. They successfully validated their results in a different subset of 40 patients from the same cohort. Significant changes in AD, MCI or dementia of four lipids from the Mapstone panel (PC aa C36:6, PC aa C38:6, LysoPC 18:2 and C3) were also reported in other studies [16–21] (see Table 1) but sometimes in the opposite direction [16].

Three studies from two other research groups evaluated the Mapstone panel of blood markers in different longitudinal cohorts. These included subjects converting to AD or MCI during the follow-up and the use of the same MRM kit [16, 17, 22]. Casanova et al. investigated samples from two large cohorts of patients, the Baltimore Longitudinal Study of Aging (BLSA) and the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS) comprising 392 patients in total [22]. They could not reproduce the high predictive performance of the Mapstone panel in blood for preclinical AD. In their much larger cohort of preclinical AD converters ($n = 93$ in BLSA cohort, $n = 100$ in AGES-RS cohort vs. $n = 28$ in Mapstone cohort), they observed sensitivities and specificities of 52% and 66% (BLSA cohort) and 47% and 36% (AGES-RS cohort). The use of different sample matrices (plasma vs serum) has been discussed as a possible reason for the discrepancy between these two studies. However, Casanova et al. also

Table 1 Panel of ten lipid biomarkers suggested by Mapstone et al. [15] for detection of preclinical AD

Biomarker	Observation of significant changes in studies
Phospholipids	
PC aa C36:6	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15] OR Dem vs Con < 1 [17]
PC aa C38:0	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]
PC aa C38:6	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15], OR AD < 1 [21]
PC aa C40:1	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]
PC aa C40:2	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]
PC aa C40:6	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]
PC ae C40:6	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]
LysoPC a C18:2	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15], AD vs Con↓ [19], OR MCI vs Con > 1 [16], OR MCI/Dem vs Con > 1 [16], Concentration increase during follow-up in AD-converters vs Con↑ [18]
Acylcarnitines	
C3	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15], MCI vs Con↓ [20]
C16:1-OH	Converter(Pre) vs Con↓ [15], aMCI/AD vs Con↓ [15]

AD Alzheimer's disease, *aMCI* amnestic MCI, *Con* control patients, *Dem* dementia, *MCI* mild cognitive impairment, *OR* odds ratio, *Pre* samples before conversion to aMCI/AD
↑ Significantly increased, ↓ significantly decreased

provided data that plasma and serum levels of the Mapstone panel are highly correlated [22].

Li and colleagues investigated plasma samples from the Atherosclerosis Risk in Communities Neurocognitive Study (ARIC-NCS). A large majority of these samples were from African Americans (> 96%) and their aim was to evaluate the predictive performance of the Mapstone panel (established in a white population) in African American [16]. Their cohort included 95 MCI/dementia converters with a mean follow-up time of 7.3 years, which is considerably longer than in the Mapstone study (2.1 years). In agreement with the study by Casanova et al., they observed neither a large added value of the Mapstone panel for prediction of MCI/dementia [16] nor for detection of MCI/dementia in a cross-sectional comparison (n = 441) [17]. However, the MCI/dementia patients in the studies by Li et al. [16, 17] are not solely AD patients which might weaken the discriminatory performance of the (potentially AD-specific) biomarker panel. Changes of several other phospholipids in serum/plasma were reported in single studies and are listed in Table 2.

Sphingolipids are another class of lipids which are part of the plasma membrane and involved in a myriad of different biological processes such as signal transduction and cell–cell interaction [23]. Changes of different sphingolipids in AD blood using MS techniques have been reported in single studies and are listed in Table 2.

Apolipoprotein E (ApoE)

ApoE is the most prevalent lipoprotein in the brain and it exists as three major isoforms, ApoE2, ApoE3 and ApoE4 [24]. ApoE4 is the most important genetic risk factor for AD [1] and might be an interesting biomarker candidate. Han and colleagues used two orthogonal methods to measure total ApoE in serum

Table 2 Summary of molecules measured by MS techniques and with a reported change in AD

