

Title: Blood-based ATN biomarkers of Alzheimer's disease: A meta-analysis

Running title: Blood biomarkers meta-analysis

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Abstract

Background

The Amyloid Tau Neurodegeneration (ATN) framework was proposed to define the biological state underpinning Alzheimer's disease (AD). Blood-based biomarkers offer a scalable alternative to the costly and invasive currently available biomarkers.

Objective

In this meta-analysis we sought to assess the diagnostic performance of plasma amyloid ($A\beta_{40}$, $A\beta_{42}$, $A\beta_{42/40}$ ratio), tangle (p-tau181) and neurodegeneration (total tau [t-tau], neurofilament light [NfL]) biomarkers.

Methods

Electronic databases were screened for studies reporting biomarker concentrations for AD and control cohorts. Biomarker performance was examined by random-effect meta-analyses based on the ratio between biomarker concentrations in patients and controls.

Results

83 studies published between 1996 and 2020 were included in the analyses. $A\beta_{42/40}$ ratio as well as $A\beta_{42}$ discriminated AD patients from controls when using novel platforms such as Immunomagnetic Reduction, IMR. We found significant differences in ptau-181 concentration for studies based on single molecule array (Simoa), but not for studies based on IMR or ELISA. T-tau was significantly different between AD patients and control in IMR and Simoa but not in

ELISA-based studies. In contrast, NfL differentiated between groups across platforms. Exosome studies showed strong separation between patients and controls for $A\beta_{42}$, t-tau and p-tau181.

Conclusion

Currently available assays for sampling plasma ATN biomarkers appear to differentiate between AD patients and controls. Novel assay methodologies have given the field a significant boost for testing these biomarkers, such as IMR for $A\beta$, Simoa for p-tau181. Enriching samples through extracellular vesicles shows promise but requires further validation.

Keywords: Alzheimer's disease, ATN framework, meta-analysis, fluid biomarkers, diagnosis

Introduction

The current standard of diagnosing Alzheimer's disease (AD) clinically is confined to establishing 'probable' or 'possible' AD depending on the level of certainty. It relies on data gathered through clinical examination, patient and carer interview, with differential diagnosis guided by structural and/or glucose metabolism imaging [1]. The limitations of this approach have been highlighted by the demonstration that a significant proportion of patients diagnosed with AD have their diagnosis changed through in-vivo amyloid positron emission tomography [2] or post-mortem studies [3, 4]. Syndrome-based AD definition is particularly problematic in the context of preclinical or prodromal disease which is where the major efforts of disease-modification are currently focused [5]. Biomarker results from CSF, PET as well as structural imaging have allowed to largely close this gap for the purposes of clinical research and define AD pathophysiologically in both its clinical and preclinical phases [6]. This ATN (amyloid, tangle and neurodegeneration) framework has the potential to revolutionise the practice of dementia diagnosis and risk monitoring. However the expense, invasiveness and dependence on relevant infrastructure limit severely the utility of these methods for standard clinical practice or large-scale screening.

The ease of use and analysis of blood biomarkers have established them as a low-cost standard method of modern medicine for the diagnosis and monitoring of a wide gamut of disease processes. The development of blood-based biomarkers of AD would represent a major advance in the field and the past several decades has seen a number of analytes being tested for this purpose [7]. However, there are several challenges in the development of blood biomarkers for central nervous system (CNS) disorders. First, the target proteins tend to be of

much lower concentration in blood relative to CSF and that an analyte expressed peripherally may reflect systemic rather than CNS changes [8]. Second, the target proteins exist within a matrix of other proteins which are of higher concentration in orders of magnitude, (e.g. albumin and immunoglobulins) making the investigation of lower abundant proteins extremely challenging [7, 9]. Third, the target analyte may undergo proteolytic degradation by proteases in plasma and it may also be metabolised through the liver or excreted by the renal system [10, 11]. A final consideration is that blood may contain host antibodies against the antibodies of the assay which interfere with the reliability of the test [12]. Because of this, there is an increasing number of studies attempting to identify biomarkers in neuronally derived exosomes isolated from blood as exosomes can protect their contents from degradation [13, 14]. Nevertheless, the measurement of targeted biomarkers in both blood and exosomes requires high sensitivity of the analytical platform.

The recent advent of ultrasensitive measurement techniques such as the immunomagnetic reduction (IMR) and single-molecule array (Simoa) methods, has generated new enthusiasm in the blood biomarker field. Despite these advances, individual studies of blood biomarker validity show great variability. This meta-analysis aims to systematically examine the level of evidence supporting the use of individual blood biomarkers as diagnostic tools to differentiate AD patients from healthy subjects. Here, we focused on analytes relating to the three key AD biomarker (ATN) in blood as well as in neuronally derived exosomes including: amyloid ($A\beta_{40}$, $A\beta_{42}$ and $A\beta_{42/40}$ ratio), tangle pathology (p-tau181) and neurodegeneration (total tau and neurofilament light, abbreviated as t-tau and NfL respectively). We also compared the

performance across different platform including traditional methods such as ELISA and new generation analytical techniques such as IMR and Simoa.

Methods

Literature search

This meta-analysis was conducted according to the PRISMA guidelines [15]. For the meta-analysis of blood biomarkers, the databases PubMed and Web of Science were searched for studies published in English from all years of publication until April 2020 with a combination of the search term (Alzheimer disease OR Alzheimer's disease) AND (biomarker* OR biological marker*) AND (plasma OR serum OR blood) and search terms specific to each biomarker, e.g., AND ($A\beta_{42}$ OR $A\beta_{-42}$ OR Abeta42 OR Abeta42 OR Abeta-42 OR A^{*42} OR $A\beta$). All search terms are included in Appendix A.

For the meta-analysis of biomarkers in extracellular vesicles, we identified references from a recent systematic review for which a comprehensive search had been undertaken [16].

Additional references from the databases PubMed and Web of Science were identified with the search term “Alzheimer’s disease AND extracellular vesicles”. Since Badwhar and Haqqani had searched for studies published until October 2019, we searched for studies which had been published after that.

Inclusion and exclusion criteria

For inclusion, studies had to:

- 1) Report data for one of the following blood biomarkers relevant to AD pathology: $A\beta_{40}$, $A\beta_{42}$, $A\beta_{42/40}$ ratio, t-tau, p-tau181, neurofilament light (NfL).
- 2) Include measurements of the respective biomarker

- a. in blood plasma or serum or extracellular vesicles extracted from plasma or serum in
 - b. a group of patients with Alzheimer's disease and a healthy control group, each with at least 10 individuals
 - c. using a quantitative method to assess biomarker concentrations (such as ELISA, IMR, Simoa).
- 3) Report mean and standard deviation or standard errors of these measurements.
 - 4) Report the diagnostic criteria used to diagnose AD patients.

Studies fulfilling these criteria were excluded if

- 1) the control group contained participants with inflammatory, neurological or psychiatric diagnoses that might affect biomarker concentrations
- 2) The study reported biomarker data from cellular blood fractions other than extracellular vesicles.

When two studies reported data from the same sample, only data from the more comprehensive study were included to avoid duplication. If studies reported that they had used a cognitive healthy control group (defined as healthy or cognitively normal or cognitively unimpaired or non-demented) but did not give further information, it was deemed eligible. If all criteria were fulfilled, but means and standard deviations or standard errors were not given, the authors of the paper were contacted and kindly requested to provide the respective data.

Study selection

The search for studies on biomarkers in blood yielded 2290 studies. After removal of duplicates, 1148 studies remained which were screened for eligibility by reading titles and abstracts. In addition, relevant meta-analyses and reviews were identified and screened for additional references, yielding 58 potentially eligible studies. Out of those 1206 studies, 933 were excluded due to not meeting inclusion criteria. Of the remaining 273 studies, 83 studies were found to be eligible for the present meta-analysis after a careful assessment of accordance with the in- and exclusion criteria (see Figure 1A).

For extracellular vesicles biomarkers, 8 and 61 studies were identified from a recent review [16] and database respectively. After removal of duplicates and criteria check, a total number of 9 studies were included in the final meta-analysis (Figure 1B).

Coding of variables

Data were extracted using a predefined coding scheme. First, general information about the study such as the authors, year of publication and the country in which the study was conducted were coded. Sample sizes of AD and control group as well as the mean (M) and standard deviation (SD) of plasma or serum levels of the respective biomarker were extracted for all included studies. If only standard errors (SE) were reported, SD was calculated using the formula $SD = \sqrt{n} \cdot SE$. In addition, M and SD of participant age were coded for both groups, again applying the abovementioned formula whenever necessary. When only the range of participant age was given, the range rule of thumb ($SD = (max - min)/4$) was applied to obtain standard deviations. Furthermore, we coded whether participants had undergone

systematic psychiatric and physical health evaluation at study baseline, and if those with major comorbidities were excluded.

Meta-analytic strategy

All analyses were carried out using the R package *metafor* by Viechtbauer (2010) [22]. As an effect size, the ratio of the mean biomarker concentration in the AD vs. control group was calculated for each study, and transformed by calculating its natural logarithm

$$\ln\left(\frac{M_{AD}}{M_{control}}\right)$$

The logarithm was calculated to obtain a symmetric measure where values below 0 (corresponding to a ratio below 1) indicate that the mean biomarker concentration was lower in the AD group and values above 0 (corresponding to a ratio above 1) indicate that mean biomarker concentration was lower in the control group. A measure based on the ratio of means was chosen since biomarker concentrations vary across laboratories and assays.

The variance of the log-ratio was estimated using the delta method [23]:

$$Var\left[\ln\left(\frac{M_{AD}}{M_{control}}\right)\right] = \frac{1}{n_{AD}}\left(\frac{SD_{AD}}{M_{AD}}\right)^2 + \frac{1}{n_{control}}\left(\frac{SD_{control}}{M_{control}}\right)^2$$

Random-effect models using the REML method [17] were calculated separately for each biomarker. Random-effect models build on the assumption that the true effect size varies across studies according to a normal distribution with mean μ and variance τ^2 (heterogeneity). This means that each study has its own true effect size θ_i (with i being the study subscript). Estimated

effect sizes y_i are assumed to be normally distributed with mean θ_i and variance v_i , which is the study-specific sampling variance that arises due to measurement error. Whenever an analysis contained studies in which biomarker concentration was measured in more than one study group (e.g., if a study included two pairs of AD and control group), dependencies between these groups were taken into account by calculating a mixed-effect model with groups nested in studies [18].

For mixed-effects models, τ can be calculated using the formula: $\tau = \sqrt{\tau_{between}^2 + \tau_{within}^2}$,

where $\tau_{between}^2$ is the variance of the effect size estimate between studies, while τ_{within}^2 is the variance of the estimate between samples within studies. Heterogeneity was assessed by a Q -test [19, 20]. Furthermore, prediction intervals were calculated. Prediction intervals must be distinguished from confidence intervals: The former are based mainly on the variance of true effect sizes across studies, while the latter are based exclusively on the standard error of the overall effect size estimate. Significance of the estimated overall effect size was assessed using confidence intervals, while heterogeneity of the effect sizes across studies was examined using prediction intervals. We decided to calculate random-effect models instead of fixed-effect models even when the Q -test indicated homogeneity because of the methodological variability of the studies included.

Subgroup analyses for the most common assay methods (ELISA, IMR and Simoa) were conducted. Also, subgroup analyses were carried out for studies in which the mean ages of the AD and control group did not differ significantly (according to a two-sided Welch two-sample t -test at an alpha level of 5 %) and for studies in which control subjects were excluded if they were found to have significant relevant comorbidities (as described above).

Publication bias was investigated using funnel plots, the trim and fill method and the Egger test [19]. Since neither of these methods is implemented for mixed-effect models in R, we decided to base all analyses of publication bias on random-effect models, which were estimated for the analysis of publication bias if they had not been estimated for meta-analytical comparisons.

The alpha level for all tests was set to 5 % for all tests reported below, and all confidence and prediction intervals reported are 95 % intervals, except it is otherwise specified.

