


## RESEARCH ARTICLE

# Subsequent correlated changes in complement component 3 and amyloid beta oligomers in the blood of patients with Alzheimer's disease

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## Abstract

**INTRODUCTION:** Alzheimer's disease (AD) involves the complement cascade, with complement component 3 (C3) playing a key role. However, the relationship between C3 and amyloid beta (A $\beta$ ) in blood is limited.

**METHODS:** Plasma C3 and A $\beta$  oligomerization tendency (A $\beta$ Ot) were measured in 35 AD patients and 62 healthy controls. Correlations with cerebrospinal fluid (CSF) biomarkers, cognitive impairment, and amyloid positron emission tomography (PET) were analyzed. Differences between biomarkers were compared in groups classified by concordances of biomarkers.

Kyu Hwan Shim and Dan Yeong Kim contributed equally to this study.

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**RESULTS:** Plasma C3 and A $\beta$ Ot were elevated in AD patients and in CSF or amyloid PET-positive groups. Weak positive correlation was found between C3 and A $\beta$ Ot, while both had strong negative correlations with CSF A $\beta$ <sub>42</sub> and cognitive performance. Abnormalities were observed for A $\beta$ Ot and CSF A $\beta$ <sub>42</sub> followed by C3 changes.

**DISCUSSION:** Increased plasma C3 in AD are associated with amyloid pathology, possibly reflecting a defense response for A $\beta$  clearance. Further studies on A $\beta$ -binding proteins will enhance understanding of A $\beta$  mechanisms in blood.

**KEYWORDS**

Alzheimer's disease, amyloid beta, biomarker, complement component 3, oligomer, plasma

**1 | BACKGROUND**

Neuroinflammation is generally considered a key pathogenic contributor to Alzheimer's disease (AD) pathogenesis.<sup>1</sup> Clinical and laboratory studies have supported changes in inflammation in the early stages of AD prior to amyloid beta (A $\beta$ ) deposition in the brain tissue and the progression of neurodegeneration.<sup>2,3</sup> These inflammatory changes in the early stages of AD could occur through the complement system, a pivotal part of the inflammatory and immune systems.<sup>4,5</sup> The complement cascade can be triggered by the conventional, alternative, or lectin pathways. All three processes result in the activation of complement component 3 (C3) followed by cleaving/activating complement component 5 (C5). In the AD brain, aggregated A $\beta$  activates the complement system.<sup>1</sup> Complement component 1q (C1q) bound to A $\beta$  activates other complement components, and the activated complement system recruits and activates microglia at the locations of A $\beta$  deposition.<sup>6-8</sup> Although the role of complement C3 in AD is controversial, the final consequence would be determined by the balance of complement activation and inhibition, and the disturbance of this balance could lead to neuroinflammation and disease progression.<sup>9</sup>

Currently, positron emission tomography (PET) imaging with radioligands binding to fibrillar A $\beta$ , such as 11C-Pittsburgh compound B, and assays of cerebrospinal fluid (CSF) A $\beta$ , total tau (t-tau), and phosphory-

lated tau (p-tau) are valuable methods for accessing levels of brain amyloid burden and tauopathies.<sup>10,11</sup> Because of the high economic burden for patients, it would be necessary to develop a low-cost, non-invasive screening technique that could provide an early and precise diagnosis of AD. Blood biomarkers for the early neuropathological changes would have potential usability in both clinical and research studies, which would be of high clinical value, especially if the new disease-modifying AD therapies gain more widespread use.<sup>12</sup> Blood-based biomarkers for AD have made remarkable advances in recent years. Considerable efforts have revealed high possibilities for implementing key pathological biomarkers (A $\beta$  species and p-tau), blood markers of neurodegeneration (neurofilament light chain [NfL]), and astrocyte biomarkers (glial fibrillary acidic protein [GFAP]) in clinical laboratory practice.<sup>13-15</sup> On the other hand, inflammatory biomarkers, including complement C3, have been investigated to improve the diagnosis of AD, but no significant difference between groups or inconsistent results have been reported so far. In several CSF studies, complement C3 levels have been conflicting, with some reporting increased,<sup>16-19</sup> decreased,<sup>20</sup> or unchanged<sup>21</sup> levels in patients with AD compared to healthy controls (HC). Blood complement C3 levels have also revealed different results.<sup>22-28</sup> Although a meta-analysis reported that complement C3 concentrations in AD compared to HC were not significantly different in peripheral blood,<sup>29</sup> recent studies have revealed elevated levels of plasma complement C3 in mild cognitive impairment,<sup>30</sup> as

well as in AD.<sup>31,32</sup> Some of the discrepancies could be due to different co-morbidities, including peripheral inflammation across studies, and it is clear that more studies are needed to better understand the relationship between immune mechanisms and A $\beta$  homeostasis in humans.

