

Featured Article

Concordance between the assessment of A β 42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid

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Abstract

Introduction: Neuronal-derived exosomal A β 42, T-tau, and P-T181-tau have been demonstrated to be biomarkers of Alzheimer's disease (AD). However, no study has assessed the association of A β 42, T-tau, and P-T181-tau between exosomes and CSF.

Methods: This was a multicenter study with two-stage design. The subjects included 28 AD patients, 25 aMCI patients, and 29 controls in the discovery stage; the results of which were confirmed in the validation stage (73 AD, 71 aMCI, and 72 controls).

Results: The exosomal concentrations of A β 42, T-tau, and P-T181-tau in AD group were higher than those in aMCI and control groups (all $P < .001$). The level of each exosomal biomarker was highly correlated with that in CSF.

Discussion: This study verified the agreement between CSF and blood exosomal biomarkers and confirmed that exosomal A β 42, T-tau, and P-T181-tau have the same capacity as those in CSF for the diagnosis of AD and aMCI.

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Keywords:

Alzheimer's disease; Mild cognitive impairment; Exosome; Biomarker; A β ; tau

1. Introduction

A diagnosis of Alzheimer's disease (AD) requires a combination of symptoms, signs, psychological tests, and biomarker measurements. Positron emission tomography (PET) imaging and A β 42, T-tau, and P-T181-tau concentrations in cerebrospinal fluid (CSF) are recommended as diagnostic biomarkers for the disease in clinical practice and

research [1]. However, the expensive costs of PET limit its application in the clinic. CSF is considered the optimal source of AD biomarkers because its direct contact with the brain allows it to reflect pathophysiological changes that occur in the central nervous system (CNS). A β 42 in CSF has shown a high correlation with pathological changes in postmortem and PET images of the brain [2–4], confirming its validation for the diagnosis of AD. However, lumbar puncture is an invasive procedure, and repeated CSF collection is challenging. Compared with CSF, blood measurements are advantageous for AD biomarker screening because blood collection is easier and

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less invasive. As such, biomarkers obtained from blood samples have become increasingly common, but the findings have been contradictory. A meta-analysis including more than 5000 records showed that A β 42 in the blood is not a biomarker for AD [5], whereas a more recent article provided convincing evidence that A β 42 combined with other peptides in plasma has a very high ability to predict AD [6]. These findings suggest that peripheral blood may still be a promising source for screening AD biomarkers. Moreover, studies demonstrated that A β 42, T-tau, and P-T181-tau in blood neuronal-derived exosomes can differentiate patients with AD from controls [7], suggesting that exosomes may be an ideal biomarker carrier for AD screening. However, the concordance of these biomarkers in blood neuronal-derived exosomes with their presence in the CSF has not yet been validated. Given that the levels of exosomal biomarkers for AD are highly correlated in the blood and CSF, these biomarkers reflect brain pathological changes, suggesting that exosomal proteins in peripheral blood can potentially be used to diagnose AD in the clinic.

In the present study, we aimed to (1) explore the diagnostic capacity of blood exosomal A β 42, T-tau, and P-T181-tau on AD and amnesic mild cognitive impairment (aMCI), (2) verify the exosomal biomarker results in CSF for all subjects, and (3) validate the results from a discovery stage in a validation stage with more samples. In this study, all necessary measures were taken to ensure the objectivity of the final results.

2. Methods and materials

2.1. Participants

This study included subjects from a Beijing center for the discovery stage and subjects from other centers (Guizhou, Shandong, Henan, Jilin, Inner Mongolia, Guangxi, and Hebei provinces) for the validation stage from September 2016 to July 2018. A discovery stage examining 28 individuals with AD, 25 individuals with aMCI, and 29 healthy controls (82 in total) as well as a validation stage including 73 individuals with AD, 71 individuals with aMCI, and 72 healthy controls (216 in total) were conducted. The diagnosis of AD was based on the criteria of the National Institute on Aging and Alzheimer's Association (NIA-AA) [1]. The diagnosis of aMCI was made according to published criteria [8]. Written informed consent was obtained from all participants or their legal guardians. This study was approved by the Institutional Review Board of Xuanwu Hospital, Capital Medical University.

2.2. Collection of neuronal-derived exosomes from blood

Blood samples were collected in the morning after a 12-h fast. Twenty milliliters of whole blood were drawn from each subject and stored in a polypropylene tube containing EDTA. Whole blood samples collected at the Beijing center (Xuanwu Hospital) were immediately processed to obtain neuronal-derived exosomes. At the other centers, whole

blood collected from each local hospital was immediately centrifuged at $4200 \times g$ for 10 min to obtain the plasma. The plasma samples were then shipped on dry ice to the Beijing center within 12 h. Upon arrival of the plasma samples from the other centers, specific neuronal-derived exosomes were immediately separated for consistency according to a published protocol [7]. In brief, one-half milliliter of plasma was incubated with 0.15 ml thromboplastin-D (Thermo Fisher Scientific, MA) for 60 min, and 0.35 ml calcium- and magnesium-free Dulbecco's phosphate-buffered saline (DPBS, Thermo Fisher Scientific) with protease and phosphatase inhibitor cocktails (Thermo Fisher Scientific) was then added. Next, 0.5 ml of the obtained serum was mixed with 0.5 ml DPBS, and the mixed solution was centrifuged at $1500 \times g$ for 20 min. The supernatants were then mixed with ExoQuick exosome precipitation solution (EXOQ; System Biosciences, CA) and incubated for 1 h on ice. After centrifugation at $1500 \times g$ for 30 min, the pellets were resuspended in 250 μ l DPBS (Santa Cruz, CA). Each sample was mixed with 100 μ l 3% bovine serum albumin (BSA, Thermo Fisher Scientific) and then incubated for 1 h on ice with a mouse anti-human neural cell adhesion molecule (NCAM) antibody (2 pg/ml, Santa Cruz); the antibody was labeled with biotin using the EZ-Link sulfo-NHS-biotin system (Thermo Fisher Scientific). Then, 25 μ l of streptavidin-agarose resin (Thermo Fisher Scientific) containing 50 μ l of 3% BSA was added. After centrifugation at $200 \times g$ for 10 min at 4°C and removal of the supernatant, each sample was resuspended in 50 μ l 0.05 M glycine-HCl (pH = 3.0) by vortexing for 10 seconds and mixed with 0.45 ml DPBS containing 2 g/100 ml BSA, 0.10% Tween 20, and inhibitor cocktails. The samples were then incubated for 10 min at 37°C with vortex mixing and stored at -80°C . The removed supernatants were collected and recentrifuged to obtain the nonimmunoprecipitated exosomes. Exosomes were resuspended in 0.25 ml of 0.05 M glycine-HCl (pH = 3.0) on ice and centrifuged at $200 \times g$ for 15 min. The pH of the supernatant was then adjusted to 7.0 with 1 M Tris-HCl (pH = 8.6).