Results

Description of studies

A total of 82 studies, published between 1996 and 2020, were included in the meta-analysis of biomarkers in blood [21-103]. Twenty three percent ($n = 21$) of the studies were conducted in the USA. Nine studies, published between 2015 and 2020, were included in the meta-analysis of biomarkers in extracellular vesicles [13, 72, 98, 104-109]. Five studies were conducted in the USA, 3 studies were conducted in China, and one study was conducted in South Korea. More comprehensive descriptions of the samples will be given in the sections below.

Meta-analyses of blood biomarkers

Results of overall meta-analytical comparisons for all blood biomarkers as well as subgroup analyses on assay method are displayed in Table 1.

Amyloid

For $A\beta_{40}$ the overall results were based on a total number of 3092 subjects in the AD group and 5219 subjects in the control group. An overall mean ratio of 1.03 was estimated. Since the CI contained the neutral value 1, $A\beta_{40}$ levels were not significantly different in AD vs. control subjects. From studies using ELISA, a significantly higher overall mean ratio of 1.10 was obtained (CI = [1.03, 1.17]), indicating $A\beta_{40}$ levels were higher in AD than in control subjects. In contrast, results for studies using IMR indicated lower $A\beta_{40}$ levels in AD patients relative to

controls (ratio = 0.77, CI = [0.68, 0.87]). Only one study was based on Simoa, and indicated no difference in $A\beta_{40}$ levels between AD and control subjects (ratio = 1.17, CI = [0.90, 1.52]). Large heterogeneity, as indicated by a significant Q -test ($p_Q < .05$), was found in both the overall analysis as well as in subgroup analyses of studies using ELISA or IMR, which was also reflected in large prediction intervals (see Table 1). Forest plots for the analyses described above are displayed in Figure 2.

Overall results for $A\beta_{42}$ (3513 AD and 5642 controls), indicated no significant difference between AD and control subjects (ratio = 1.01, CI = [0.94, 1.08]). The results did not reach significant when restricting the analysis to studies using ELISA (ratio = 0.98, CI = [0.89, 1.08]) or Simoa (ratio = 0.90, CI = [0.78, 1.05]). In comparison, $A\beta_{42}$ was significantly higher in AD when restricting the analysis to studies using IMR (ratio = 1.15, CI = [1.07, 1.24]). Significant heterogeneity was found for both the overall analysis and the subgroup analyses, with all prediction intervals containing the neutral value of 1 (Table 1). Forest plots for $A\beta_{42}$ are displayed in Figure 3.

For $A\beta_{42/40}$, no significant difference was observed for the overall analysis between AD ($n=1818$) and control ($n=4023$) (ratio = 1.12, CI = [0.97, 1.30]) as well as in subgroup analysis of studies using ELISA (ratio = 0.98, CI = [0.88, 1.09]). In contrast, $A\beta_{42/40}$ levels were significantly higher in AD in studies using IMR (ratio = 1.88, CI = [1.37, 2.58]). For the one Simoa-based study, no significant difference in $A\beta_{42/40}$ levels was found (ratio = 0.72, CI = [0.32, 1.59]).

Heterogeneity was significant for the overall analysis and the subgroup analyses of studies using ELISA and IMR, with very large heterogeneity when all studies were included (prediction

interval [0.54, 2.23]) and when only IMR studies were included (prediction interval [0.88, 4.05]).

Forest plots for $A\beta_{42/40}$ are displayed in Figure 4.

Tangle pathology

P-tau181 levels were significantly higher in AD (n=783) relative to control (n=1143) subjects (ratio = 1.75, CI = [1.43, 2.14]) in the overall analysis. No significance was found in subgroup analyses (ELISA: ratio = 1.43, CI = [0.94, 2.19]; IMR: ratio = 1.47, CI = [0.89, 2.44]). However, p-tau181 concentration was significantly higher in AD patients in studies using Simoa (ratio= 2.26, CI = [1.56, 3.28]). Heterogeneity was significant for all analyses and large prediction intervals were observed (e.g., the overall prediction interval was [0.81, 3.76]; see Table 1). Forest plots for p-tau181 are displayed in Figure 5.

Neurodegeneration

NfL levels were significantly higher in AD patients (n=1249) relative to controls (n=1585) (ratio = 1.65, CI = [1.46, 1.85]). Similar results were obtained for studies using Simoa (ratio = 1.58, CI = [1.37, 1.82]) and ELISA (ratio = 1.83, CI = [1.62, 2.06]). Significant heterogeneity was found for the overall analysis and the subgroup analysis of studies using Simoa but not for ELISA. Lower bounds of the prediction intervals were above 1 for all NfL analyses (Table 1). Forest plots for NfL are displayed in Figure 6.

T-tau levels were also significantly higher in AD (n =1591) relative to control subjects (n = 2454) (ratio = 1.52, CI = [1.25, 1.84]). Similar results was also observed in subgroup analyses using IMR (ratio = 2.29, CI = [1.74, 3.01]) and Simoa (ratio = 1.28, CI = [1.18, 1.38]) although the overall mean ratio was markedly higher when using IMR. In contrast, no significant difference was

observed for studies using ELISA (ratio = 1.10, CI = [0.81, 1.50]). Heterogeneity was significant for the overall analysis as well as the subgroup analysis of ELISA and IMR studies. Only for IMR studies, the lower bound of the prediction interval was above 1. No significant heterogeneity was found for Simoa studies, which was reflected in a relatively narrow prediction interval (Table 1). Forest plots for t-tau are displayed in Figure 7.

Subgroup analyses: Age differences, medical history of control group subjects

For all biomarkers, we performed two additional analyses. First, we only included the studies in which mean ages were not significantly different between AD and control group. As shown in Table 2, the levels of NfL, t-tau, and p-tau181 were significantly higher in AD relative to controls. However, heterogeneity was significant for all biomarkers. Forest plots of these subgroup analyses are included in Appendix B. Second, we only included the studies in which control group participants had undergone systematic psychiatric and physical health evaluation at study baseline. Results showed that $A\beta_{42}$, $A\beta_{42/40}$, NfL and p-tau181 were significantly higher in AD subjects (Table 3, Appendix B). Heterogeneity was large for all biomarkers except NfL. It should be noted that some of the analyses were based on a small number of studies and therefore have to be interpreted with caution.

Meta-analyses of biomarkers in extracellular vesicles

The studies which had measured biomarkers in extracellular vesicles only contained data for $A\beta_{42}$, NfL, t-tau, and p-tau181. As shown in Table 4, all biomarkers showed significantly higher levels in AD relative to control subjects. Heterogeneity was significant for all analyses, with very large prediction intervals especially for $A\beta_{42}$ and p-tau181. NfL was only measured in one study and the ratio was 1.31 (CI = [1.19, 1.46]). For forest plots, please refer to Appendix C. However, it should be noted that only a small number of studies were available for such analyses and thus, these results have to be interpreted carefully.

Publication Bias

Publication bias was assessed separately for each meta-analytical comparison.

For blood biomarkers, the Egger's test was significant for $A\beta_{40}$ ($p = .003$), and p-tau181 ($p = .002$), indicating that publication bias was present for these biomarkers. The test did not reveal signs of publication bias for the other biomarkers ($A\beta_{42}$: $p = .802$; $A\beta_{42/40}$: $p = .606$; NfL: $p = .090$, t-tau: $p = .217$). Note that the results for NfL were based on a small number of studies, for which the Egger's test is not well suited. Thus, trim and fill analyses were applied in addition. The respective funnel plots are included in Appendix D. The trim and fill method suggested publication bias for $A\beta_{40}$, $A\beta_{42/40}$, NfL, and p-tau181. For $A\beta_{40}$, the trim and fill method added 11 studies, which decreased the estimate from 1.03 to 0.94, but did not affect significance (CI = [0.88, 1.01]). For $A\beta_{42/40}$, three studies were imputed, resulting in an increase of the overall mean ratio to 1.19 (CI = [1.03, 1.38]). For NfL, two studies were added, which decreased the

estimate to 1.58 (CI = [1.40, 1.78]). Finally, for p-tau181, two studies were added and the overall mean ratio decreased to 1.69 (CI = [1.39, 2.05]).

Since the number of studies included in the meta-analyses of biomarkers in extracellular vesicles was small, we used only the trim and fill method to investigate publication bias for these analyses. The trim and fill method suggested publication bias only for t-tau. Adding one study decreased the overall mean ratio to 1.50, but did not affect significance (CI = [1.22, 1.84]).

Funnel plots are included in Appendix E.

Discussion

In this meta-analytic study we sought to evaluate the evidence for the diagnostic value of blood-based biomarkers for AD and the extent to which it is affected by choice of analytical platforms and study design factors. We found that overall there was evidence for improved diagnostic performance of amyloid, tau and neurodegeneration blood biomarkers through novel analytical platforms. Also, there was no evidence for stringent inclusion criteria (age matching, systematic exclusion of neurological and psychiatric diseases in controls) affecting the results.

Plasma A β

We found that the strongest separation of AD and controls in terms of A β came from using IMR based approaches to determine A β_{42} /A β_{40} ratio. In contrast, our combined result across studies for A β_{42} and A β_{40} alone did not demonstrate a significant difference between AD patients and controls. The finding is consistent with a previous meta-analysis demonstrating large heterogeneity and overall negative results of the published literature [110]. In our analysis the A β_{42} statistically non-significant result appears to have been driven by ELISA- and Simoa-based experiments as IMR studies demonstrated a significantly higher level in patients. This result may be due to the known limitations of ELISA in detecting A β levels in plasma which has been shown to lose its sensitivity for detecting narrow differences between biological samples [29]. ELISA performance in detecting plasma A β is also further affected by the high affinity of albumin and immunoglobulins in plasma to bind which are known as A β -binding proteins and thus that can interfere with accurate detection of A β [29]. Endogenous immunoglobulins and

autoantibodies can also interfere with performance of ELISAs [30]. This may explain why studies using ELISA do not generally replicate the findings of CSF; that IMR-based studies show an increase in A β 42 is intriguing given the consistently found reduction of CSF A β 42 and A β 42/40 ratio in AD [8].

Interestingly for A β 40 we found significant but contradictory results for ELISA and IMR (higher and lower levels in patients respectively). Others had found no significant change of A β 40 in CSF as well as previous blood-based biomarker meta-analyses. The lack of agreement between ELISA and IMR in our study may similarly reflect a lack of significant change in this biomarker. The use of A β isoform ratios (A β 42/40 and A β 42/38) has been motivated by the observation that the shorter isoforms are less prone to plaque aggregation relative to A β 42 and therefore their fluid levels reflect better the rate of A β production. Methodologically they can serve as internal controls to harmonise the large variations in absolute A β 42 concentrations in individual patients. The clinical relevance of the A β 42/A β 40 ratio has been demonstrated by studies demonstrating its closer relationship with cerebral amyloid burden [111, 112] as well as gait disturbance in AD [113]. In addition, studies in CSF have pointed to a role of the ratio in differentiating AD from other dementias [114] although data supporting this utility in plasma is currently lacking.

Tangle pathology

The evidence supporting the use of the best validated plasma biomarker of tangle pathology, p-tau181, is growing. Our overall analysis showed significantly increased levels of the analyte in AD patients relative to controls with significant results obtained by Simoa-based studies. These results build on recent data demonstrating the utility of this biomarker in differentiating AD

from non-AD neurodegenerative processes [79, 95]. In addition, the first study demonstrated that plasma and CSF ptau-181 are correlated and that plasma ptau-181 levels track with disease progression starting from preclinical AD [95]. The report by Thijssen et al. in turn provided early data on the correlation of plasma ptau-181 with both amyloid and tau burden on PET [79].

While our meta-analysis results support the use of ptau-181 for screening and diagnosing AD, it may be that tau proteins phosphorylated at other sites than 181 prove to be higher yield and replace it in future. For example, a study comparing p-tau181 and ptau-217 derived from CSF showed that ptau-217 outperforms ptau-181 in its sensitivity and specificity for both AD patients as well as cognitively unimpaired amyloid-positive individuals [115]. Another recent report showed that CSF ptau-217 correlates stronger with tau PET, CSF and PET amyloid and differentiates more accurately AD from non-AD dementias [116]. Despite these promising results, work to validate tests to detect ptau-217 in plasma continues

(<https://www.alzforum.org/news/conference-coverage/blood-tests-phospho-tau-av42-track-brain-amyloid>) and in the meantime ptau-181 remains the more extensively validated tangle pathology analyte in both CSF and plasma.

