Biomarkers with Plasma Amyloid β and Tau Protein Assayed by Immunomagnetic Reduction in Patients with Amnestic Mild Cognitive Impairment and Alzheimer's Disease

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Abstract

This review addresses recent developments in the analyses of plasma amyloid beta (Aβ) and total tau (t-tau) protein levels as biomarkers for discriminating amnestic mild cognitive impairment (aMCI) from Alzheimer disease (AD), using immunomagnetic reduction (IMR). Recent studies have focused on the differential diagnosis of normal controls (NCs) with aMCI or AD. Results of 15 clinical studies have demonstrated decrease in plasma Aβ1–40 and increase in plasma Aβ1–42 and t-tau levels in patients with aMCI and AD. For a given biomarker, effect size is determined by comparing the mean ratios of biomarker levels between two diagnostic groups. Effect sizes are <1 for Aβ1–40 (0.606–1.032) but >1 for Aβ1–42 (1.018–2.167) and t-tau (1.030–4.147) in aMCI and AD compared with NCs. The effect size of the plasma tau significantly increases the most as aMCI progresses to AD. Studies into the application of IMR to determine plasma Aβ and tau levels as biomarkers for aMCI or AD have recently progressed. Future investigations should validate recently published results, preferably in patients with pathologically confirmed AD. In addition, effort should be directed toward standardizing the design of such studies and data analysis.

Keywords: amyloid beta, plasma tau, Alzheimer disease, biomarker, mild cognitive impairment.

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INTRODUCTION

Alzheimer disease (AD) has become a widespread health issue with global increases in population size and life expectancy[(1)]. Dementia confers an enormous burden on patients, their families and caregivers, and on health

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and social care systems[2]. The pathological hallmarks of Alzheimer disease (AD) are amyloid β (Aβ) plaques and tau neurofibrillary tangles[3–6]. The accumulation of pathological Aβ plaques and tau protein tangles causes neuronal damage, which subsequently lead to hippocampal atrophy, cortical thinning, and brain damage[7,8]. Amnestic mild cognitive impairment (aMCI) is a heterogeneous, symptomatic pre-dementia phase that represents a transitional state between normal aging and dementia, and often AD[9]. The annual conversion rate from aMCI to dementia ranges from <5% to 20% depending on the population investigated[10].

Over the past decade, substantial effort has been directed toward the early diagnosis of aMCI or dementia using various biomarkers. Current biomarkers comprise apolipoprotein E ε4 (APOE ε4) carrier status, atrophy confirmed by structural magnetic resonance imaging (MRI), hypometabolism confirmed by fluorodeoxyglucose F 18-positron emission tomography (FDG-PET), and cerebrospinal fluid (CSF) biomarkers, such as amyloid β 1–42 peptide (Aβ1–42), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau181)[11]. Positive positron emission (PET) imaging of the brain uses a tracer that specifically binds Aβ or tau to identify amyloid plaques and tau protein tangles[12–15]. However, the high cost of PET and low availability of Aβ or tau tracers limit its clinical application[16–19]. However, concentrations of AD-related biomarkers in body fluids are altered due to the formation of Aβ plaques and tau protein in the brain as cognitive impairment develops in patients with AD. Hence, diagnosing AD based on measurements of potential biomarkers in CSF such as Aβ and tau, and their derivatives has become popular[20,21]. Although Aβ and tau levels in CSF significantly correlate with standard uptake value ratios (SUVR) with Aβ or tau tracers[22–24], CSF sampling is invasive and comparatively uncomfortable for patients. Moreover, side effects, such as headache, spinal or epidural bleeding, minor nerve damage due to lumbar puncture, have prevented the detection of CSF biomarkers for broad screening, or resampling to monitor long-term disease progression or treatment effects. Consequently, neuropsychological examinations remain the most popular means of diagnosing Alzheimer disease[25,26]. Therefore, biomarkers in biological specimens other than CSF are needed.