2.3. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed according to a published protocol with minor modifications [9]. In brief, after immunoprecipitation, neuronal-derived exosomes were stored in 1% paraformaldehyde, dehydrated via an ethanol series and embedded in Epon. Sections (65 nm) were stained with uranyl acetate and Reynold's lead citrate. A JEM-1400plus transmission electron microscope was used for imaging.

2.4. Western blot

Western blot was performed to detect an exosomal marker, Alix, using a monoclonal anti-human Alix antibody according to the manufacturer's instructions (1:1000, Cell

Table 1
Characteristics of participants

Characteristic	Total sample (n = 298)	Discovery (n = 82)			Validation (n = 216)		
		Controls (n = 29)	aMCI (n = 25)	AD (n = 28)	Controls (n = 72)	aMCI (n = 71)	AD (n = 73)
Age, mean (SD), y	65 (6)	63 (5)	65 (5)	66 (6)	64 (5)	66 (7)	65 (6)
Education, mean (SD), y	11.5 (1.5)	12.1 (1.5)	11.5 (1.5)	10.8 (1.4)	12.3 (1.6)	11.8 (1.6)	10.6 (1.5)
Women, No. (%)	162 (54)	15 (51.7)	13 (52.0)	16 (57.1)	37 (51.4)	39 (54.9)	42 (57.5)
APOE ϵ 4 positive, No. (%)	90 (30.2)	5 (17.2)	7 (28.0)*	11 (39.2)* [†]	14 (19.4)	22 (31.0)*	31 (42.5)* [†]
MMSE score, mean (SD)	25.2 (1.6)	29.2 (1.2)	26.5 (0.4)*	20.3 (3.2)* [†]	29.3 (1.2)	26.2 (0.4)*	19.6 (3.1)* [†]
CDR score of 0/0.5/1.0/2.0, No.	142/85/52/19	28/1/0/0	11/14/0/0*	0/9/14/5* [†]	70/2/0/0	33/38/0/0*	0/21/38/14* [†]

Abbreviations: AD, Alzheimer's disease; APOE ϵ 4, apolipoprotein ϵ 4; CDR, clinical dementia rating; aMCI, amnesic mild cognitive impairment; MMSE, Mini-Mental State Examination.

* $P < .05$ compared to controls.

[†] $P < .05$ compared to aMCI.

Signaling Technology, MA). Immunoprecipitated and centrifuged samples were used to measure neuronal-derived exosomes, and supernatants were used as negative controls.

2.5. Collection of CSF

CSF was collected immediately after the blood draw according to international guidelines [10]. In brief, the subjects were positioned in a left lateral position for lumbar puncture. Fifteen milliliters of CSF were collected from each subject, centrifuged at $2000 \times g$ for 10 min at room temperature and stored in a polypropylene tube at -80°C . After lumbar puncture, the subject was monitored for any signs of discomfort for at least 12 h.

2.6. Protein measurements

The levels of A β 42, T-tau, and P-T181-tau in neuronal-derived exosomes and CSF were measured with an enzyme-linked immunosorbent assay (ELISA). The amount of CD81 protein was measured to normalize the exosomal content. The mean value of CD81 levels in each group was set to 1.00, and the relative values for each sample were used to normalize their recovery [7]. In addition, to confirm the neuronal-derived enrichment, the L1 cell adhesion molecule (L1CAM) levels in nonimmunoprecipitated exosomes were measured. The ELISA kits used in this research are listed in Supplementary Table 1. All measurements were performed in a blinded manner.

2.7. Statistical analysis

Statistical analyses were performed using SPSS v.22 and R v.3.3.0 with the rms package. Data from the discovery and validation stages were calculated independently. For categorical data, such as gender, clinical subgroups, and apolipoprotein ϵ 4 (APOE ϵ 4) carrier distributions, group differences were analyzed using the χ^2 test. For numerical data, such as concentrations of biomarkers and group differences were analyzed by using Welch's t-test or ANOVA. Correlative analysis was performed using a linear regression model. Af-

ter generating an adjusted receiver operating characteristic (ROC) curve, the predicted values were calculated using a binary logistic regression model in which age, gender, and APOE status were used as covariates [6]. The composite biomarker was generated by combining normalized scores of A β 42, T-tau, and P-T181-tau. A DeLong test was used to compare the area under the curve (AUC) between groups [11]. All tests were two-tailed, and the significant difference was set at $P < .05$.

3. Results

3.1. Participant characteristics

Table 1 lists the characteristics of the participants. There were no differences in the ages or ratios of males/females among the AD, aMCI, and control groups in either the discovery stage or the validation stage. The percentages of APOE ϵ 4, Mini-Mental State Examination (MMSE), and clinical dementia rating (CDR) scores were significantly different ($P < .01$) between AD patients and controls, AD and aMCI patients, and aMCI patients and controls.

3.2. Confirmation of exosomal collection

The neuronal-derived exosomes were confirmed by TEM and Western blot (Fig. 1A). The representative TEM image of an AD patient's exosomes clearly shows the exosomes. Western blot analysis showed that Alix was expressed in the exosomal samples but not in the supernatants or negative controls (Fig. 1B). In the discovery stage, the L1CAM content in immunoprecipitated exosomes was increased by approximately 10-fold compared with that in nonimmunoprecipitated exosomes (Fig. 1C). Based on these data, we confirmed that neuronal-derived exosomes were successfully collected.