Neurodegeneration

We analysed the evidence for two analytes relevant to neurodegeneration: total tau and NfL. While overall analysis showed higher total tau in AD versus controls, these results were driven by significant effects in IMR and Simoa but not ELISA. The results for IMR-based studies were also notable however for their high heterogeneity which potentially limits the usefulness of

plasma total tau. In contrast, the Simoa-based studies had low heterogeneity thus pointing to their likely higher utility relative to IMR in this context. NfL appeared to be consistently different between groups across platforms, including ELISA. The NfL results are consistent with a trend for rapid expansion in the use of this biomarker across neurodegenerative disorders with evidence for increases in patients with amyotrophic lateral sclerosis, multiple sclerosis to the extent that NfL is now a recognised outcome measure in therapeutic trials [117]. In Alzheimer's disease NfL has been shown to be raised at the stage of preclinical AD (i.e. amyloid-positive individuals) as well as Mild Cognitive Impairment among carriers of autosomally dominant AD mutations and to correlate with imaging markers of neurodegeneration as well as cognitive impairment [118]. The relevance of NfL to cognitive decline in the earliest stages of disease is highlighted by a study demonstrating an association between its levels and cortical hypometabolism in areas vulnerable to AD [119] as well as a strong relationship with longitudinal changes to neuroimaging markers of neurodegeneration and cognition in healthy aging adults [120]. Overall, the less variable results obtained with NfL relative to total tau as well as the mounting evidence for its usefulness in tracking AD disease progression as well as other neurodegenerative disorders argues strongly for its use as a biomarker of neurodegeneration.

In addition to the main analyses we explored the effect to which design factors affect the strength of the results. We did not find evidence that strict matching of controls by age impacts the direction or magnitude of the results. However, across the majority of biomarkers (amyloid, NfL and p-tau181), the lack of stringent control of somatic and psychiatric morbidity among the control groups associated with weaker differentiation of AD from controls. This could be

interpreted in the light of the known link between significant physical health, cardiovascular in particular, morbidity with AD risk [121]. In addition, mental health morbidity (e.g. depression) can also be part of the dementia prodrome [121]. These results therefore suggest that the lack of exclusion criteria relevant to morbidity known to associate with AD may lead to the inclusion of significant preclinical AD pathology with resulting attenuation in biomarker sensitivity when differentiation between AD and controls.

Furthermore, enriching the analytical samples by focusing on extracellular vesicles appeared to offer early evidence for stronger associations for the main biomarkers of interest ($A\beta_{42}$, p-tau181, total tau, NfL). Replication studies are required to determine the usefulness of this analytical approach to determine whether it should be preferred to testing plasma directly.

Limitations

Several limitations to this meta-analysis exist. Firstly, it is inherent in all systematic searches that despite every attempt for exhaustiveness, some eligible studies may have been missed. In addition, some studies reported in a format unsuitable for analyses (e.g. median and range instead of mean and SD) which points to the need to establish a common way to report methods and results in order to enhance standardisation across the field. For plasma NfL and some subgroup analyses, the number of included studies was small which warrants caution in interpreting the results. It is notable that our results showed large unexplained heterogeneity for a number of primary analyses as well as secondary analyses which necessitates further work to identify the source of this variability and implement measures to limit it in future studies. While we found promising data regarding exosome enrichment, significant work is needed to

improve standardisation between methods. Also a critical limitation is that it is yet unclear what proportion of exosomes are CNS- versus peripherally derived. Finally, our study only focused on the differentiation of cases from controls in developed Alzheimer's dementia. This associates with at least two issues. Firstly, patients with syndrome-based AD diagnosis are known not to have an underlying AD pathology in up to a third of cases while conversely controls may have preclinical AD. Secondly, by focusing on clinical cases we cannot draw conclusions about any changes of the various biomarkers specific to the preclinical and prodromal stages of the disease which may have implications for their utility as screening tools.

Conclusions

We demonstrate that the new analytical assays of IMR and Simoa have led to a significant improvement in the reliability of detection of key AD ATN analytes in blood. Our evidence supports the use of A β _{42/40} using the IMR platform, p-tau181 (the best assay for it remains to be clarified) as well as total tau and NfL using the IMR and Simoa platforms. While further work is required to validate the use of blood biomarkers as screening tools to evidence ATN status in clinical settings, the current meta-analysis points to this being a realistic aim.

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Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to report

References

- [1] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (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.
- [2] Barthel H, Sabri O (2017) Clinical Use and Utility of Amyloid Imaging. *J Nucl Med* **58**, 1711-1717.
- [3] Selvackadunco S, Langford K, Shah Z, Hurley S, Bodi I, King A, Aarsland D, Troakes C, Al-Sarraj S (2019) Comparison of clinical and neuropathological diagnoses of neurodegenerative diseases in two centres from the Brains for Dementia Research (BDR) cohort. *J Neural Transm (Vienna)* **126**, 327-337.
- [4] Beach TG, Monsell SE, Phillips LE, Kukull W (2012) Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J Neuropathol Exp Neurol* **71**, 266-273.
- [5] Sperling RA, Jack CR, Jr., Aisen PS (2011) Testing the right target and right drug at the right stage. *Sci Transl Med* **3**, 111cm133.
- [6] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R, Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.
- [7] Blennow K (2017) A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood. *Neurol Ther* **6**, 15-24.
- [8] Zetterberg H (2019) Blood-based biomarkers for Alzheimer's disease-An update. *J Neurosci Methods* **319**, 2-6.
- [9] Apweiler R, Aslanidis C, Deufel T, Gerstner A, Hansen J, Hochstrasser D, Kellner R, Kubicek M, Lottspeich F, Maser E, Mewes HW, Meyer HE, Mullner S, Mutter W, Neumaier M, Nollau P, Nothwang HG, Ponten F, Radbruch A, Reinert K, Rothe G, Stockinger H, Tarnok A, Taussig MJ, Thiel A, Thiery J, Ueffing M, Valet G, Vandekerckhove J, Verhuven W, Wagener C, Wagner O, Schmitz G (2009) Approaching clinical proteomics: current state and future fields of application in fluid proteomics. *Clin Chem Lab Med* **47**, 724-744.
- [10] Zetterberg H, Blennow K (2018) From Cerebrospinal Fluid to Blood: The Third Wave of Fluid Biomarkers for Alzheimer's Disease. *J Alzheimers Dis* **64**, S271-S279.
- [11] Yoshimura T, Fujita K, Kawakami S, Takeda K, Chan S, Beligere G, Dowell B (2008) Stability of pro-gastrin-releasing peptide in serum versus plasma. *Tumour Biol* **29**, 224-230.
- [12] Bolstad N, Warren DJ, Nustad K (2013) Heterophilic antibody interference in immunometric assays. *Best Pract Res Clin Endocrinol Metab* **27**, 647-661.
- [13] Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL, Goetzl EJ (2015) Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement* **11**, 600-607 e601.
- [14] Ying LW, Bai DW, Ao Z, Xin XS, Ping ZJ (2019) Role of exosomes in central nervous system diseases. *Frontiers in molecular neuroscience* **12**, 240.
- [15] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* **339**, b2700.

- [16] Badhwar A, Haqqani AS (2020) Biomarker potential of brain-secreted extracellular vesicles in blood in Alzheimer's disease. *Alzheimers Dement (Amst)* **12**, e12001.
- [17] Sidik K, Jonkman JN (2007) A comparison of heterogeneity variance estimators in combining results of studies. *Stat Med* **26**, 1964-1981.
- [18] Van den Noortgate W, Lopez-Lopez JA, Marin-Martinez F, Sanchez-Meca J (2013) Three-level meta-analysis of dependent effect sizes. *Behav Res Methods* **45**, 576-594.
- [19] Egger M, Smith GD, Phillips AN (1997) Meta-analysis: principles and procedures. *BMJ* **315**, 1533-1537.
- [20] Song F, Sheldon TA, Sutton AJ, Abrams KR, Jones DR (2001) Methods for exploring heterogeneity in meta-analysis. *Eval Health Prof* **24**, 126-151.
- [21] Abdullah L, Paris D, Luis C, Quadros A, Parrish J, Valdes L, Keegan AP, Mathura V, Crawford F, Mullan M (2007) The influence of diagnosis, intra- and inter-person variability on serum and plasma Abeta levels. *Neurosci Lett* **428**, 53-58.
- [22] Ait-ghezala G, Abdullah L, Volmar CH, Paris D, Luis CA, Quadros A, Mouzon B, Mullan MA, Keegan AP, Parrish J, Crawford FC, Mathura VS, Mullan MJ (2008) Diagnostic utility of APOE, soluble CD40, CD40L, and Abeta1-40 levels in plasma in Alzheimer's disease. *Cytokine* **44**, 283-287.
- [23] Akatsu H, Ogawa N, Kanesaka T, Hori A, Yamamoto T, Matsukawa N, Michikawa M (2011) Higher activity of peripheral blood angiotensin-converting enzyme is associated with later-onset of Alzheimer's disease. *J Neurol Sci* **300**, 67-73.
- [24] Arvanitakis Z, Lucas JA, Younkin LH, Younkin SG, Graff-Radford NR (2002) Serum creatinine levels correlate with plasma amyloid Beta protein. *Alzheimer Dis Assoc Disord* **16**, 187-190.
- [25] Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobagyi T, Hoglund K, Aarsland D, Lovestone S, Scholl M, Blennow K, Zetterberg H, Hye A (2019) Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. *Acta Neuropathol Commun* **7**, 5.
- [26] Buerger K, Frisoni G, Uspenskaya O, Ewers M, Zetterberg H, Geroldi C, Binetti G, Johannsen P, Rossini PM, Wahlund LO, Vellas B, Blennow K, Hampel H (2009) Validation of Alzheimer's disease CSF and plasma biological markers: the multicentre reliability study of the pilot European Alzheimer's Disease Neuroimaging Initiative (E-ADNI). *Exp Gerontol* **44**, 579-585.
- [27] Chen TB, Lai YH, Ke TL, Chen JP, Lee YJ, Lin SY, Lin PC, Wang PN, Cheng IH (2019) Changes in Plasma Amyloid and Tau in a Longitudinal Study of Normal Aging, Mild Cognitive Impairment, and Alzheimer's Disease. *Dement Geriatr Cogn Disord* **48**, 180-195.
- [28] Chiu MJ, Chen TF, Hu CJ, Yan SH, Sun Y, Liu BH, Chang YT, Yang CC, Yang SY (2020) Nanoparticle-based immunomagnetic assay of plasma biomarkers for differentiating dementia and prodromal states of Alzheimer's disease - A cross-validation study. *Nanomedicine* **28**, 102182.
- [29] Chiu MJ, Chen YF, Chen TF, Yang SY, Yang FP, Tseng TW, Chieh JJ, Chen JC, Tzen KY, Hua MS, Horng HE (2014) Plasma tau as a window to the brain-negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease. *Hum Brain Mapp* **35**, 3132-3142.
- [30] Chiu MJ, Lue LF, Sabbagh MN, Chen TF, Chen HH, Yang SY (2019) Long-Term Storage Effects on Stability of Abeta1-40, Abeta1-42, and Total Tau Proteins in Human Plasma Samples Measured with Immunomagnetic Reduction Assays. *Dement Geriatr Cogn Dis Extra* **9**, 77-86.
- [31] Chiu MJ, Yang SY, Chen TF, Chieh JJ, Huang TZ, Yip PK, Yang HC, Cheng TW, Chen YF, Hua MS, Horng HE (2012) New assay for old markers-plasma beta amyloid of mild cognitive impairment and Alzheimer's disease. *Curr Alzheimer Res* **9**, 1142-1148.
- [32] Corzo L, Zas R, Rodriguez S, Fernandez-Novoa L, Cacabelos R (2007) Decreased levels of serum nitric oxide in different forms of dementia. *Neurosci Lett* **420**, 263-267.