Blood proteins can be conveniently measured and serve as biomarkers. However, biomarkers of AD circulate in the bloodstream at pg/mL levels. Ultrasensitive analytical assays, such as immunomagnetic reduction (IMR), immunoprecipitation mass spectrometry (IP-MS), and single-molecule array (SIMOA) have shown potential for more accurately quantifying biomarkers in blood samples. Plasma Aβ and tau can be accurately quantified at levels of 1–10 pg/mL using IMR[27–30]. The protocol for IMR includes magnetic nanoparticles that are functionalized with antibodies and homogeneously suspended in phosphate-buffered saline (PBS). Under external alternative-current (ac) magnetic fields, the nanoparticles oscillate to generate an ac magnetic signal. Magnetic nanoparticles that associate with a target biomarker expand, which causes a reduction in the ac magnetic signal. A target biomarker is then quantified as ac magnetic signal attenuation[30]. Recent findings have revealed significant correlations between plasma biomarker levels and cognitive impairment[31–33], brain atrophy, and Aβ accumulation in the brain[34–37], which provide evidence that assays of plasma Aβ1–40, Aβ1–42 or tau using IMR are promising for facilitating an early diagnosis of AD. This review focuses on the results of 15 clinical studies in which plasma Aβ and tau were determined using IMR[31,32,34–36,38–42,43–46]. One each of these studies were from the USA, Europe, Japan, and China, and 11 were from Taiwan. We compared the effect sizes of plasma Aβ1–40, Aβ1–42, and tau among normal controls (NCs), aMCI and AD as described in these studies.

BRIEF DEMOGRAPHIC INFORMATION OF REPORTED CASES

Patients were diagnosed in the studies in Taiwan and the USA based on the guidelines issued by the National Institute on Aging-Alzheimer's Association (NIA-AA) workgroups in 2011. In addition to these guidelines, Aβ plaque accumulation in the patients with AD was confirmed using Pittsburgh compound B (PiB)-PET in the Chinese study. Neuropsychological tests and FDG-PET were compulsory for all patients in the Japanese study. The European patients were diagnosed with AD based on Aβ1–42 and tau levels in CSF. Table 1 shows the numbers, age, gender, mini-mental state examination (MMSE)

findings of patients in various diagnostic groups among the studies. The average age and MMSE findings of individual patients in the diagnostic groups were provided in each study.

A total of 658, 303, and 478 reported controls and patients with aMCI and AD were aged 63.0–81.9, 68.0–75.6, and 64.9–82.5 years, respectively. The ages of the patients with aMCI and AD did not significantly differ among the studies. The ratios (%) of females in the NC, aMCI and AD groups were 32.6%–80.8%, 38.2%–75.0%, and 42.9%–84.2%, respectively, and the MMSE scores were 28.2–29.3, 24.2–26.9 and 12.7–21.6, respectively, indicating a significant decrease as dementia progressed.

PLASMA PREPARATION AND BIOMARKER ASSAY

Non-fasting venous blood samples (6 or 9 mL) were collected into lavender-topped tubes containing EDTA. Plasma was separated from blood samples within 3 h by centrifugation at 2,500 × g for 15 min, then portioned into cryotubes for storage at -80°C. Thawed plasma samples (40/60/40 μL) were mixed with 80/60/80 μL of the IMR reagents MF-Aβ0-0060, MF-Aβ2-0060, MF-TAU-0060 to assay Aβ1–40/Aβ1–42/tau in duplicate using a XacPro-S IMR analyzer (MagQu Co., Ltd., New Taipei City, Taiwan). The results are expressed as the average concentrations of biomarkers in duplicate plasma samples.

PLASMA Aβ1-40

The mean concentrations of plasma Aβ1–40 levels in the NC, aMCI, and AD groups were 50.70–65.84, 40.60–52.81, and 36.90–53.21 pg/mL, respectively (Table 1). The overall plasma Aβ1–40 levels were significantly lower in aMCI and AD, than in NC . A ratio-based method was applied to identify effect sizes (ES) as mean biomarker

Table 1. Brief demographic information and measured plasma biomarker levels in different diagnostic groups.