Molecule	Observed changes	Molecule	Observed changes
Acyl carnitines		*Cholesterol metabolism*	
Carnitine	MCI vs Con↓ [20], AD vs Con↓ [35]	24S-OH cholesterol esters	AD vs Con↓ [53], $MCI_{non\text{-}conv}$ vs MCI_{conv}↑ [53]
	AD vs MCI↓ [35]		MCI vs AD↑ [53]
C5-OH	AD vs Con↓ [20]	27-OH cholesterol	AD vs Con↓ [41]↔[42]
C10:0	AD vs Con↑ [36]↓ [20, 35]		MCI vs Con↓ [41]↔[42]
	MCI vs Con↑ [36]↓ [20]	Desmosterol	AD vs Con ↓ [54], MCI vs Con↓ [54]
	AD vs MCI↓ [35]		
C14:2	AD vs Con↓ [35], AD vs MCI↓ [35]	*Steroids*	
C10:1	AD vs Con↓ [36], MCI vs Con↓ [36]	5α-Androstane-3α,17β-diol sulfate/ 5α-Androstane-3α,17β-diol	AD vs Con↓ [55]
	MCI vs AD↑ [35]	5α-Androstane-3β,17β-diol sulfate/ 5α-Androstane-3β,17β-diol	AD vs Con↓ [55]
C16:1	AD vs Con↓ [20], MCI vs Con↓ [20]	Free 7α-Hydroxy-DHEA	AD vs Con↑ [56]
C6:1	AD vs Con↓ [20]	7α-Hydroxy-DHEA-S	AD vs Con↑ [56]
C18:2	AD vs Con↓ [20]	7α-Hydroxy-DHEA-FA esters	AD vs Con↑ [56]
C3-DC	AD vs Con↓ [20]	Androsterone sulfate/androsterone	AD vs Con↓ [55]
C14	AD vs Con↓ [20]	Cortisol	OR AD vs Con↑ [57]
C16	AD vs MCI↓ [20]	DHEA-S	AD vs Con↓ [58]
C18:1	AD vs Con↓ [20], MCI vs Con↓ [20]	DHEA-S/DHEA	AD vs Con↓ [55]
	AD vs MCI↓ [35]	Epiandrosterone sulfate/ epiandrosterone	AD vs Con↓ [55]
C18	AD vs Con↓ [20], MCI vs Con↓ [20]		
		Ox. stress-med. DHEA-increase	AD vs Con↓ [59], MCI vs Con↑ [59], MCI vs AD↑ [59]
Fatty acids/DAG/ TG			
C14:0	AD vs Con↓ [46]	*Vitamin D*	

Table 2 continued

Molecule	Observed changes	Molecule	Observed changes
C16:0	AD vs Con↓ [46]	25(OH) Vitamin D	HR AD with low levels↑ [60]↔[61]
C16:1	AD vs Con↓ [46]		
C18:0	AD vs Con↓ [46]	*Amino acids and derivatives*	
C18:1	AD vs Con↓ [46]	Aspartate	AD vs Con↓ [36], MCI vs Con↓ [36]
C18:2	AD vs Con↓ [46]	Carnosine	AD vs Con↓ [62]
γ-C18:3	AD vs Con↓ [46]	Cysteine	MCI vs Con↑ [63]
C18:3	AD vs Con↑ [46]	DOPA	AD vs Con↑ [62]
C20:2	AD vs Con↓ [46]	Glutamic acid	HR AD↑ [64]
C20:5	AD vs Con↓ [47]	Hcy-Cys	AD vs Con↑ [36]
C22:6	AD vs Con↓ [46]	Histidine	AD vs Con↓ [36], AD vs MCI↓ [36]
MMA	AD vs Con [48]	Isoaspartate peptides	AD/MCI vs Con↑ [65]
DAG (34:3)	MCI vs Con↑ [49], Dem vs Con↑ [49]	Methionine	AD vs Con↓ [36], AD vs MCI↓ [36]
DAG (36:3)	MCI vs Con↑ [49], Dem vs Con↑ [49]	Methyldopa	AD vs Con [48]
DAG (36:4)	MCI vs Con↑ [49], Dem vs Con↑ [49]	N-acetylglutamine	AD vs Con↓ [19]
DAG (38:6)	MCI vs Con↑ [49], Dem vs Con↑ [49]	Phe Phe	AD vs Con↑ [36], MCI vs Con↑ [36]
DAG (34:2)	MCI vs Con↑ [49], Dem vs Con↑ [49]	O-Acetylserine	AD vs Con [48]
DAG (36:2)	MCI vs Con↑ [49], Dem vs Con↑ [49]	Tyrosine	AD vs Con [48]
DAG (38:4)	MCI vs Con↑ [49], Dem vs Con↑ [49]	Valine	AD vs Con [48]
TG (48:0)	AD vs Con [48]		
TG (50:4)	AD vs Con [48]	*Metals*	
TG (48:2)	AD vs Con [48]	Al	AD vs Con↓ [44]↑ [66], MCI vs Con↑ [66]
TG (51:3)	AD vs Con [48]	Al-Ferritin	AD vs Con↑ [67]
TG (54:6)	AD vs Con [48]	Ca	DLB vs AD↑ [68]