- [33] de Almeida SM, Ribeiro CE, Rotta I, Letendre S, Potter M, Tang B, Batistela M, Vaida F, Ellis RJ, Group HIVNRC (2020) Blood amyloid-beta protein isoforms are affected by HIV-1 in a subtype-dependent pattern. *J Neurovirol* **26**, 3-13.
- [34] de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ, Wermeling P, Sedaghat S, Ikram MK, Waziry R, Koudstaal W, Klap J, Kostense S, Hofman A, Anderson R, Goudsmit J, Ikram MA (2020) Plasma tau, neurofilament light chain and amyloid-beta levels and risk of dementia; a population-based cohort study. *Brain* **143**, 1220-1232.
- [35] Fagan AM, Head D, Shah AR, Marcus D, Mintun M, Morris JC, Holtzman DM (2009) Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. *Ann Neurol* **65**, 176-183.
- [36] Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM (2007) Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* **64**, 343-349.
- [37] Fan LY, Tzen KY, Chen YF, Chen TF, Lai YM, Yen RF, Huang YY, Shiue CY, Yang SY, Chiu MJ (2018) The Relation Between Brain Amyloid Deposition, Cortical Atrophy, and Plasma Biomarkers in Amnesic Mild Cognitive Impairment and Alzheimer's Disease. *Front Aging Neurosci* **10**, 175.
- [38] Feinkohl I, Schipke CG, Kruppa J, Menne F, Winterer G, Pischon T, Peters O (2020) Plasma Amyloid Concentration in Alzheimer's Disease: Performance of a High-Throughput Amyloid Assay in Distinguishing Alzheimer's Disease Cases from Controls. *J Alzheimers Dis* **74**, 1285-1294.
- [39] Figurski MJ, Waligorska T, Toledo J, Vanderstichele H, Korecka M, Lee VM, Trojanowski JQ, Shaw LM, Alzheimer's Disease Neuroimaging I (2012) Improved protocol for measurement of plasma beta-amyloid in longitudinal evaluation of Alzheimer's Disease Neuroimaging Initiative study patients. *Alzheimers Dement* **8**, 250-260.
- [40] Fisar Z, Jirak R, Zverova M, Setnicka V, Habartova L, Hroudova J, Vanickova Z, Raboch J (2019) Plasma amyloid beta levels and platelet mitochondrial respiration in patients with Alzheimer's disease. *Clin Biochem* **72**, 71-80.
- [41] Fossati S, Ramos Cejudo J, Debure L, Pirraglia E, Sone JY, Li Y, Chen J, Butler T, Zetterberg H, Blennow K, de Leon MJ (2019) Plasma tau complements CSF tau and P-tau in the diagnosis of Alzheimer's disease. *Alzheimers Dement (Amst)* **11**, 483-492.
- [42] Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Growdon JH, Irizarry MC (2003) Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. *Arch Neurol* **60**, 958-964.
- [43] Giedraitis V, Sundelof J, Irizarry MC, Garevik N, Hyman BT, Wahlund LO, Ingelsson M, Lannfelt L (2007) The normal equilibrium between CSF and plasma amyloid beta levels is disrupted in Alzheimer's disease. *Neurosci Lett* **427**, 127-131.
- [44] Han Y, Jia J, Jia XF, Qin W, Wang S (2012) Combination of plasma biomarkers and clinical data for the detection of sporadic Alzheimer's disease. *Neurosci Lett* **516**, 232-236.
- [45] Head E, Doran E, Nistor M, Hill M, Schmitt FA, Haier RJ, Lott IT (2011) Plasma amyloid-beta as a function of age, level of intellectual disability, and presence of dementia in Down syndrome. *J Alzheimers Dis* **23**, 399-409.
- [46] Huang CW, Wang SJ, Wu SJ, Yang CC, Huang MW, Lin CH, Cheng IH (2013) Potential blood biomarker for disease severity in the Taiwanese population with Alzheimer's disease. *Am J Alzheimers Dis Other Dement* **28**, 75-83.
- [47] Jiao SS, Bu XL, Liu YH, Wang QH, Liu CH, Yao XQ, Zhou XF, Wang YJ (2015) Differential levels of p75NTR ectodomain in CSF and blood in patients with Alzheimer's disease: a novel diagnostic marker. *Transl Psychiatry* **5**, e650.
- [48] Kim K, Kim MJ, Kim DW, Kim SY, Park S, Park CB (2020) Clinically accurate diagnosis of Alzheimer's disease via multiplexed sensing of core biomarkers in human plasma. *Nat Commun* **11**, 119.

- [49] Konno T, Hata S, Hamada Y, Horikoshi-Sakuraba Y, Nakaya T, Saito Y, Yamamoto T, Yamamoto T, Maeda M, Ikeuchi T, Gandy S, Akatsu H, Suzuki T, Japanese Alzheimer's Disease Neuroimaging I (2011) Coordinated increase of gamma-secretase reaction products in the plasma of some female Japanese sporadic Alzheimer's disease patients: quantitative analysis of p3-Alcalpha with a new ELISA system. *Mol Neurodegener* **6**, 76.
- [50] Lewczuk P, Ermann N, Andreasson U, Schultheis C, Podhorna J, Spitzer P, Maler JM, Kornhuber J, Blennow K, Zetterberg H (2018) Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimers Res Ther* **10**, 71.
- [51] Lin YS, Lee WJ, Wang SJ, Fuh JL (2018) Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. *Sci Rep* **8**, 17368.
- [52] Liu S, Huang Z, Zhang L, Pan J, Lei Q, Meng Y, Li Z (2020) Plasma Neurofilament Light Chain May Be a Biomarker for the Inverse Association Between Cancers and Neurodegenerative Diseases. *Front Aging Neurosci* **12**, 10.
- [53] Lopez OL, Kuller LH, Mehta PD, Becker JT, Gach HM, Sweet RA, Chang YF, Tracy R, DeKosky ST (2008) Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. *Neurology* **70**, 1664-1671.
- [54] Lue LF, Sabbagh MN, Chiu MJ, Jing N, Snyder NL, Schmitz C, Guerra A, Belden CM, Chen TF, Yang CC, Yang SY, Walker DG, Chen K, Reiman EM (2017) Plasma Levels of Abeta42 and Tau Identified Probable Alzheimer's Dementia: Findings in Two Cohorts. *Front Aging Neurosci* **9**, 226.
- [55] Lui JK, Laws SM, Li QX, Villemagne VL, Ames D, Brown B, Bush AI, De Ruyck K, Dromey J, Ellis KA, Faux NG, Foster J, Fowler C, Gupta V, Hudson P, Laughton K, Masters CL, Pertile K, Rembach A, Rimajova M, Rodrigues M, Rowe CC, Rumble R, Szeoke C, Taddei K, Taddei T, Trounson B, Ward V, Martins RN, Group AR (2010) Plasma amyloid-beta as a biomarker in Alzheimer's disease: the AIBL study of aging. *J Alzheimers Dis* **20**, 1233-1242.
- [56] Matias-Guiu JA, Gomez-Pinedo U, Forero L, Pytel V, Cano F, Moreno-Ramos T, Cabrera-Martin MN, Matias-Guiu J, Gonzalez-Rosa JJ (2019) Plasma Neurofilament Light Chain in Primary Progressive Aphasia and Related Disorders: Clinical Significance and Metabolic Correlates. *J Alzheimers Dis* **72**, 773-782.
- [57] Matsubara E, Ghiso J, Frangione B, Amari M, Tomidokoro Y, Ikeda Y, Harigaya Y, Okamoto K, Shoji M (1999) Lipoprotein-free amyloidogenic peptides in plasma are elevated in patients with sporadic Alzheimer's disease and Down's syndrome. *Ann Neurol* **45**, 537-541.
- [58] Mattsson N, Andreasson U, Zetterberg H, Blennow K, Alzheimer's Disease Neuroimaging I (2017) Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol* **74**, 557-566.
- [59] Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, Palmqvist S, Baker D, Tan Hehir CA, Jeromin A, Hanlon D, Song L, Shaw LM, Trojanowski JQ, Weiner MW, Hansson O, Blennow K, Investigators A (2016) Plasma tau in Alzheimer disease. *Neurology* **87**, 1827-1835.
- [60] Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD (1999) Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. *Ann Neurol* **46**, 412-416.
- [61] Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, Airey DC, Knopman DS, Roberts RO, Machulda MM, Jack CR, Jr., Petersen RC, Dage JL (2018) Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* **14**, 989-997.
- [62] Palmqvist S, Janelidze S, Stomrud E, Zetterberg H, Karl J, Zink K, Bittner T, Mattsson N, Eichenlaub U, Blennow K, Hansson O (2019) Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related beta-Amyloid Status. *JAMA Neurol*.

- [63] Poljak A, Crawford JD, Smythe GA, Brodaty H, Slavin MJ, Kochan NA, Trollor JN, Wen W, Mather KA, Assareh AA, Ng PC, Sachdev PS (2016) The Relationship Between Plasma Abeta Levels, Cognitive Function and Brain Volumetrics: Sydney Memory and Ageing Study. *Curr Alzheimer Res* **13**, 243-255.
- [64] Rani P, Krishnan S, Rani Cathrine C (2017) Study on Analysis of Peripheral Biomarkers for Alzheimer's Disease Diagnosis. *Front Neurol* **8**, 328.
- [65] Richens JL, Vere KA, Light RA, Soria D, Garibaldi J, Smith AD, Warden D, Wilcock G, Bajaj N, Morgan K, O'Shea P (2014) Practical detection of a definitive biomarker panel for Alzheimer's disease; comparisons between matched plasma and cerebrospinal fluid. *Int J Mol Epidemiol Genet* **5**, 53-70.
- [66] Risacher SL, Fandos N, Romero J, Sherriff I, Pesini P, Saykin AJ, Apostolova LG (2019) Plasma amyloid beta levels are associated with cerebral amyloid and tau deposition. *Alzheimers Dement (Amst)* **11**, 510-519.
- [67] Roher AE, Esh CL, Kokjohn TA, Castano EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Dausgs ID, Kuo YM, Emmerling MR, Soares H, Quinn JF, Kaye J, Connor DJ, Silverberg NB, Adler CH, Seward JD, Beach TG, Sabbagh MN (2009) Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement* **5**, 18-29.
- [68] Ruiz A, Pesini P, Espinosa A, Perez-Grijalba V, Valero S, Sotolongo-Grau O, Alegret M, Monleon I, Lafuente A, Buendia M, Ibarria M, Ruiz S, Hernandez I, San Jose I, Tarraga L, Boada M, Sarasa M (2013) Blood amyloid beta levels in healthy, mild cognitive impairment and Alzheimer's disease individuals: replication of diastolic blood pressure correlations and analysis of critical covariates. *PLoS One* **8**, e81334.
- [69] Schupf N, Tang MX, Fukuyama H, Manly J, Andrews H, Mehta P, Ravetch J, Mayeux R (2008) Peripheral Abeta subspecies as risk biomarkers of Alzheimer's disease. *Proc Natl Acad Sci U S A* **105**, 14052-14057.
- [70] Seino Y, Nakamura T, Kawarabayashi T, Hirohata M, Narita S, Wakasaya Y, Kaito K, Ueda T, Harigaya Y, Shoji M (2019) Cerebrospinal Fluid and Plasma Biomarkers in Neurodegenerative Diseases. *J Alzheimers Dis* **68**, 395-404.
- [71] Shekhar S, Kumar R, Rai N, Kumar V, Singh K, Upadhyay AD, Tripathi M, Dwivedi S, Dey AB, Dey S (2016) Estimation of Tau and Phosphorylated Tau181 in Serum of Alzheimer's Disease and Mild Cognitive Impairment Patients. *PLoS One* **11**, e0159099.
- [72] Shi M, Kovac A, Korff A, Cook TJ, Ginghina C, Bullock KM, Yang L, Stewart T, Zheng D, Aro P, Atik A, Kerr KF, Zabetian CP, Peskind ER, Hu SC, Quinn JF, Galasko DR, Montine TJ, Banks WA, Zhang J (2016) CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. *Alzheimers Dement* **12**, 1125-1131.
- [73] Shin HS, Lee SK, Kim S, Kim HJ, Chae WS, Park SA (2016) The Correlation Study between Plasma Abeta Proteins and Cerebrospinal Fluid Alzheimer's Disease Biomarkers. *Dement Neurocogn Disord* **15**, 122-128.
- [74] Sobow T, Flirski M, Kloszewska I, Liberski PP (2005) Plasma levels of alpha beta peptides are altered in amnesic mild cognitive impairment but not in sporadic Alzheimer's disease. *Acta Neurobiol Exp (Wars)* **65**, 117-124.
- [75] Sparks DL, Kryscio RJ, Sabbagh MN, Ziolkowski C, Lin Y, Sparks LM, Liebsack C, Johnson-Traver S (2012) Tau is reduced in AD plasma and validation of employed ELISA methods. *Am J Neurodegener Dis* **1**, 99-106.
- [76] Startin CM, Ashton NJ, Hamburg S, Hithersay R, Wiseman FK, Mok KY, Hardy J, Lleo A, Lovestone S, Parnetti L, Zetterberg H, Hye A, LonDown SC, Strydom A (2019) Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease. *Alzheimers Res Ther* **11**, 26.