Study No.	Site	NC							aMCI							AD						
		n	Age (yr.)	F/M	MMSE	Aβ1-40 (pg/ml)	Aβ1-42 (pg/ml)	Tau (pg/ml)	n	Age (yr.)	F/M	MMSE	Aβ1-40 (pg/ml)	Aβ1-42 (pg/ml)	Tau (pg/ml)	n	Age (yr.)	F/M	MMSE	Aβ1-40 (pg/ml)	Aβ1-42 (pg/ml)	Tau (pg/ml)
1[31]	TW	26	66.3	13/13	28.9	65.84	15.79	-	16	77.2	9/7	24.6	47.98	22.66	-	18	72.9	10/8	19.1	53.21	34.22	-
2[39]	US	16	81.9	12/4	29.3	-	15.33	20.48	-	-	-	-	-	-	-	16	82.5	9/7	16.1	--	16.8	34.52
3[40]	CN	57	67.9	31/29	28.3	-	16.92	20.65	-	-	-	-	-	-	-	40	68.1	25/15	12.7	-	18.77	25.91
4[32]	TW	67	63.3	41/26	29	60.3	15.8	13.3	34	68.8	13/21	26.9	40.6	17.6	30.5	59	71.8	32/27	21.6	43.9	23.5	47.6
5[32]	TW	134	68.4	89/45	28.2	62.2	14.65	20.2	33	72.6	23/20	24.2	52.81	18.6	30.5	73	78.1	37/36	17.1	42.73	20	40.9
6[41]	TW	13	64.4	10/3	29.5	51.8	16.7	22.5	40	72.4	30/10	26.1	51.9	17	24.5	37	77	28/11	20.2	51.7	17.4	27.1
7[42]	EU	43	66.7	14/29	-	-	15.5	-	-	-	-	-	-	--	-	66	71.0	32/34	-	-	17.9	-
8[35]	TW	20	63.7	10/10	29	60.9	15.9	13.5	11	69.2	5/6	26.5	41.4	17.2	33.5	14	64.9	10/4	20.7	36.9	18.9	46.7
9[34]	TW	30	64.4	17/13	28.8			15.6	20	71.2	11/9	26.3			32.7	10	69.3	6/4	22.7	-	-	53.9
10[36]	TW	39	63	23/16	28.8	59.2	16.1	14.3	25	68	11/14	26.6	41.5	17	29.7	16	67.6	7/9	16	39.5	19	39.4
11[38]	TW	66	64.6	-	> 26	-	-	13.37	24	71	-	24-28	-	-	33.33	29	72.2	-	10-22	-	-	55.44
12[43]	TW	97	64	66/31	28.9	59.45	15.66	16.95	41	72.9	25/16	26.8	50.28	18.3	30.93	35	75.2	21/14	18.2	49.01	21.15	38.70
13[44]	TW	26	68.7	21/5	28.4	50.7	16.9	23.8	35	75.6	23/12	26.9	51.9	17.2	26	39	77.7	29/10	21.2	52.3	17.4	27.1
14[45]	JP	14	64.9	10/4	29.3	63.24	16.38	20.1	-	-	-	-	-	-	-	7	74.3	3/4	18.4	47.53	20.1	28.6
15[46]	TW	10	64.6	7/3	28.7	53.0	16.6	23.0	24	72.8	18/6	26.8	51.4	17.0	23.7	19	77.7	16/3	22.6	52.7	17.4	26.4

AD, Alzheimer disease; aMCI, amnestic mild cognitive impairment; CN, China; EU, Europe; F, female; JP, Japan; M, male; MMSE, mini mental state examination; NC, normal control; TW, Taiwan; US, United Sates of America; y, years.

ratios to distinguish different groups. In Study No. 1[31], the effect size of Aβ1–40 between aMCI and NC was 47.98/65.84 = 0.729.

Recent IMR studies have found that the range of effect sizes of plasma Aβ1–40 between aMCI and NC is 0.673–1.024 (Figure 1 unfilled symbols). Plasma Aβ1–40 notably remained at similar levels between aMCI and NC in studies 6 (effect size: 1.002), 13 (effect size: 1.024), and 15 (effect size: 0.970) (Table 1). These values significantly differed from other findings that showed obvious decreases in plasma Aβ1–40 levels in aMCI compared with NC (effect size: 0.673–0.849, $p < 0.001$). The effect sizes of plasma Aβ1–40 were similar between AD and NC (Fig. 1a; filled symbols). The effect sizes of plasma Aβ1–40 between AD and NC in studies 6, 13 and 15 were 0.998, 1.032, and 0.994, respectively, compared with 0.606–0.824 ($p < 0.001$) in other studies. Variations in plasma sample preparation procedures might have contributed to the discrepancies. Blood samples were centrifuged at 4°C in studies 6, 13, and 15 but at room temperature (20–25°C) in the other studies. The reasons for the variations in the measured plasma Aβ1–40 levels at different centrifugation temperatures are not clear. Appropriate temperatures need to be established, and sample preparation protocols must be standardized.