Table 2 continued

Molecule	Observed changes	Molecule	Observed changes
TG (50:3)	AD vs Con [48]	Cd	severeAD vs mildAD↑ [69], AD vs Con↑ [44]
TG (48:1)	AD vs Con [48]	Cs	AD vs Con↓ [45]
TG (52:4)	AD vs Con [48]	Cu	DLB vs AD↑ [68]
TG (48:3)	AD vs Con [48]	Non-ceruloplasmin Cu	MCI vs Con↓ [70], AD vs Con↓ [70]
TG (46:2)	AD vs Con [48]	Fe	AD vs Con↓ [71]
TG (52:5)	AD vs Con [48]	Transferrin-Fe/Transferrin	AD vs Con↓ [71]
TG (58:10)	AD vs Con [48]	Mg	DLB vs AD↑ [68]
TG (56:7)	AD vs Con [48]	Mn	severeAD vs mildAD↓ [69]
TG (56:8)	AD vs Con [48]		AD vs Con↑ [45]↓ [66, 72], MCI vs Con↓ [66]
		Mo	severeAD vs mildAD↑ [69]
Phospholipids/ Sphingolipids		Se	AD vs Con↓ [45], MCI vs AD↑ [66]
Lyso-PAF (C16:0)	AD_{conv} vs Con↑ [18]	Sn	AD vs Con↑ [44]
Lyso-PAF (C18:0)	AD_{conv} vs Con↑ [18]	Zn	DLB vs AD↓ [68], AD vs Con↓ [66]
Lyso-PAF (C18:1)	AD_{conv} vs Con↑ [18]		
LysoPC (16:0)	AD_{conv} vs Con↑ [18]	*Oxidative stress markers*	
LysoPC (18:0)	AD_{conv} vs Con↑ [18], AD vs Con↓ [19]	8,12-iso-iPF$_{2\alpha}$-VI	AD vs MCI↑ [73]↔[74]
LysoPC (18:1)	AD_{conv} vs Con↑ [18]		MCI vs Con↑ [73]↔[74]
LysoPC (20:0)	AD_{conv} vs Con↑ [18]	Ent-7(RS)-7-F2t-dihomo-IsoP	AD vs Con↑ [75]
LysoPC (20:3)	AD vs Con↓ [19]	17-epi-17-F2t-dihomo-IsoP	AD vs Con↑ [75]
LysoPC (22:0)	AD_{conv} vs Con↑ [18]	15(R)-15-F2t-IsoP	AD vs Con↑ [75]
LysoPC (24:0)	AD_{conv} vs Con↑ [18]	PGF$_{2\alpha}$	AD vs Con↓ [75]
PC aa C38:4	HR MCI-AD↑ [50], HR Con-AD↓ [50]	4(RS)-F4t-NeuroP	AD vs Con↑ [75]
PC aa C38:4 (16:0, 22:4)	OR AD↑ [21]		
PC aa C38:4 (18:0, 20:4)	OR AD↑ [21]	*Clusterin glycosylation*	