3.3. Levels of A β 42, T-tau, and P-T181-tau in blood neuronal-derived exosomes and CSF

We first measured the CD81 levels in all samples to normalize the exosomal content (Fig. 1D), and no difference

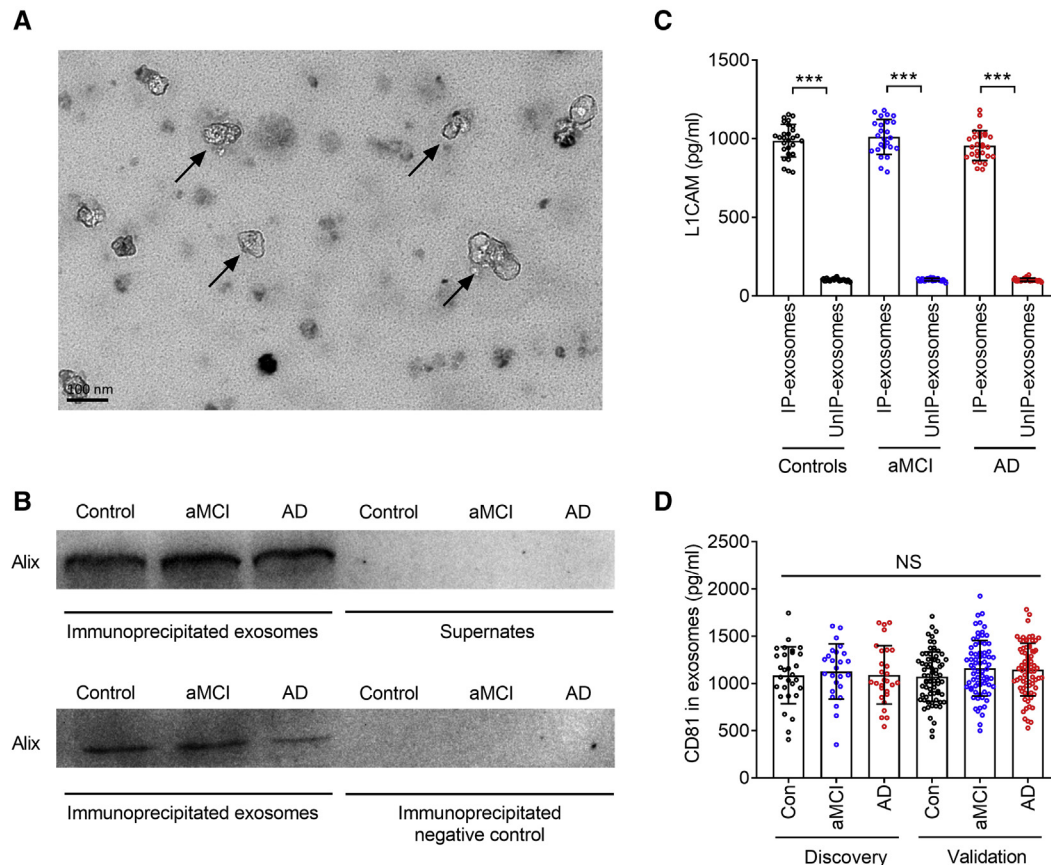


Fig. 1. Confirmation of exosomal collection by TEM and Western blot and normalization of exosomal content. Representative TEM image (A) showing neuronal-derived exosomes (black arrows) that were successfully collected. Representative Western blot images (B) showing that the exosomal marker Alix was highly expressed in exosomal samples but not detected in supernatants. Moreover, an additional negative control was set after the ExoQuick immunoprecipitation step with beads alone not linked to NCAM. Western blots (B) showing that Alix was not detected in the negative control. These data confirm that exosomal collection was effective. ELISAs (C) in controls, aMCI and, AD patients in the discovery stage show that the L1CAM concentration in immunoprecipitated exosomes was increased by approximately 10-fold compared with that in nonimmunoprecipitated exosomes. CD81 was measured to normalize the exosomal content. D shows that no differences were detectable between each group. Scale bar = 100 nm. Con = controls. NS = no significance. IP = immunoprecipitated. UnIP = nonimmunoprecipitated. *** $P < .001$.

was observed among the AD, aMCI, and control groups (all $P > .05$). The CD81 levels in each sample were used to normalize the subsequent exosomal measurements. We then measured the levels of A β 42, T-tau, and P-T181-tau in neuronal-derived exosomes in the discovery stage (Fig. 2A–C). The exosomal concentrations of A β 42, T-tau, and P-T181-tau in the AD group (4.96 ± 1.50 , 255 ± 72 , and 95 ± 25 pg/ml, respectively) were significantly higher than those in the control group (2.59 ± 0.77 , 145 ± 42 , and 44 ± 13 pg/ml, respectively, $P < .001$). Furthermore, their concentrations in the aMCI group (3.56 ± 0.89 , 191 ± 48 , and 63 ± 26 pg/ml, respectively) were significantly lower than those in the AD group ($P < .01$) and higher than those in the control group ($P < .01$). We then assessed the same measurements in the validation stage (Fig. 2A–C). The exosomal concentrations of A β 42, T-tau, and P-T181-tau in the AD group (5.02 ± 1.54 , 255 ± 81 , and 93 ± 32 pg/ml, respectively) were significantly higher than those in the control group (2.53 ± 0.70 , 137 ± 46 , 49 ± 17 pg/ml,

respectively, $P < .001$). Moreover, their concentrations in the aMCI group (3.30 ± 0.94 , 195 ± 56 , 66 ± 22 pg/ml, respectively) were significantly lower than those in the AD group ($P < .001$) and higher than those in the control group ($P < .001$). These data indicated that the biomarkers in blood neuronal-derived exosomes sufficiently distinguished AD patients from controls or aMCI patients and aMCI patients from controls in both the discovery and validation data sets, and no significant differences were observed between the two data sets. We then investigated the levels of A β 42, T-tau, and P-T181-tau in CSF (Fig. 2D–F). Patients with AD had significantly different levels of all biomarkers compared with those in aMCI patients and controls ($P < .05$ or 0.001 , respectively), and the biomarker levels in aMCI patients were significantly different ($P < .001$, respectively) from those in the controls. Furthermore, the levels of exosomal and CSF biomarkers were not different between the discovery and validation data sets ($P > .05$), indicating that the biomarkers performed the same in the