- [77] Sun HL, Li WW, Zhu C, Jin WS, Liu YH, Zeng F, Wang YJ, Bu XL (2018) The Correlations of Plasma and Cerebrospinal Fluid Amyloid-Beta Levels with Platelet Count in Patients with Alzheimer's Disease. *Biomed Res Int* **2018**, 7302045.
- [78] Tamaoka A, Fukushima T, Sawamura N, Ishikawa K, Oguni E, Komatsuzaki Y, Shoji S (1996) Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease. *J Neurol Sci* **141**, 65-68.
- [79] Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, Iaccarino L, Bourakova V, Cobigo Y, Heuer H, Spina S, VandeVrede L, Chai X, Proctor NK, Airey DC, Shcherbinin S, Duggan Evans C, Sims JR, Zetterberg H, Blennow K, Karydas AM, Teunissen CE, Kramer JH, Grinberg LT, Seeley WW, Rosen H, Boeve BF, Miller BL, Rabinovici GD, Dage JL, Rojas JC, Boxer AL, 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**, 387-397.
- [80] Tsai CL, Liang CS, Lee JT, Su MW, Lin CC, Chu HT, Tsai CK, Lin GY, Lin YK, Yang FC (2019) Associations between Plasma Biomarkers and Cognition in Patients with Alzheimer's Disease and Amnesic Mild Cognitive Impairment: A Cross-Sectional and Longitudinal Study. *J Clin Med* **8**.
- [81] Tzen KY, Yang SY, Chen TF, Cheng TW, Horng HE, Wen HP, Huang YY, Shiue CY, Chiu MJ (2014) Plasma Aβ but not tau is related to brain PiB retention in early Alzheimer's disease. *ACS Chem Neurosci* **5**, 830-836.
- [82] Tzikas S, Schlak D, Sopova K, Gatsiou A, Stakos D, Stamatelopoulos K, Stellos K, Laske C (2014) Increased myeloperoxidase plasma levels in patients with Alzheimer's disease. *J Alzheimers Dis* **39**, 557-564.
- [83] Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E (2000) Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. *Amyloid* **7**, 245-258.
- [84] Wang T, Xiao S, Liu Y, Lin Z, Su N, Li X, Li G, Zhang M, Fang Y (2014) The efficacy of plasma biomarkers in early diagnosis of Alzheimer's disease. *Int J Geriatr Psychiatry* **29**, 713-719.
- [85] Westwood S, Baird AL, Anand SN, Nevado-Holgado AJ, Kormilitzin A, Shi L, Hye A, Ashton NJ, Morgan AR, Bos I, Vos SJB, Baker S, Buckley NJ, Ten Kate M, Scheltens P, Teunissen CE, Vandenberghe R, Gabel S, Meersmans K, Engelborghs S, De Roeck EE, Sleegers K, Frisoni GB, Blin O, Richardson JC, Bordet R, Molinuevo JL, Rami L, Wallin A, Kettunen P, Tsolaki M, Verhey F, Lleo A, Sala I, Popp J, Peyratout G, Martinez-Lage P, Tainta M, Johannsen P, Freund-Levi Y, Frolich L, Dobricic V, Legido-Quigley C, Bertram L, Barkhof F, Zetterberg H, Morgan BP, Streffer J, Visser PJ, Lovestone S (2020) Validation of Plasma Proteomic Biomarkers Relating to Brain Amyloid Burden in the EMIF-Alzheimer's Disease Multimodal Biomarker Discovery Cohort. *J Alzheimers Dis* **74**, 213-225.
- [86] Wongchitrat P, Pakpian N, Kitidee K, Phopin K, Dharmasaroja PA, Govitrapong P (2019) Alterations in the Expression of Amyloid Precursor Protein Cleaving Enzymes mRNA in Alzheimer Peripheral Blood. *Curr Alzheimer Res* **16**, 29-38.
- [87] Xu W, Kawarabayashi T, Matsubara E, Deguchi K, Murakami T, Harigaya Y, Ikeda M, Amari M, Kuwano R, Abe K, Shoji M (2008) Plasma antibodies to Aβ40 and Aβ42 in patients with Alzheimer's disease and normal controls. *Brain Res* **1219**, 169-179.
- [88] Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, Yang SY (2018) Assay of Plasma Phosphorylated Tau Protein (Threonine 181) and Total Tau Protein in Early-Stage Alzheimer's Disease. *J Alzheimers Dis* **61**, 1323-1332.
- [89] Yang SY, Chiu MJ, Chen TF, Lin CH, Jeng JS, Tang SC, Lee YF, Yang CC, Liu BH, Chen HH, Wu CC (2017) Analytical performance of reagent for assaying tau protein in human plasma and feasibility study screening neurodegenerative diseases. *Sci Rep* **7**, 9304.

- [90] Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, Hansson O (2013) Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther* **5**, 9.
- [91] Zhang J, Jia J, Qin W, Wang S (2013) Combination of plasma tumor necrosis factor receptors signaling proteins, beta-amyloid and apolipoprotein E for the detection of Alzheimer's disease. *Neurosci Lett* **541**, 99-104.
- [92] Andersson E, Janelidze S, Lampinen B, Nilsson M, Leuzy A, Stomrud E, Blennow K, Zetterberg H, Hansson O (2020) Blood and cerebrospinal fluid neurofilament light differentially detect neurodegeneration in early Alzheimer's disease. *Neurobiol Aging* **95**, 143-153.
- [93] Benussi A, Karikari TK, Ashton N, Gazzina S, Premi E, Benussi L, Ghidoni R, Rodriguez JL, Emersic A, Simren J, Binetti G, Fostinelli S, Giunta M, Gasparotti R, Zetterberg H, Blennow K, Borroni B (2020) Diagnostic and prognostic value of serum NfL and p-Tau181 in frontotemporal lobar degeneration. *J Neurol Neurosurg Psychiatry* **91**, 960-967.
- [94] Callahan CM, Apostolova LG, Gao S, Risacher SL, Case J, Saykin AJ, Lane KA, Swinford CG, Yoder MC (2020) Novel Markers of Angiogenesis in the Setting of Cognitive Impairment and Dementia. *J Alzheimers Dis* **75**, 959-969.
- [95] Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, Chai X, Proctor NK, Eichenlaub U, Zetterberg H, Blennow K, Reiman EM, Stomrud E, Dage JL, Hansson O (2020) Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* **26**, 379-386.
- [96] Jiao F, Yi F, Wang Y, Zhang S, Guo Y, Du W, Gao Y, Ren J, Zhang H, Liu L, Song H, Wang L (2020) The Validation of Multifactor Model of Plasma Abeta 42 and Total-Tau in Combination With MoCA for Diagnosing Probable Alzheimer Disease. *Front Aging Neurosci* **12**, 212.
- [97] Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, Chamoun M, Savard M, Kang MS, Theriault J, Scholl M, Massarweh G, Soucy JP, Hoglund K, Brinkmalm G, Mattsson N, Palmqvist S, Gauthier S, Stomrud E, Zetterberg H, Hansson O, Rosa-Neto P, Blennow K (2020) Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* **19**, 422-433.
- [98] Nam E, Lee YB, Moon C, Chang KA (2020) Serum Tau Proteins as Potential Biomarkers for the Assessment of Alzheimer's Disease Progression. *Int J Mol Sci* **21**.
- [99] Nyberg L, Lundquist A, Nordin Adolfsson A, Andersson M, Zetterberg H, Blennow K, Adolfsson R (2020) Elevated plasma neurofilament light in aging reflects brain white-matter alterations but does not predict cognitive decline or Alzheimer's disease. *Alzheimers Dement (Amst)* **12**, e12050.
- [100] Sugarman MA, Zetterberg H, Blennow K, Tripodis Y, McKee AC, Stein TD, Martin B, Palmisano JN, Steinberg EG, Simkin I, Budson AE, Killiany R, O'Connor MK, Au R, Qiu WWQ, Goldstein LE, Kowall NW, Mez J, Stern RA, Alosco ML (2020) A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging* **94**, 60-70.
- [101] Tatebe H, Kasai T, Ohmichi T, Kishi Y, Kakeya T, Waragai M, Kondo M, Allsop D, Tokuda T (2017) Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener* **12**, 63.
- [102] Tsai CL, Liang CS, Yang CP, Lee JT, Ho TH, Su MW, Lin GY, Lin YK, Chu HT, Hsu YW, Yang FC (2020) Indicators of rapid cognitive decline in amnesic mild cognitive impairment: The role of plasma biomarkers using magnetically labeled immunoassays. *J Psychiatr Res* **129**, 66-72.
- [103] Lantero Rodriguez J, Karikari TK, Suarez-Calvet M, Troakes C, King A, Emersic A, Aarsland D, Hye A, Zetterberg H, Blennow K, Ashton NJ (2020) Plasma p-tau181 accurately predicts Alzheimer's

- disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol* **140**, 267-278.
- [104] Goetzl EJ, Mustapic M, Kapogiannis D, Eitan E, Lobach IV, Goetzl L, Schwartz JB, Miller BL (2016) Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. *FASEB J* **30**, 3853-3859.
 - [105] Guix FX, Corbett GT, Cha DJ, Mustapic M, Liu W, Mengel D, Chen Z, Aikawa E, Young-Pearse T, Kapogiannis D, Selkoe DJ, Walsh DM (2018) Detection of Aggregation-Competent Tau in Neuron-Derived Extracellular Vesicles. *Int J Mol Sci* **19**.
 - [106] Jia L, Qiu Q, Zhang H, Chu L, Du Y, Zhang J, Zhou C, Liang F, Shi S, Wang S, Qin W, Wang Q, Li F, Wang Q, Li Y, Shen L, Wei Y, Jia J (2019) Concordance between the assessment of Abeta42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid. *Alzheimers Dement* **15**, 1071-1080.
 - [107] Li F, Xie XY, Sui XF, Wang P, Chen Z, Zhang JB (2020) Profile of Pathogenic Proteins and MicroRNAs in Plasma-derived Extracellular Vesicles in Alzheimer's Disease: A Pilot Study. *Neuroscience* **432**, 240-246.
 - [108] Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, Galasko D, Masliah E, Rissman RA (2016) Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement (Amst)* **3**, 63-72.
 - [109] Gu D, Liu F, Meng M, Zhang L, Gordon ML, Wang Y, Cai L, Zhang N (2020) Elevated matrix metalloproteinase-9 levels in neuronal extracellular vesicles in Alzheimer's disease. *Ann Clin Transl Neurol*.
 - [110] Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, Holtta M, Rosen C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* **15**, 673-684.
 - [111] Doecke JD, Perez-Grijalba V, Fandos N, Fowler C, Villemagne VL, Masters CL, Pesini P, Sarasa M, Group AR (2020) Total Abeta42/Abeta40 ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. *Neurology* **94**, e1580-e1591.
 - [112] Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High performance plasma amyloid-beta biomarkers for Alzheimer's disease. *Nature* **554**, 249-254.
 - [113] Koychev I, Galna B, Zetterberg H, Lawson J, Zamboni G, Ridha BH, Rowe JB, Thomas A, Howard R, Malhotra P, Ritchie C, Lovestone S, Rochester L (2018) Abeta42/Abeta40 and Abeta42/Abeta38 Ratios Are Associated with Measures of Gait Variability and Activities of Daily Living in Mild Alzheimer's Disease: A Pilot Study. *J Alzheimers Dis* **65**, 1377-1383.
 - [114] Hansson O, Lehmann S, Otto M, Zetterberg H, Lewczuk P (2019) Advantages and disadvantages of the use of the CSF Amyloid beta (Abeta) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimers Res Ther* **11**, 34.
 - [115] Barthelemy NR, Bateman RJ, Hirtz C, Marin P, Becher F, Sato C, Gabelle A, Lehmann S (2020) Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. *Alzheimers Res Ther* **12**, 26.
 - [116] Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, Proctor NK, Chai X, Shcherbinin S, Sims JR, Triana-Baltzer G, Theunis C, Slemmon R, Mercken M, Kolb H, Dage JL, Hansson O (2020) Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun* **11**, 1683.