Table 2 continued

Molecule	Observed changes	Molecule	Observed changes
PC ae C34:2	HR Con-AD↑ [50]	β64N_SA$_1$-(HexNAc-Hex)$_2$-core	AD low atrophy vs AD high atrophy↑ [76]
PC aa C38:5 (18:0, 20:5)	OR AD↓ [21]	β64N_SA$_2$-(HexNAc-Hex)$_2$-core	AD low atrophy vs AD high atrophy↑ [76]
PC aa C40:4 (18:0, 22:4)	OR AD↑ [21]	β64N_SA$_1$-(HexNAc-Hex)$_3$-core	AD low atrophy vs AD high atrophy↑ [76]
PC aa C40:5 (18:0, 22:5)	OR AD↑ [21]	β64N_SA$_2$-(HexNAc-Hex)$_3$-core	AD low atrophy vs AD high atrophy↑ [76]
PC aa C40:5 (18:1, 22:4)	OR AD↑ [21]	β64N_SA$_1$-(HexNAc-Hex)$_4$-core	AD low atrophy vs AD high atrophy↑ [76]
PlsCho (18:0-18:1)	AD$_{conv}$ vs Con↑ [18]	β64N_SA$_3$-(HexNAc-Hex)$_3$-core	AD low atrophy vs AD high atrophy↑ [76]
PlsCho (18:0-22:6)	AD$_{conv}$ vs Con↑ [18]	β64N_SA$_2$-(HexNAc-Hex)$_4$-core	AD low atrophy vs AD high atrophy↑ [76]
Cer16:0	MCI vs AD↓ [51]	β64N_SA$_3$-(HexNAc-Hex)$_4$-core	AD low atrophy vs AD high atrophy↑ [76]
Cer18:0	MCI vs AD↓ [51]		
Cer20:0	MCI vs AD↓ [51]	*Other Metabolites*	
Cer22:0	MCI vs AD↓ [51], MCI vs Con↓ [51]	2-Aminoadipic acid	AD vs Con [48]
Cer24:0	MCI vs AD↓ [51], MCI vs Con↓ [51]	3-Hydroxyisovaleric acid	AD vs Con [48]
Cer26:0	MCI vs AD↓ [51], MCI vs Con↓ [51]	8-iso-PGF2a (15-F2t-IsoP)	AD vs Con [48]
SM (d18:1/20:1)	AD vs Con [48]	Anthranilic acid	HR AD↑ [64]
SM (d18:1/23:0)	AD vs Con [48]	Asymetric dimethyl-Arginine	AD vs Con↑ [36]
SM (C18:1)	HR MCI-AD↑ [50], HR Con-AD↑ [50]	β-Hydroxy butyric acid	AD vs Con↓ [35], MCI vs Con↓ [35]
SM (C16:0)	HR Con-AD↑ [50]	Choline	AD vs Con↑ [36], MCI vs Con↑ [36]
SM (C16:1)	HR Con-AD↑ [50]	Creatine	AD vs MCI↓ [36], AD vs Con↓ [77]
SM (OH) (C14:1)	HR Con-AD↑ [50]	Creatinine	AD vs con↑ [36]
SM (OH) (C22:1)	OR Dem vs Con↓ [17]	Dihydrosphingosine	AD vs Con↓ [19]
		Hypoxanthine	HR AD↓ [64]

Table 2 continued

Molecule	Observed changes	Molecule	Observed changes
Bile acids		Palmitic amide	AD vs Con↑ [19]
Cholic acid	OR AD vs Con↓ [52]	Ornithine	AD vs Con [48]
	$MCI_{non\text{-}conv}$ vs MCI_{conv} ↓ [52]	S-3-Hyxroxyisobutyric acid	AD vs Con [48]
Deoxycholic acid,	OR AD vs Con↑ [52]	S-adenosylhomocysteine	MCI vs Con↑ [63]
Glucodeoxycholic acid	OR AD vs Con↑ [52]		
Taurodeoxycholic acid	OR AD vs Con↑ [52]		
Glycolithocholic acid	OR AD vs Con↑ [52]		
Taurolithocholic acid	OR AD vs Con↑ [52]		

AD Alzheimer's disease, AD_{conv} AD converter, *DAG* diacylglycerols, *Dem* dementia, *DLB* dementia with Lewy bodies, *HR* hazard ratio, *MCI* mild cognitive impairment, MCI_{conv} MCI converter, $MCI_{non\text{-}conv}$ MCI non-converter, *MMA* methylmalonic acid, *OR* odds ratio, *Ox.* stress-med., oxidative stress-mediated, *TG* triglycerides, ↑ significantly increased, ↓ significantly decreased, ↔ unchanged

including MRM and observed lower ApoE levels in AD with both methods [25], Two other studies did not find a difference [26, 27]. Inconsistent results of ApoE levels in AD blood were also reported in studies using immunassays [28–31]. In addition, total ApoE levels in blood are different between carriers of different ApoE genotypes [26, 27, 32] which renders ApoE quite unsuitable as a disease biomarker. However, it turned out that MRM seems to be a valuable method for ApoE genotyping. Several groups reported the successful isoform identification of ApoE in blood by quantifying allele-specific peptides with up to 100% concordance to classical genotyping [32, 33].