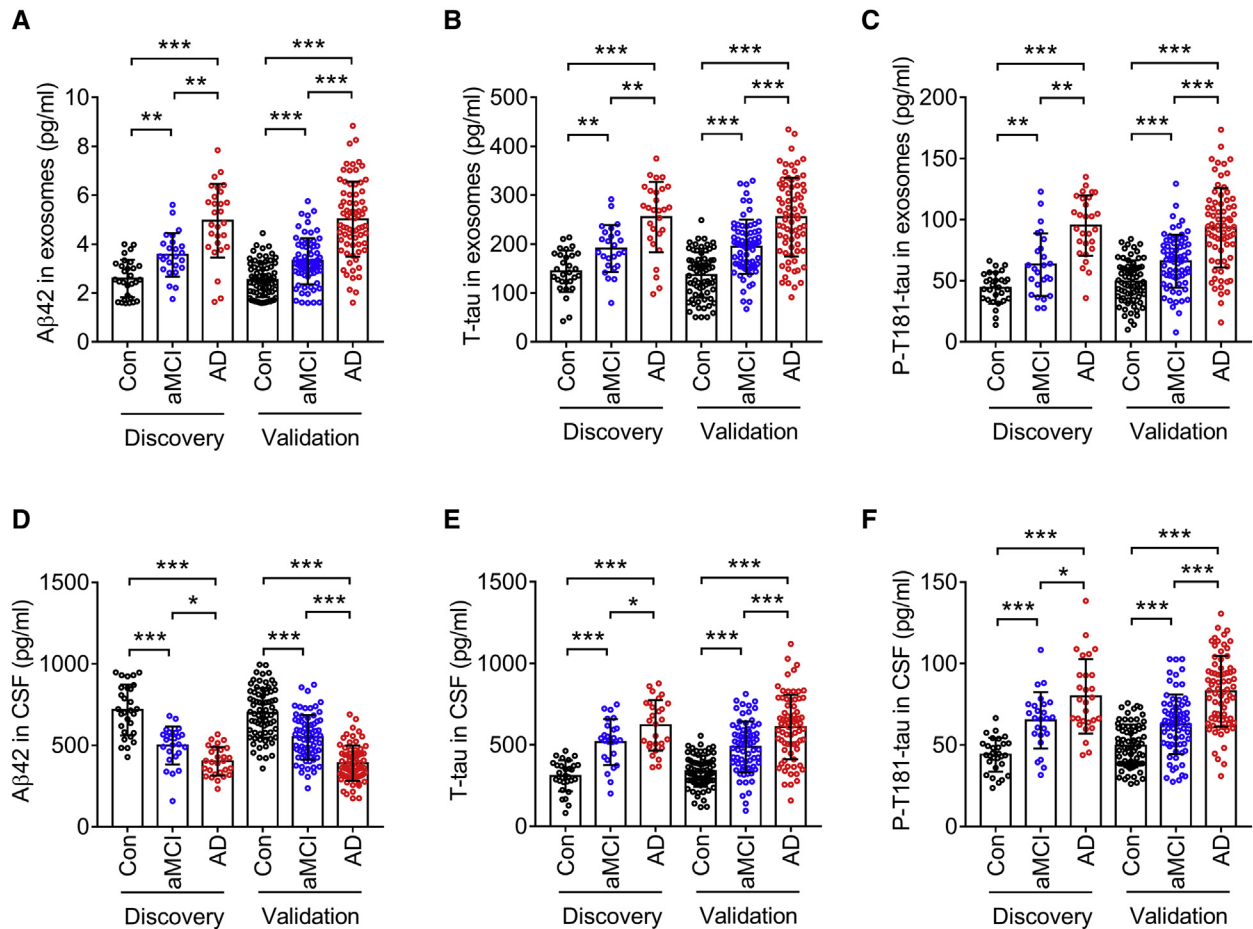


Fig. 2. Biomarker values in the discovery and validation data sets. A-C show levels of neuronal-derived exosomal Aβ42 (A), T-tau (B), and P-T181-tau (C). D-F show levels of CSF Aβ42 (D), T-tau (E), and P-T181-tau (F). In the discovery stage, $n = 28$ (AD), 25 (aMCI), and 29 (controls). In the validation stage, $n = 73$ (AD), 71 (aMCI), and 72 (controls). Con = controls. *** $P < .001$, ** $P < .01$, * $P < .05$.

two stages. Moreover, additional ELISA kits were used to confirm the levels of exosomal Aβ42 and P-T181-tau. These data did not differ from those obtained using the corresponding kits (Supplementary Fig. 1 and 2), indicating that the measurements were convincing.

3.4. Biomarker correlation analysis between blood neuronal-derived exosomes and CSF

We performed correlation analysis between exosomal and CSF biomarkers and found that the levels of Aβ42, T-tau, and P-T181-tau in neuronal-derived exosomes were highly correlated with their levels in CSF (Fig. 3). In detail, in the discovery data set, the Aβ42 levels in neuronal-derived exosomes were inversely correlated with those in the CSF in the AD ($R^2 = 0.76$, $P < .0001$, Fig. 3A), aMCI ($R^2 = 0.78$, $P < .0001$, Fig. 3B), and control ($R^2 = 0.80$, $P < .0001$, Fig. 3C) groups. The levels of T-tau were positively correlated between the exosomes and CSF in the AD ($R^2 = 0.82$, $P < .0001$, Fig. 3D), aMCI ($R^2 = 0.81$, $P < .0001$, Fig. 3E), and control ($R^2 = 0.85$, $P < .0001$,

Fig. 3F) groups. Similarly, P-T181-tau levels were positively correlated between exosomes and CSF in the AD ($R^2 = 0.79$, $P < .0001$, Fig. 3G), aMCI ($R^2 = 0.81$, $P < .0001$, Fig. 3H), and control ($R^2 = 0.82$, $P < .0001$, Fig. 3I) groups. We then confirmed the correlation analysis in the validation stage and found the same correlations between exosomal and CSF biomarkers (Fig. 3). Our findings revealed high correlations of Aβ42, T-tau, and P-T181-tau between exosomes and CSF, suggesting that exosomal biomarkers may reflect pathological changes in the brain and can be used for the diagnosis of AD.