- [117] Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, Barro C, Kappos L, Comabella M, Fazekas F, Petzold A, Blennow K, Zetterberg H, Kuhle J (2018) Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol* **14**, 577-589.
- [118] Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K (2019) Association Between Longitudinal Plasma Neurofilament Light and Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol* **76**, 791-799.
- [119] Benedet AL, Ashton NJ, Pascoal TA, Leuzy A, Mathotaarachchi S, Kang MS, Therriault J, Savard M, Chamoun M, Scholl M, Zimmer ER, Gauthier S, Labbe A, Zetterberg H, Blennow K, Neto PR (2019) Plasma neurofilament light associates with Alzheimer's disease metabolic decline in amyloid-positive individuals. *Alzheimers Dement (Amst)* **11**, 679-689.
- [120] Mielke MM, Syrjanen JA, Blennow K, Zetterberg H, Vemuri P, Skoog I, Machulda MM, Kremers WK, Knopman DS, Jack C, Jr., Petersen RC, Kern S (2019) Plasma and CSF neurofilament light: Relation to longitudinal neuroimaging and cognitive measures. *Neurology* **93**, e252-e260.
- [121] Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, Brayne C, Burns A, Cohen-Mansfield J, Cooper C, Costafreda SG, Dias A, Fox N, Gitlin LN, Howard R, Kales HC, Kivimaki M, Larson EB, Ogunniyi A, Orgeta V, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbaek G, Teri L, Mukadam N (2020) Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet* **396**, 413-446.

Table 1. Overall meta-analytical results and subgroup analyses of different assay methods for blood biomarkers. Abbreviations: k = number of studies, s = number of samples, Ratio = ratio of mean biomarker concentration in AD vs. control group, CI = confidence interval, Q = Cochran Q-statistic, p_Q = p value assessing the significance of the Q-statistic, PI = prediction interval.

Biomarker	<i>k</i> (<i>s</i>)	Ratio	95 % CI	<i>Q</i> (df)	p_Q	τ	95 % PI
$A\beta_{40}$	48 (51)	1.03	[0.97, 1.10]	1101.63 (50)	< .001	0.22	[0.67, 1.60]
ELISA	37 (37)	1.10	[1.03, 1.17]	272.63	< .001	0.18	[0.77, 1.56]
IMR	7 (9)	0.77	[0.68, 0.87]	(36)272.35	< .001	0.18	[0.53, 1.13]
		0.94		(8)			
$A\beta_{42}$	50 (53)	1.01	[0.94, 1.08]	1270.94 (52)	< .001	0.23	[0.64, 1.58]
ELISA	37 (37)	0.98	[0.89, 1.08]	313.33 (36)	< .001	0.26	[0.58, 1.67]
IMR	7 (9)	1.15	[1.07, 1.24]	180.97 (8)	< .001	0.09	[0.95, 1.40]
Simoa	2 (2)	0.90	[0.78, 1.05]	2.20 (1)	.138	0.09	[0.72, 1.14]
$A\beta_{42/40}$	26 (28)	1.12	[0.97, 1.30]	1246.39 (27)	< .001	0.36	[0.54, 2.31]
ELISA	17 (17)	0.98	[0.88, 1.09]	148.75 (16)	< .001	0.18	[0.67, 1.43]
IMR	5 (6)	1.88	[1.37, 2.58]	301.74 (5)	< .001	0.36	[0.88, 4.05]
NfL	11	1.65	[1.46, 1.85]	55.75 (10)	< .001	0.17	[1.16, 2.34]
ELISA	(11)	1.83	[1.62, 2.06]	0.00 (1)	.995	0.00	[1.62, 2.06]
Simoa	2 (2)	1.58	[1.37, 1.82]	44.14 (7)	< .001	0.18	[1.08, 2.31]
	8 (8)						

t-tau	28 (31)	1.52	[1.25, 1.84]	1084.25 (30)	< .001	0.51	[0.55, 4.19]
ELISA	9 (9)	1.10	[0.81, 1.50]	195.78 (8)	< .001	0.45	[0.43, 2.82]
IMR	10	2.29	[1.74, 3.01]	441.30 (11)	< .001	0.48	[0.87, 6.04]
Simoa	(12)	1.28	[1.18, 1.38]	11.45 (6)	.075	0.05	[1.12, 1.46]
	7 (7)						
p-tau181	15	1.75	[1.43, 2.14]	509.15 (15)	< .001	0.38	[0.81, 3.76]
ELISA	(16)	1.43	[0.94, 2.19]	42.99 (3)	<.001	0.41	[0.58, 3.54]
IMR	4 (4)	1.47	[0.89, 2.44]	44.79 (2)	< .001	0.44	[0.54, 4.00]
Simoa	3 (3)	2.26	[1.56, 3.28]	24.23 (4)	< .001	0.33	[1.07, 4.74]
	4 (5)						

Table 2. Subgroup analysis: Effect of age differences between groups. Abbreviations: k = number of studies, s = number of samples, Ratio = ratio of mean biomarker concentration in AD vs. control group, CI = confidence interval, Q = Cochran Q-statistic, p_Q = p value assessing the significance of the Q-statistic, PI = prediction interval.

Biomarker	k (s)	Ratio	95 % CI	Q (df)	p_Q	τ	95 % PI
$A\beta_{40}$	18 (18)	1.02	[0.92, 1.15]	567.13 (17)	< .001	0.24	[0.64, 1.65]
$A\beta_{42}$	17 (17)	0.98	[0.86, 1.13]	689.09 (16)	< .001	0.26	[0.58, 1.67]
$A\beta_{42/40}$	7 (7)	0.94	[0.72, 1.23]	397.49 (6)	< .001	0.35	[0.45, 1.97]
NfL	4 (4)	1.58	[1.34, 1.85]	8.81 (3)	.032		[1.17, 2.13]
						0.13	
t-tau	10 (10)	1.84	[1.37, 2.47]	394.43	< .001	0.46	[0.72, 4.73]
p-tau181	5 (5)	2.13	[1.43, 3.18]	36.76	< .001	0.40	[0.89, 5.14]

Table 3. Subgroup analysis: Control group subjects assessed for neurological and systemic health. Abbreviations: k = number of studies, s = number of samples, Ratio = ratio of mean biomarker concentration in AD vs. control group, CI = confidence interval, Q = Cochran Q-statistic, p_Q = p value assessing the significance of the Q statistic, PI = prediction interval.

biomarker	k (s)	ratio	95 % CI	Q (df)	p_Q	τ	95 % PI
A β ₄₀	16 (17)	1.04	[0.93, 1.16]	382.22 (16)	< .001	0.21	[0.68, 1.59]
A β ₄₂	15 (17)	1.14	[1.02, 1.28]	303.52 (16)	< .001	0.20	[0.76, 1.72]
A β _{42/40}	10 (11)	1.34	[1.02, 1.75]	799.90 (10)	< .001	0.43	[0.56, 3.22]
NfL	2 (2)	1.56	[1.32, 1.84]	1.82 (1)	.178	0.08	[1.24, 1.97]
t-tau	9 (11)	1.38	[0.99, 1.93]	346.96 (10)	< .001	0.53	[0.47, 4.10]
p-tau181	4 (4)	1.40	[1.02, 1.91]	40.44 (3)	<.001	0.31	[0.70, 2.78]

Table 4. Meta-analysis of biomarkers measured in extracellular vesicles. Abbreviations: k = number of studies, s = number of samples, Ratio = ratio of mean biomarker concentration in AD vs. control group, CI = confidence interval, Q = Cochran Q-statistic, p_Q = p value assessing the significance of the Q statistic, PI = prediction interval.

Biomarker	k (s)	Ratio	95 % CI	Q (df)	p_Q	τ	95 % PI
A β ₄₂	7 (8)	2.66	[1.15, 6.17]	568.15 (7)	< .001	1.13	[0.25, 28.45]
t-tau	6 (7)	1.55	[1.22, 1.96]	67.53 (6)	< .001	0.27	[0.87, 2.76]
p-tau181	8 (9)	2.55	[1.37, 4.74]	1604.81 (8)	< .001	0.89	[0.40, 16.14]

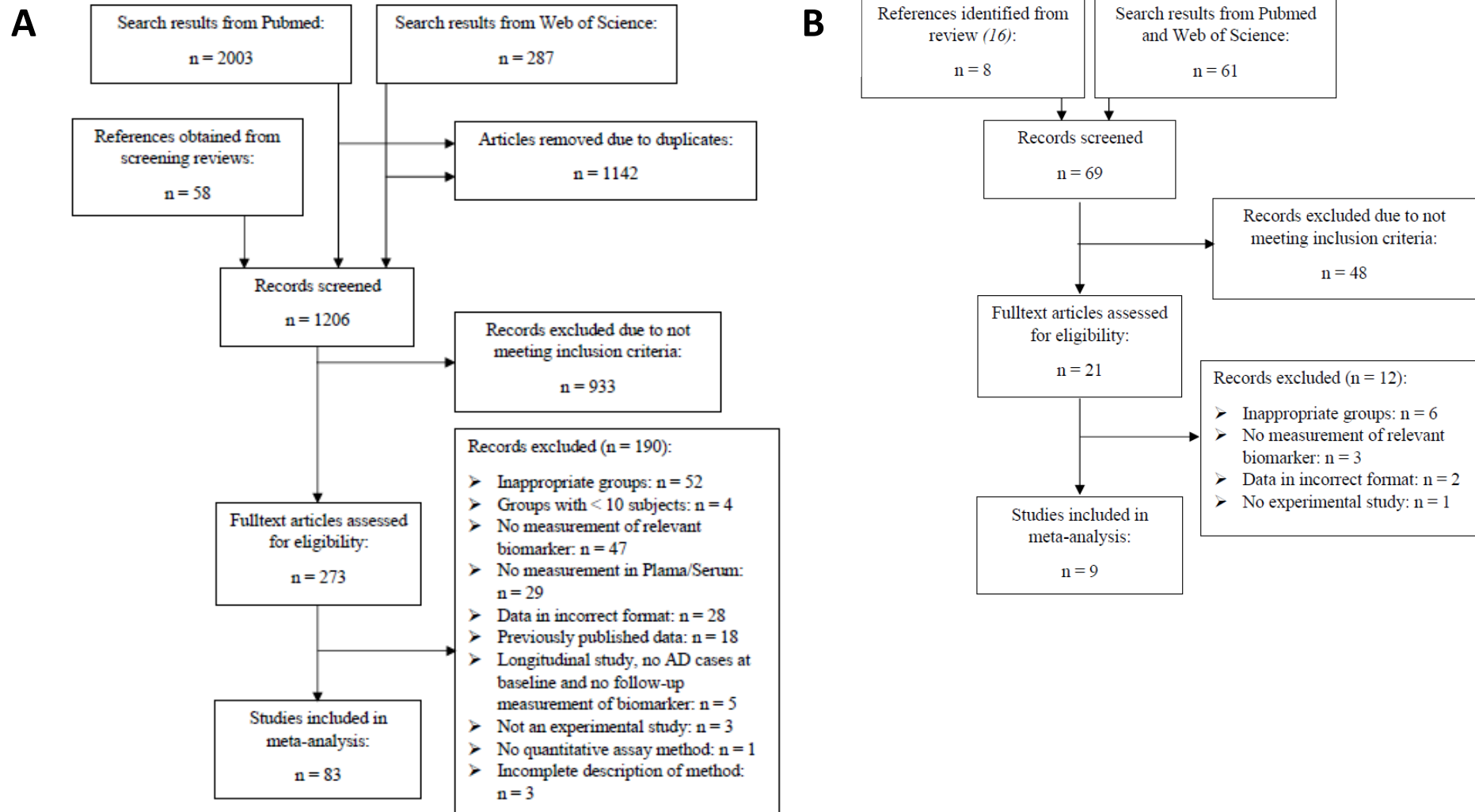


Figure 1. Flowchart of search results, screening and eligibility assessment of studies for the main (A) as well as the exosome-focused meta-analyses (B).