Plasma Aβ1–42

The plasma Aβ1–42 levels ranges from 14.65–16.92, 17.00–22.66, and 16.80–34.22 pg/ml in NC, aMCI, and AD, respectively (Table1). All studies found increased plasma Aβ1–42 levels in patients with aMCI and AD, compared with NC. The effect sizes of plasma Aβ1–42 between aMCI and NC and between AD and NC were 1.018–1.435, and 1.030–2.167 (Figure 1b unfilled and filled symbols, respectively). Increased plasma Aβ-1–42 concentrations in aMCI and AD using IMR are inconsistent with previous findings using other analytical methods such as single molecule assays or mass spectrometry. These different methodological approaches with variable capacity with respect to Aβ aggregation or Aβ binding to other proteins might partly explain the variances[42].

The consistent findings across all studies that plasma Aβ1–42 is significantly elevated in aMCI and AD implies that plasma Aβ1–42 could serve as a useful biomarker to help differentiate aMCI/AD from NC. Chiu et al.[32] validated the cut-off plasma Aβ1–42 concentration required to discriminate aMCI and AD as 16.41 pg/mL. The corresponding sensitivity, specificity, and accuracy were 0.882, 0.952, and 0.919, respectively. Furthermore, the findings of longitudinal studies[47] associated elevated plasma Aβ1–42 levels >16.8 pg/mL with a 17-fold higher risk of cognitive decline in patients with aMCI, and another study[46] found a significant correlation between plasma Aβ1–42 and a rapid cognitive decline in aMCI during an average followup of 1.2 years.

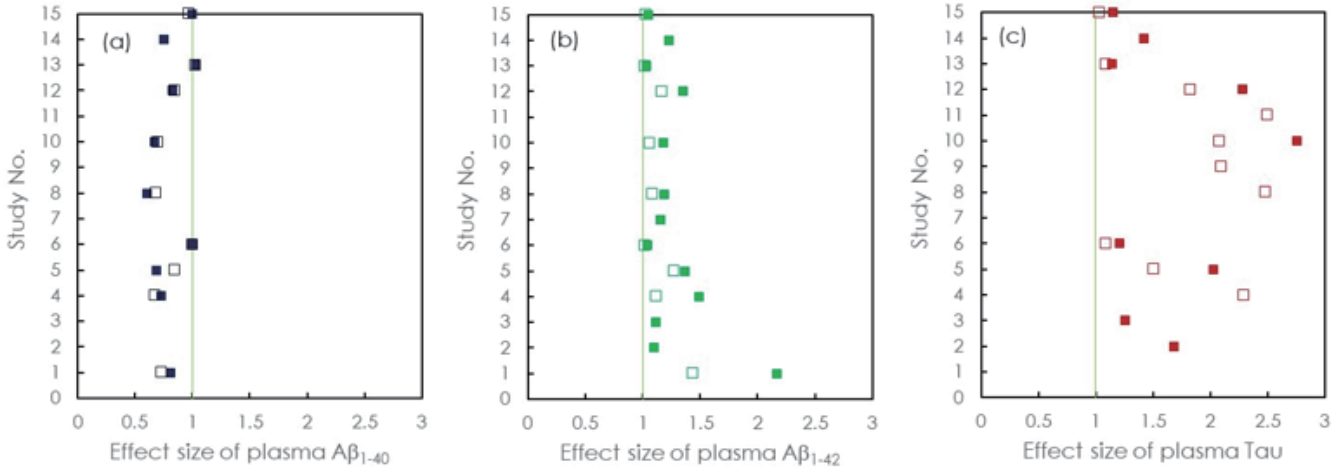

Fig. 1. Effect sizes of plasma (a) Aβ1–40 (b) Aβ1–42 (c) tau levels between aMCI and NC (unfilled symbols), between AD and NC (filled symbols).