3.5. Diagnostic power of each biomarker in blood neuronal-derived exosomes and CSF

We calculated the ROCs of Aβ42, T-tau, and P-T181-tau in exosomes and CSF, and the results showed significantly high AUCs. In detail, in the discovery stage (data not shown in figures), for Aβ42 in neuronal-derived exosomes, the AUC for the AD/controls comparison was 0.93 ($P < .0001$), while the AUC for the aMCI/controls

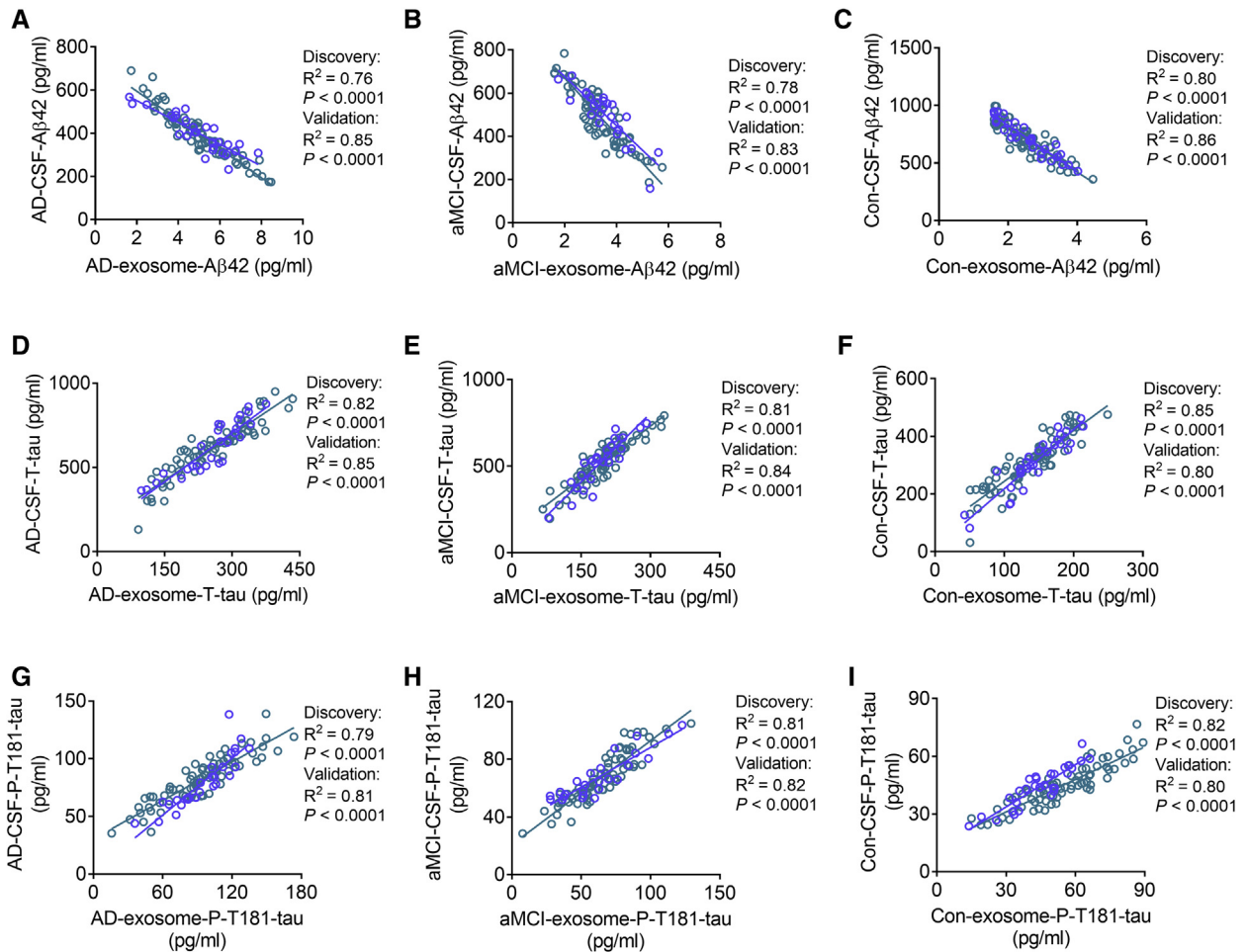


Fig. 3. The levels of exosomal biomarkers are significantly correlated with the levels of CSF biomarkers. A-C show robust correlations of A β 42 from exosomes and CSF in AD patients (A), aMCI patients (B), and controls (C). D-F show significant correlations of T-tau from exosomes and CSF in AD patients (D), aMCI patients (E), and controls (F). G-I show high correlations of P-T181-tau from exosomes and CSF in AD patients (G), aMCI patients (H), and controls (I). The blue circles and lines indicate the discovery data set. The green circles and lines indicate the validation data set. In the discovery stage, $n = 28$ (AD), 25 (aMCI), and 29 (controls). In the validation stage, $n = 73$ (AD), 71 (aMCI), and 72 (controls). Con = controls. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

comparison was 0.72 ($P < .0001$). For A β 42 in CSF, the AUCs for the AD/controls and aMCI/controls comparisons were 0.93 ($P < .0001$) and 0.72 ($P < .0001$), respectively. Similarly, T-tau and P-T181-tau also showed high AUCs in the comparisons of AD/controls (T-tau in neuronal-derived exosomes, 0.87, $P < .0001$; T-tau in CSF, 0.86, $P < .0001$; P-T181-tau in neuronal-derived exosomes, 0.89, $P < .0001$; P-T181-tau in CSF, 0.88, $P < .0001$) and aMCI/controls (T-tau in neuronal-derived exosomes, 0.78, $P < .001$; T-tau in CSF, 0.78, $P < .0001$; P-T181-tau in neuronal-derived exosomes, 0.72, $P < .0001$; P-T181-tau in CSF, 0.71, $P < .0001$). In the validation data set (Fig. 4), for A β 42 in neuronal-derived exosomes (Fig. 4A), the AUC for the AD/controls comparison was 0.93 ($P < .0001$), while the AUC for the aMCI/controls comparison was 0.74 ($P < .0001$). For A β 42 in CSF (Fig. 4D), the AUCs for the AD/controls and aMCI/controls comparisons were 0.93 ($P < .0001$) and 0.76 ($P < .0001$), respectively.