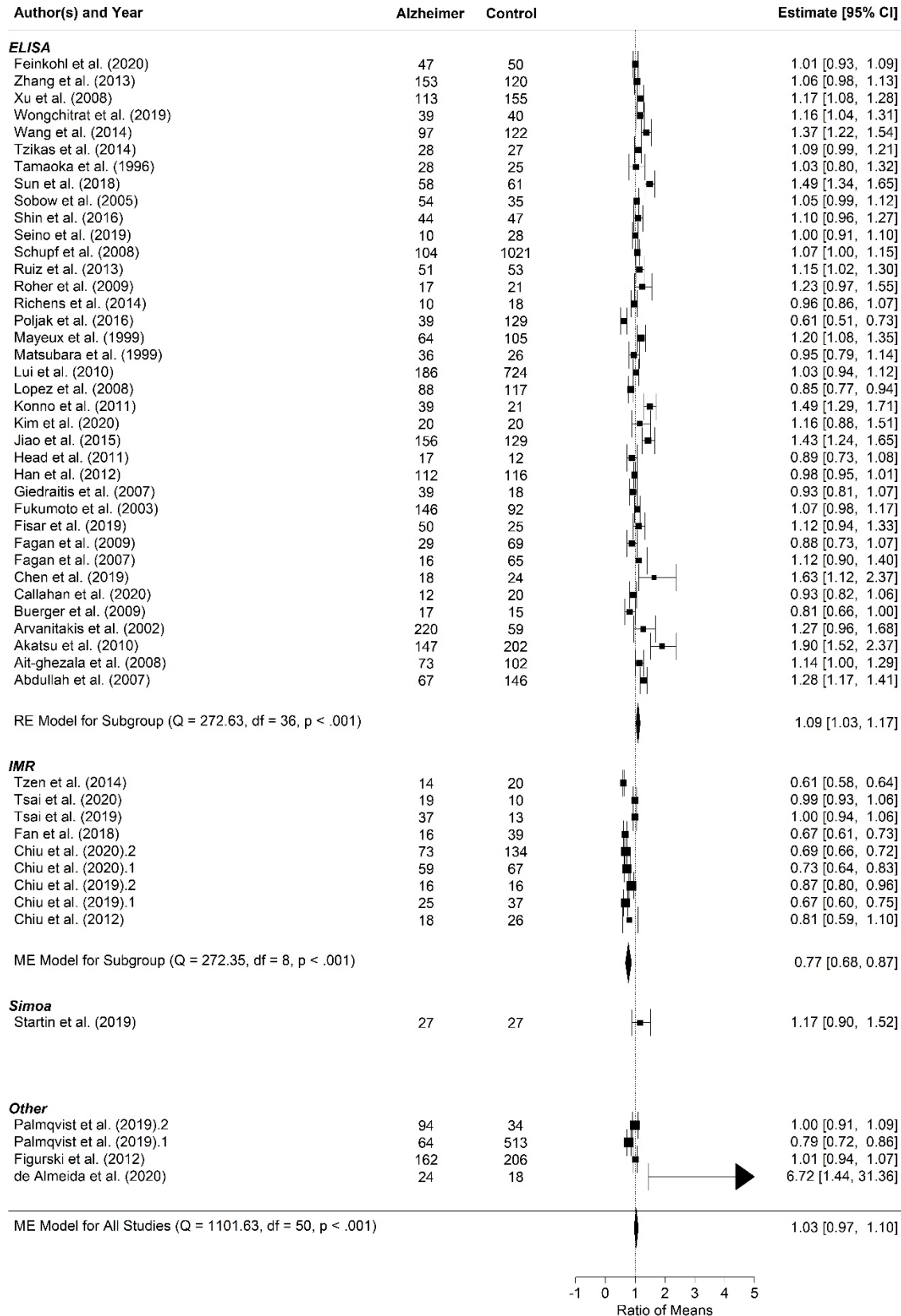


Figure 2. Forest plot of $A\beta_{40}$ studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.

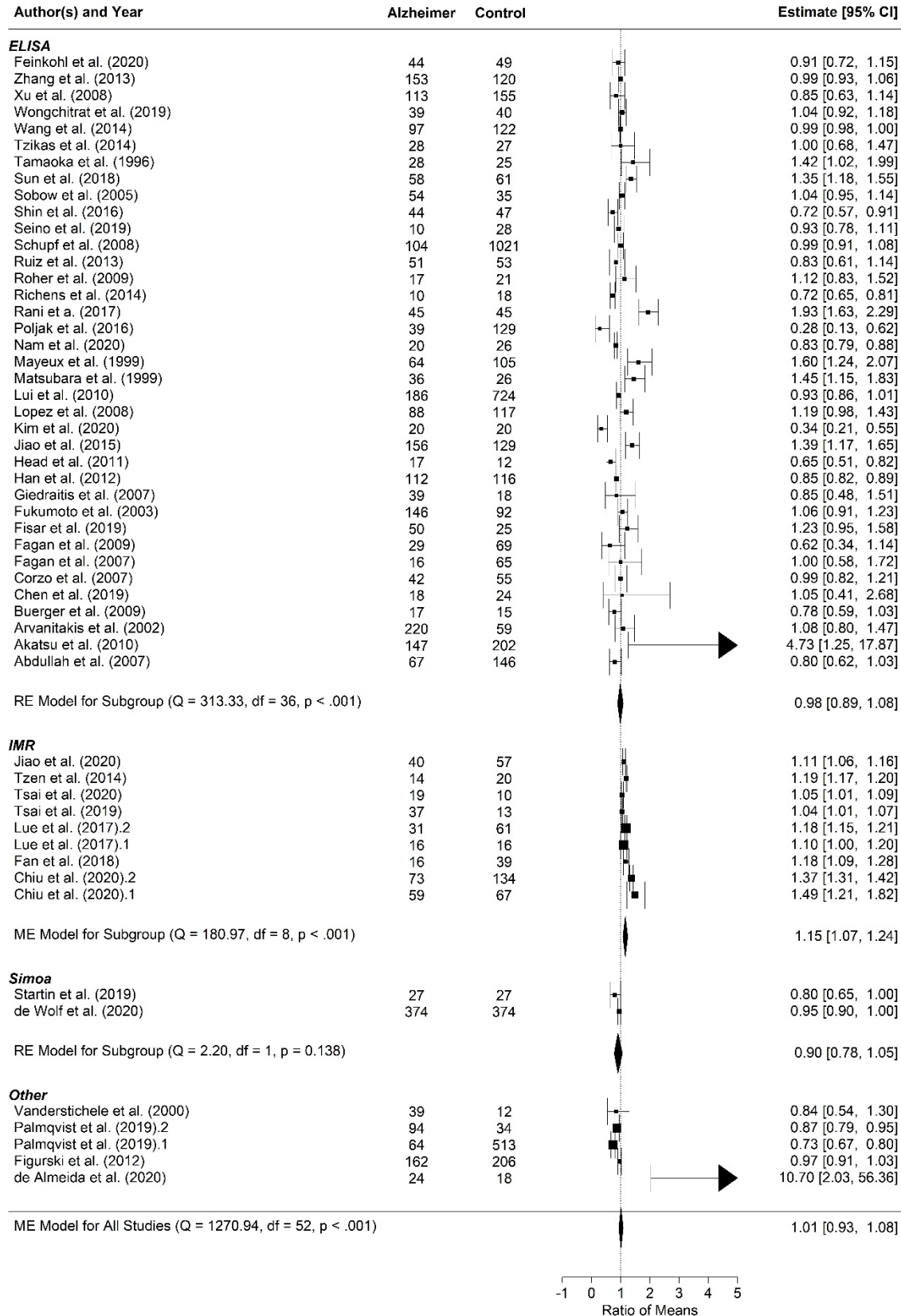


Figure 3. Forest plot of $A\beta_{42}$ studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.

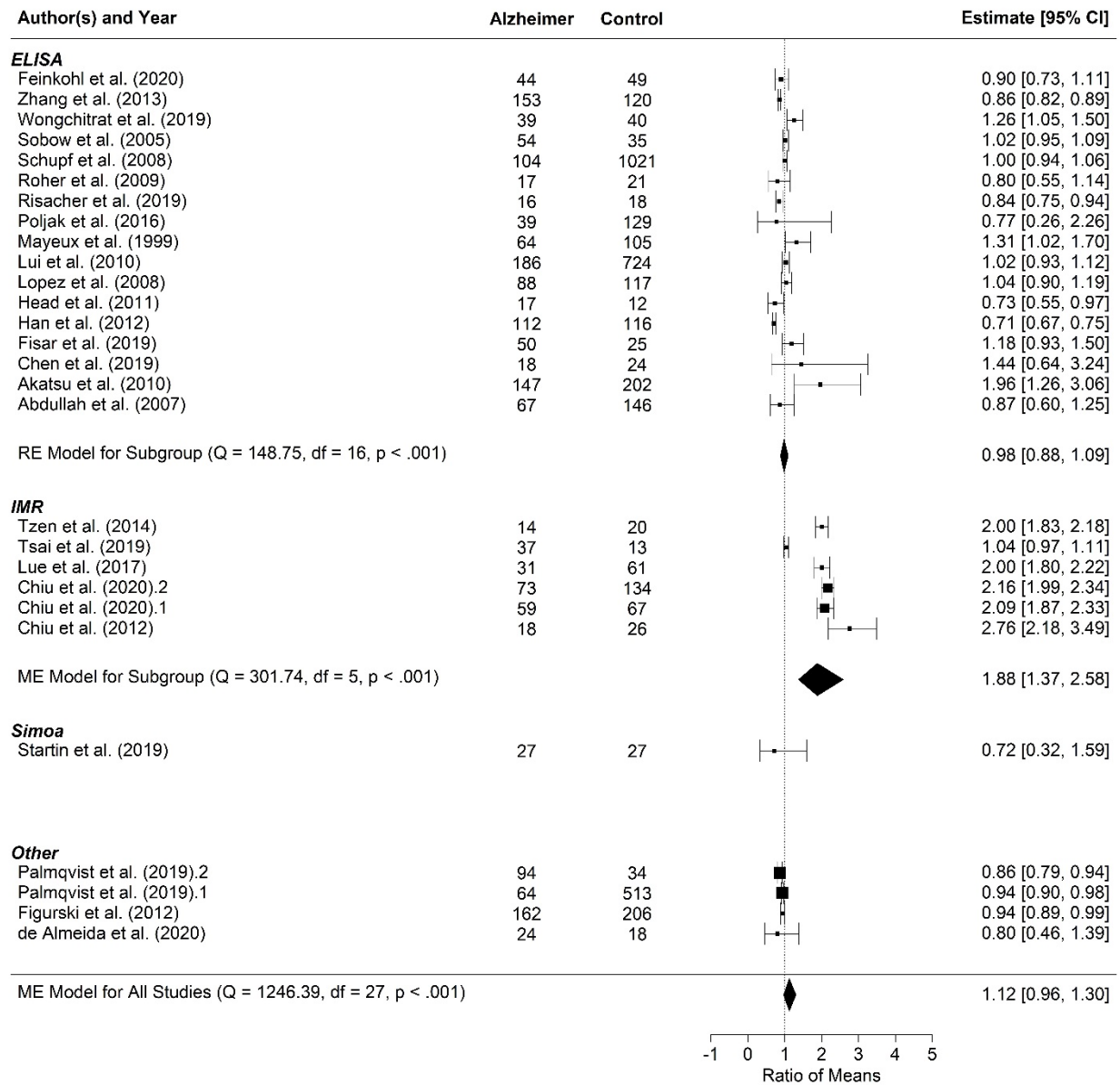


Figure 4. Forest plot of $A\beta_{42/40}$ studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.