Acylcarnitines/Fatty acids/Di-and Triglycerides

L-Carnitine and acylcarnitines are essential players in energy metabolism but also other functions e.g. in the brain are reported. This includes protein modulation, neuromodulation, protection from excitotoxicity, antioxidant and anti-apoptotic functions all of which can be linked to neurodegenerative diseases [34]. Consistent changes in AD in two independent studies have been reported for five acylcarnitines. Acetyl-carnitine (C2), dodecanoyl-carnitine (C12), dodecenoyl-carnitine (C12:1) and tetradecenyl-carnitine (C14:1) were reduced in plasma [35] and serum [20] of AD patients compared with controls and MCI patients. C2, C12:1 and C14:1 were already reduced in MCI [20]. Both studies used MRM for acylcarnitine measurement. In contrast, octanoyl-carnitine (C8) was increased in AD in two studies analyzing plasma [35] or serum [36] samples. Reports on serum C8 levels in MCI were inconsistent [20, 36]. Changes of other acylcarnitines in blood of AD patients were reported only by single studies and are listed in Table 2. Free fatty acids and diacyl- and triglycerides measured with MS-based methods are also depicted in Table 2 because changes in AD were described in single studies only.

Cholesterol and Related Metabolites

A link between cholesterol and AD comes from epidemiological studies indicating that high cholesterol levels in blood are associated with a higher risk for the development of AD. In addition, cholesterol can influence APP processing and Aβ aggregation [37]. In the brain, cholesterol is converted to the more hydrophilic 24S-hydroxycholesterol to eliminate it from the brain because cholesterol itself cannot cross the blood–brain-barrier. 24S-hydroxycholesterol is an important metabolite in the regulation of cholesterol homeostasis in the brain [38]. Several studies investigated 24S-hydroxycholesterol levels in blood of AD patients using MS but with conflicting results. Lütjohann et al. [39] and Zuliani et al. [40] observed significantly increased 24S-hydroxycholesterol levels in plasma of AD patients compared with controls although with a substantial overlap between groups. In contrast, Solomon and colleagues found reduced levels in AD [41] and another study observed no difference between AD and controls [42]. Thus, 24S-hydroxycholesterol might not be an optimal biomarker candidate for AD. Two precursors of cholesterol synthesis, lanosterol and lathosterol, have consistently been reported to be lower in AD patients in two studies [41, 43] but also with a high variation within groups. This might limit their use as a biomarker. Several other cholesterol-related metabolites such as bile acids, steroids and vitamin D were measured in blood of AD patients in single studies or with conflicting results and they are listed in Table 2.

Other Molecules

Several other molecules were investigated in blood of AD patients using MS methods and were suggested as potential biomarker candidates. For a majority of them, significant changes in AD were reported in single studies only or with conflicting results and are therefore summarized in this section and listed in Table 2. This includes the group of metal ions. Here, consistent changes in AD in more than one study were described only for cobalt (down-regulated) and mercury (up-regulated) [44, 45]. Furthermore, markers of oxidative stress, amino acids and their derivatives and other metabolites or proteins have been described to be affected in blood of AD patients. However, their suitability as AD biomarkers is difficult to assess due to the single reports and also the question of specificity for AD.

CONCLUSION

To date, MS-based blood biomarkers for AD are still sparse. Although many studies used MS to identify AD biomarker candidates in blood, most candidates were reported in single studies only and need further validation. In addition, fold-changes in AD patients were often low with substantial overlap of blood levels with the control group which makes them unsuitable as a biomarker. There is also the question of specificity, especially for changes of many metabolites such as lipids since short- and long-term nutritional behavior significantly influences blood levels. Fasting before sample collection alone might not account for these effects. Thus, they might be risk factors for AD but not useful biomarker candidates. The most promising MS-based blood biomarkers for AD at the moment seem to be the determination of Aβ peptides by IP- MS [12, 13] and the "genotyping" of ApoE by MRM [33].

The major strength of MS-based techniques is their high specificity for the target analyte and against matrix effects. Current efforts to measure the core AD biomarkers, Aβ42, total-tau and phospho-tau, in blood with immunoassays struggle with the high complexity of this matrix and the low abundance of the proteins. This could be the place for MS techniques, eventually in combination with immunological methods, to overcome this problems as has successfully been shown for Aβ peptides by Nakamura and colleagues [12]. The improvement of the diagnostic performance of tau determination in blood and the so far unsuccessful measurement of a synaptic blood marker for AD could be promising applications of MS in the current biomarker development. Definitely, MS will be essential in the definition

of reference methods for the measurement of AD biomarkers in blood.

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