Similarly, T-tau and P-T181-tau also showed high AUCs for the comparisons of AD/controls (T-tau in neuronal-derived exosomes, 0.89, $P < .0001$; T-tau in CSF, 0.88, $P < .0001$; P-T181-tau in neuronal-derived exosomes, 0.88, $P < .0001$; P-T181-tau in CSF, 0.90, $P < .0001$) and aMCI/controls (T-tau in neuronal-derived exosomes, 0.79, $P < .0001$; T-tau in CSF, 0.78, $P < .0001$; P-T181-tau in neuronal-derived exosomes, 0.73, $P < .0001$; P-T181-tau in CSF, 0.72, $P < .0001$) (Fig. 4B-C, and E-F). In the validation stage, we also analyzed the AUCs of AD/aMCI comparisons and found that A β 42, T-tau, and P-T181-tau in neuronal-derived exosomes and CSF had good performance in identifying the diseases (AUCs and P values shown in Fig. 4). We further compared the ROCs of each biomarker between the discovery and validation data sets and found no significant differences between the two groups. These data suggested that the biomarkers had the same diagnostic efficiencies in the discovery and validation stages. In

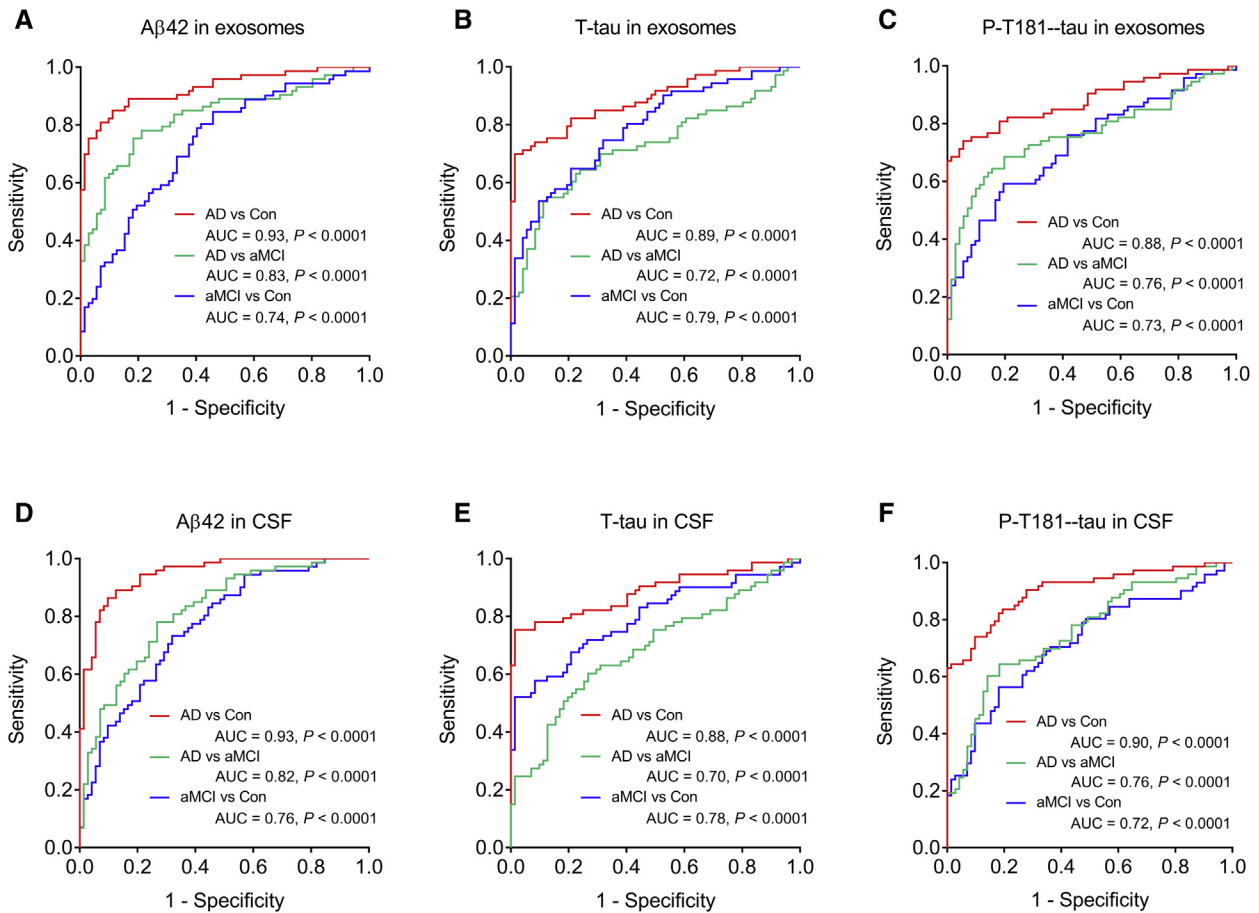


Fig. 4. High performance of the exosomal biomarkers in the validation stage. A-C show ROC analyses of Aβ42 (A), T-tau (B), and P-T181-tau (C) in blood exosomes in AD patients, aMCI patients, and controls. D-F show AUCs for Aβ42 (D), T-tau (E), and P-T181-tau (F) in CSF in AD patients, aMCI patients, and controls. $n = 73$ (AD), 71 (aMCI), and 72 (controls). Con = controls.

addition, we compared the diagnostic powers of the biomarkers between neuronal-derived exosomes and CSF and found that Aβ42, T-tau, and P-T181-tau in neuronal-derived exosomes had the same predictive capacities as their counterparts in CSF as evaluated by the DeLong test (Supplementary Fig. 3). These data indicate that Aβ42, T-tau, and P-T181-tau in blood neuronal-derived exosomes perform at the same levels as those in CSF and are potential clinical candidates for the diagnosis of AD.