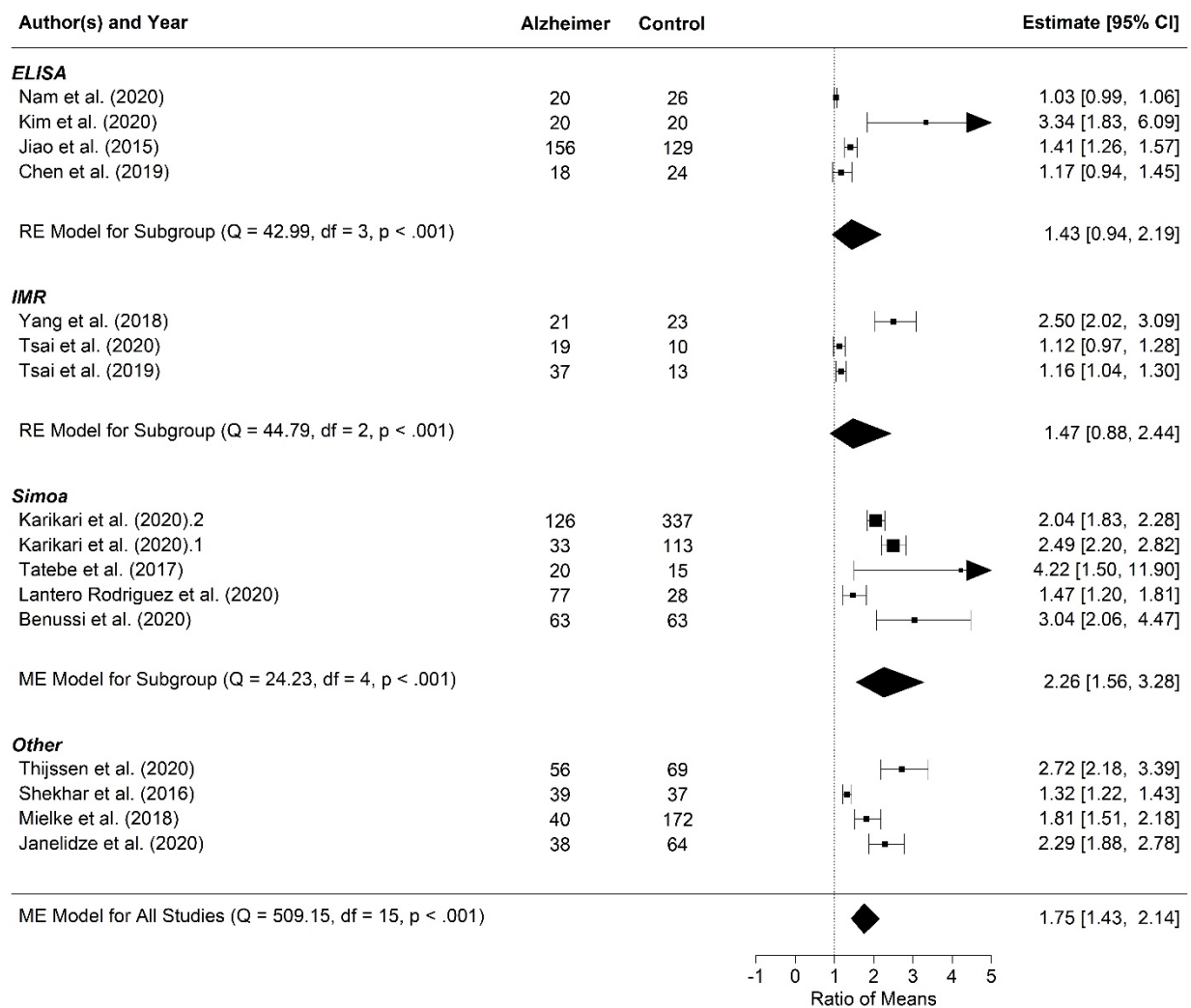


Figure 5. Forest plot of ptau-181 studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.

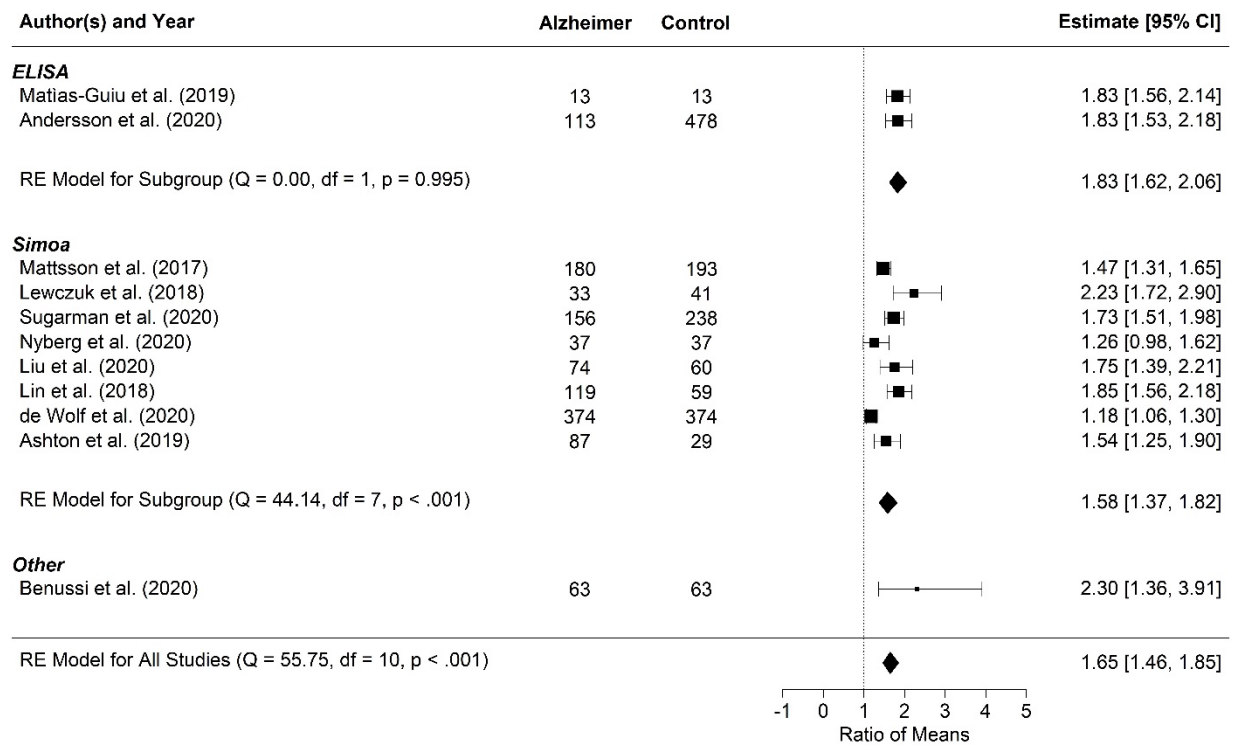


Figure 6. Forest plot of neurofilament light (NfL) studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.

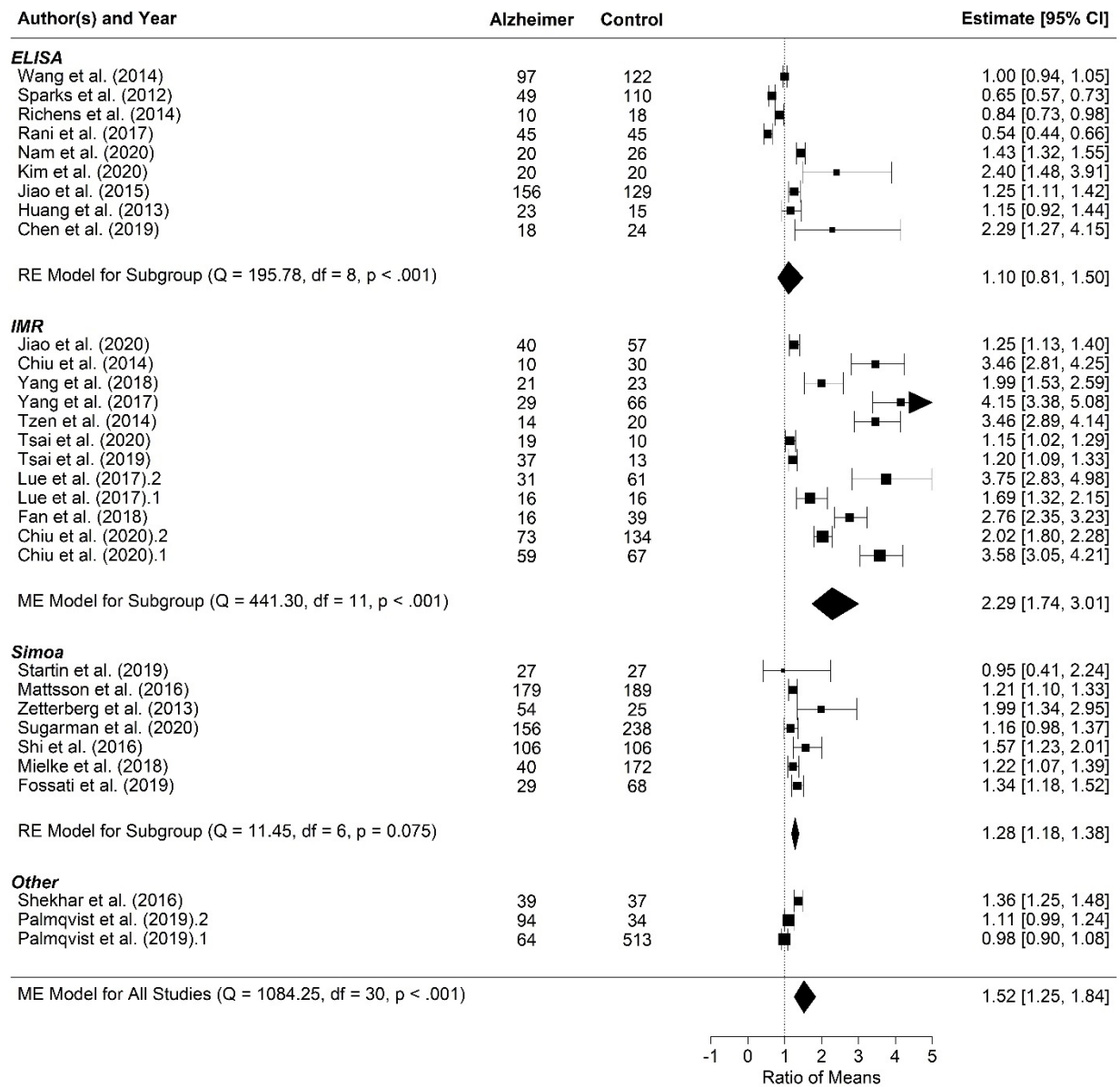


Figure 7. Forest plot of total tau (t-tau) studies. Abbreviations: RE = random effect, ME = mixed effect, df = degrees of freedom, CI = confidence interval, Q = Cochran Q-statistic.