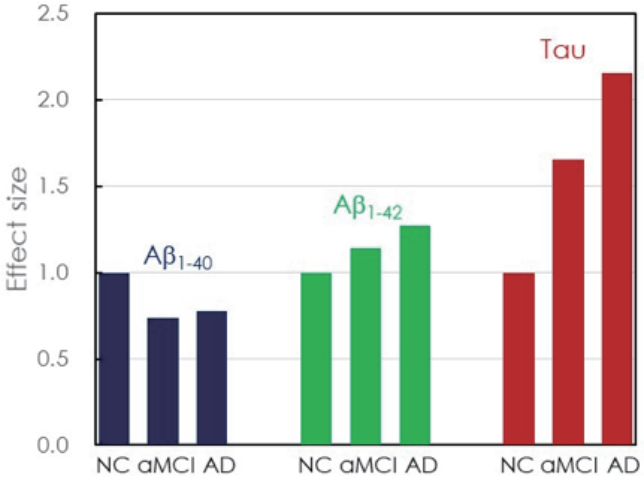

Fig. 2. Averaged effect sizes of different plasma biomarkers in NC, aMCI, and AD, among several studies.

Plasma tau

The reported ranges of plasma tau concentrations are 13.30–23.80, 26.00–33.50, and 25.91–55.44 pg/mL in NC, aMCI, and AD, respectively (Table1). All reports described higher plasma tau levels in aMCI and AD than in NC, which agrees with the plasma Aβ1–42 values. The findings indicated that plasma tau could also serve as a biomarker to differentiate aMCI/AD from NC. The feasibility of this has been demonstrated[32]. At a plasma tau cut-off of 24.9 pg/mL, the sensitivity, specificity, and accuracy of discriminating aMCI/AD from NC were 0.892, 0.955, and 0.961, respectively[32]. The ranges of effect sizes of plasma tau levels between aMCI and NC and between AD and NC were 1.030–2.493 and 1.139– 4.147, respectively (Figure 1c; unfilled and filled symbols, respectively). Plasma tau has a larger effect size than plasma Aβ1–42 (Figure 1b and c), indicating more significant changes in plasma tau than plasma Aβ1–42 concentrations in patients with aMCI and AD. However, elevated plasma tau levels are not specific to aMCI or AD. Increased plasma tau levels are also found in frontotemporal dementia[38], Parkinson disease[38][48], and vascular cognitive impairment[49]. Therefore, plasma Tau is not ideal as a singular biomarker with which to differentially diagnose aMCI and AD.

Severity-dependent biomarkers

The filled and unfilled symbols in Figure 1a almost overlap, which implies no significant changes in plasma Aβ1–40 concentrations between AD and aMCI. In contrast, plasma Aβ1–42 and tau levels were elevated in AD compared with aMCI (Figure 1b and c), which consistently supported the severity dependence of plasma Aβ1–42 and tau levels in AD. Furthermore, Figure 2 shows that concentrations of plasma Aβ1–42 and tau, but not Aβ1–40, vary from NC to aMCI to AD, indicating that combined plasma Aβ1–42 and tau levels could potentially discriminate aMCI from NC, and AD from aMCI. To enhance plasma biomarker levels in aMCI and AD, some authors have suggested that measuring Aβ1–42xtau, instead of individual Aβ1–42 or tau, offers better sensitivity, specificity and accuracy in differentiating aMCI from NC, AD from aMCI[32,33,40,50].

CONCLUSIONS

Following the standard procedures to prepare plasma samples for IMR assays described in 15 studies published between 2012 and 2020, the results consistently showed significant decreases in plasma levels of Aβ1–40 and increases in those of Aβ1–42 and tau in aMCI and AD. Furthermore, plasma Aβ1–42 and tau levels are related to the severity of AD. To achieve clear discrimination among NC, aMCI, and AD, a combination of Aβ1–42xtau biomarkers should be measured in clinics. However, more detailed analyses are necessary to comprehend their advantages as potential diagnostic, monitoring, or prognostic biomarkers of aMCI, and AD. A triage algorithm based on current blood biomarkers is crucial to identify patients who might be eligible for further assessment using the current gold standard for identifying AD pathology (CSF and/or amyloid PET) and for inclusion in clinical trials. After validation, a triage algorithm would facilitate AD drug discovery and effective disease-modifying strategies for treating AD.

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