3.6. Composite biomarkers

We further tested whether exosomal and CSF Aβ42, T-tau, and P-T181-tau in combination as composite biomarkers were more powerful for the diagnosis. Three composite biomarkers were evaluated by logistic regression analysis and compared by ROC analyses. Using this model, we calculated the performances of Aβ42, T-tau, and P-T181-tau in either neuronal-derived exosomes or CSF as composite-E or composite-C, respectively. In the discovery stage (data not shown in figures), composite-E showed

high AUCs for the AD/controls, AD/aMCI, and aMCI/controls comparisons (0.97, 0.86, and 0.88, $P < .0001$), which was very similar to the AUCs of composite-C for the AD/controls, AD/aMCI, and aMCI/controls comparisons (0.97, 0.87, and 0.89, $P < .0001$). There was no difference between the diagnostic powers of composite-E and composite-C as determined by the DeLong test (all $P > .05$). Similarly, in the validation data set, composite-E showed high AUCs for the AD/controls, AD/aMCI, and aMCI/controls comparisons (0.98, 0.88, and 0.85, $P < .0001$) (Fig. 5A). The AUCs of composite-C for the AD/controls, AD/aMCI, and aMCI/controls comparisons were also very high (0.98, 0.89, and 0.86, $P < .0001$) (Fig. 5B). Furthermore, the AUCs of composite-C and composite-E were significantly higher than those of each single biomarker (all $P < .01$) (Supplementary Fig. 4A-F). Importantly, comparisons of the AUCs between composite-E and composite-C showed no differences ($P > .05$). We further compared the AUCs between the discovery and validation data sets and found no differences in the AUCs of each biomarker. These data indicated that the combination of exosomal biomarkers had

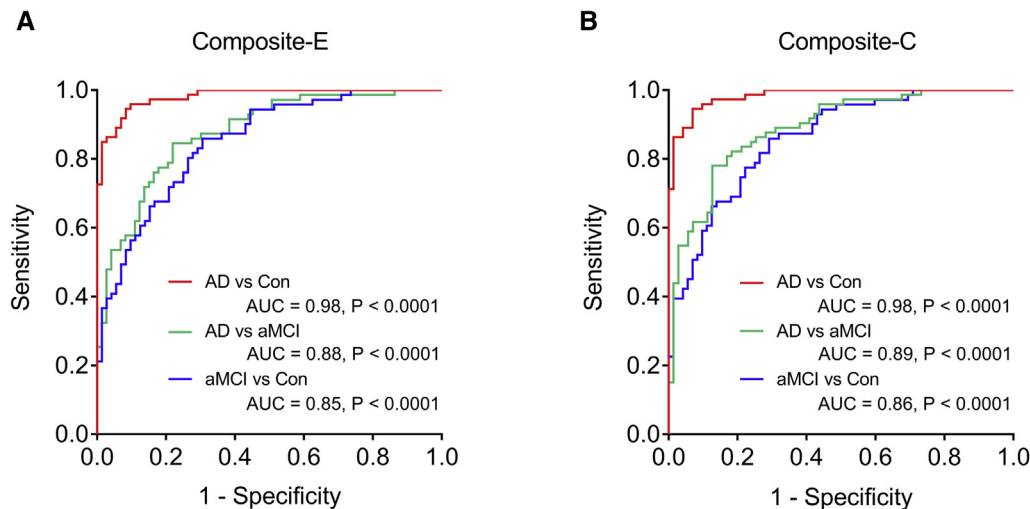


Fig. 5. Higher performance of the combined biomarkers in the validation stage. A shows ROC analyses of the combination of exosomal A β 42, T-tau, and P-T181-tau (composite-E). B shows ROC analyses of the combination of CSF A β 42, T-tau, and P-T181-tau (composite-C). $n = 73$ (AD), 71 (aMCI), and 72 (controls). Con = controls.

higher diagnostic efficiency than the individual biomarkers and that the exosomal biomarkers had the same diagnostic power as the CSF biomarkers.

4. Discussion

In the present study, we demonstrated that the blood exosomal concentrations of A β 42, T-tau, and P-T181-tau were significantly different in AD, aMCI, and control groups. We also found that the levels of these biomarkers were highly correlated with their levels in CSF. To our knowledge, this is the first time that blood neuronal-derived exosomal biomarkers were validated in CSF in a multiple center study.

We first measured the levels of A β 42, T-tau, and P-T181-tau in blood neuronal-derived exosomes to test their diagnostic power, revealing significantly different levels between AD and healthy controls. Our findings are consistent with previously published data. In a single-center study, Fiandaca et al. [7] reported that blood exosomal A β 42, T-tau, and P-T181-tau could differentiate AD and predict the disease up to 10 years before clinical onset. These data suggest that blood neuronal-derived exosomes are an ideal biomarker carrier for AD screening. However, most of these studies were conducted at a single center without CSF confirmation. In the present study, we confirmed the diagnostic performances of exosomal A β 42, T-tau, and P-T181-tau using a multicenter design with a relatively large number of samples to provide more support before wide application in the clinic. To make the results more convincing, we included two independent data sets from a Beijing center and other centers to verify the findings from the Beijing center using a cohort comprising the other centers, guaranteeing the reproducibility of our results. We then measured the levels of A β 42, T-tau, and P-T181-tau in CSF and conducted a correlation analysis. Our data revealed that the concentrations

of these biomarkers in neuronal-derived exosomes were strongly correlated with those in the CSF of AD, aMCI, and control patients. In addition, comparisons of the AUCs showed no differences between biomarkers in neuronal-derived exosomes and CSF, suggesting that exosomal biomarkers have the same power for diagnosing AD and aMCI. As previous studies have demonstrated that the levels of A β 42, T-tau, and P-T181-tau in CSF are highly correlated with postmortem pathological changes in the CNS [2,3] and quantitative amyloid PET imaging [12–14], these results demonstrate that exosomal A β 42, T-tau, and P-T181-tau reflect changes in the brain induced by AD and are therefore confirmed AD biomarkers. Our findings suggest that exosomal A β 42, T-tau, and P-T181-tau can potentially be alternatives to CSF or PET scans, facilitating the clinical diagnosis of AD and making the recruitment of subjects easier for clinical trials. Furthermore, when combining A β 42, T-tau, and P-T181-tau in neuronal-derived exosomes and CSF, ROC analyses showed very high AUCs, indicating that the composite biomarker had a greater power for diagnosing AD than the individual biomarkers.

The mechanisms underlying the correlations of these biomarkers between exosomes and CSF are complex. Exosomes are natural transport microparticles (30–100 nm) secreted by numerous cell types and can be collected from bodily fluids, such as blood. AD-associated proteins, such as A β peptides, are secreted in exosomes during their formation [15], and exosomes may spread throughout the brain by synaptic delivery [16]. The features of exosomes, including their very small size and cell membrane-like structure, allow them to easily move across the blood–brain barrier (BBB) [17]. For example, intravenously injected exosomes can cross the BBB and deliver biological materials to cells in the brain, resulting in specific changes [18]. It has been demonstrated that the BBB is broken down in AD brains

[19]. In this pathological condition, it is more evident that exosomes can easily carry A β and tau into blood through the BBB. In addition, the bulk flow of CSF may play a role in the diffusion of exosomes from the CNS to peripheral blood. CSF is produced mainly by the choroid plexuses in the brain [20], and is reabsorbed through arachnoid villi [21], cervical lymphatics [22], and cerebral lymphatic system [23]. A decrease in CSF turnover [24] and clearance of A β are reportedly associated with AD [25], and these events may allow exosomes to uptake more A β and tau and therefore increase the peripheral exosomal levels of A β and tau upon the release of exosomes from CSF. In this study, we enriched neural exosomes in peripheral blood by immunoabsorption of the NCAM antibody, which mainly represents changes in the nervous system. This procedure could partially explain the correlations of biomarker levels between the neuronal-derived exosomes and CSF.

One potential limitation of this study is its cross-sectional nature. Although we confirmed that blood exosomal A β 42, T-tau, and P-T181-tau are diagnostic biomarkers for AD, longitudinal designs would be better suited for an in-depth examination of the performance of these biomarkers. In addition, longitudinal studies should investigate the relationship between the levels of biomarkers and the decline in cognitive functions of patients. Another limitation of this study is that the participants in the aMCI group were not stratified into those with aMCI converting to AD or stable aMCI, thereby limiting the extensive application of our study to predict the progression from prodromal to probable AD.

In summary, the present study verified that peripheral blood neuronal-derived exosomal A β 42, T-tau, and P-T181-tau may reflect AD pathological changes in the brain and therefore have the capacity to diagnose AD and aMCI. However, these findings need further confirmation in longitudinal studies.

Acknowledgments

This study was supported by the Key Project of the National Natural Science Foundation of China (81530036); the National Key Scientific Instrument and Equipment Development Project (31627803); Mission Program of Beijing Municipal Administration of Hospitals (SML20150801); Beijing Scholars Program; Beijing Brain Initiative from Beijing Municipal Science & Technology Commission (Z161100000216137); CHINA-CANADA Joint Initiative on Alzheimer's Disease and Related Disorders (81261120571) and Beijing Municipal Commission of Health and Family Planning (PXM2018_026283_000002). Disclosures: The authors report no biomedical financial interests or potential conflicts of interest.

Authors' Contributions: L.J. and J.J. initiated, designed and supervised the study; Q.Q., H.Z., Q.W., Q.W., Y.L., Y.L., L.S., Y.W., Q.G.W., and F.L. performed acquisition and anal-

ysis of data; L.J., Q.Q., and J.J. drafted the manuscript; L.C., Y.D., J.Z. C.Z. F.L. S.S., and S.W. revised the manuscript.

Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jalz.2019.05.002>.

RESEARCH IN CONTEXT

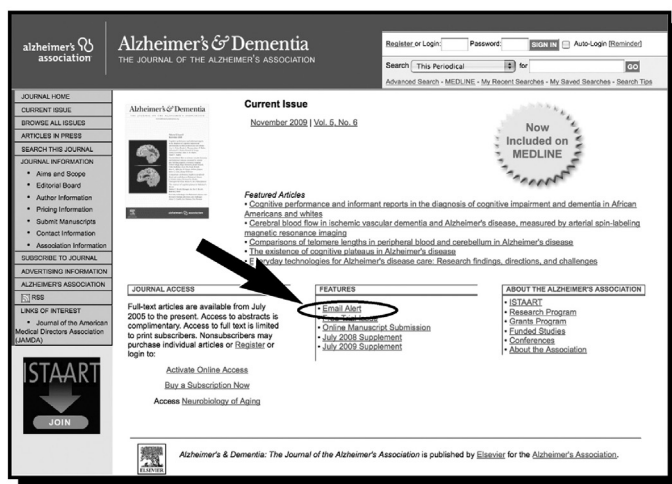
1. Systematic review: We searched PubMed using the terms “Alzheimer's disease,” “biomarker,” “exosomes,” and “CSF” since January 1, 1990. However, the concordance of these biomarkers in blood neuronal-derived exosomes with their presence in the CSF has not yet been validated.
2. Interpretation of results: Our study revealed that the concentrations of A β 42, T-tau, and P-T181-tau in neuronal-derived exosomes were closely correlated with those in CSF in AD, aMCI, and controls. Comparison of the AUCs showed no differences between biomarkers in peripheral blood neuronal-derived exosomes and CSF, suggesting that exosomal biomarkers have the same diagnostic power for AD and aMCI.
3. Future directions: The clinical usefulness of exosomal A β 42, T-tau, and P-T181-tau for AD biomarkers will be enhanced by prospective longitudinal studies. In addition, extensive samples in multiple centers cross countries will provide more support before wide application in the clinic.

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