A $\beta$  oligomers, known as the main toxic forms of AD, could be evaluated for their risk by measuring their oligomerization tendency through multimer detection system (MDS-OA $\beta$ ) technology, which would be used for the blood diagnosis. MDS-OA $\beta$  is a method for detecting the tendency of A $\beta$  oligomerization tendency (A $\beta$ Ot) by inducing oligomerization in blood samples with synthetic A $\beta$  spikes and then combining antibodies with overlapping epitopes. Accumulating evidence supports the importance of measuring blood A $\beta$ Ot by MDS-OA $\beta$  as a biomarker,<sup>33–40</sup> which reinforces the importance of their presence in the blood of AD patients. Thus, the quantification of complement C3 along with A $\beta$  oligomerization tendency in plasma could address the association between the two markers in the peripheral system. Changes in plasma complement C3 levels in patients with AD were examined, and the correlations between complement C3 and A $\beta$  oligomerization tendency were analyzed. In addition, CSF biomarker data and amyloid PET images were also used to investigate the association with complement C3.

## 2 | METHODS

### 2.1 | Participants and sampling

As part of the Alzheimer's Disease All Markers (ADAM) study, participants were recruited from multiple centers in South Korea from 2015 to 2017.<sup>41</sup> The AD group followed the NIA-AA (National Institute on Aging–Alzheimer's Association workgroups, 2011) criteria and met the following conditions: (1) between the ages of 50 and 80, (2) with a minimum of 6 years of education, and (3) at least 6 months of follow-up by a trained neurologist. Criteria for HC groups were: (1) a community-based population; (2) no concerns associated with memory complaints; (3) no abnormalities on the Health Screening Questionnaire; (4) within score-1 standard deviation of the age- and education-adjusted norms and a score greater than 26 of the Mini-Mental State Examination (MMSE); (5) Korean Dementia Screening Questionnaire  $\leq 6$ ; (6) intact daily living activities (Korean Instrumental Activities of Daily Living  $\leq 0.42$ ); (7) no depression (Simple Geriatric Depression Scale  $\leq 7$ ); and (8) no prior history of thyroid diseases, vitamin B12 deficiency, or folic acid deficiency.

### 2.2 | Collection and processing of CSF and plasma

Between 8:00 and 12:00 am, a routine lumbar puncture was performed at the L3/L4 or L4/L5 inter-space to acquire CSF. Within 4 hours of collecting CSF, the samples were centrifuged at 2000 x g for 10 minutes, and the supernatant was aliquoted into polypropylene tubes and

### RESEARCH IN CONTEXT

- 1. Systematic review:** In the available scientific literature, we found that some early studies have reported a significant association between complement component 3 (C3) and amyloid beta (A $\beta$ ) in Alzheimer's disease (AD). In biomarker studies, changes in blood complement C3 in patients with AD presented conflicting findings. Previous studies of examining blood complement C3 in AD were limited by the absence of a subgroup categorization, based on cerebrospinal fluid (CSF) biomarkers and correlation analysis with other biomarkers, especially A $\beta$ .
- 2. Interpretation:** The increased plasma complement C3 in the amyloid positron emission tomography and CSF biomarker-positive group, along with its correlations with plasma A $\beta$  oligomerization tendency, CSF A $\beta$ 42, and cognitive decline, supported the involvement of complement cascade in AD progression. These findings provide valuable insights into the role of blood complement C3 related to A $\beta$  in AD.
- 3. Future directions:** Longitudinal studies will be needed to assess the temporal changes in C3 levels and elucidate potential associations with disease trajectories and treatment responses.

stored at  $-80^{\circ}\text{C}$  until use. Blood was collected in 10 mL ethylenediaminetetraacetic acid tubes and centrifuged at 1500 x g for 10 minutes at room temperature. The plasma was aliquoted into polypropylene tubes and stored at  $-80^{\circ}\text{C}$  until further analysis.

### 2.3 | Amyloid PET acquisition and processing

Amyloid (fluorine 18 [18F]–florbetaben) PET was executed on consenting individuals.<sup>41</sup> Briefly, a PET scan was conducted 90 minutes after an injection of approximately 300 MBq of 18F-florbetaben. Amyloid PET was defined as positive when the visual assessment of PET was scored as 2 or 3 on the Brain A $\beta$  Plaque Load (BAPL) grading system.

### 2.4 | CSF and plasma analysis

Commercial enzyme-linked immunosorbent assay (ELISA) kits (INNOTEST  $\beta$ -AMYLOID[1-42], INNOTEST hTAU-Ag, and INNOTEST PHOSPHO-TAU[181P], Fujirebio Europe) were used to quantify the amounts of CSF A $\beta$ <sub>42</sub>, t-tau, and p-tau at threonine 181 (p-tau<sub>181</sub>). Plasma complement C3 was measured using a commercial human C3 ELISA kit (Abcam, ab108822) according to the manufacturer's instructions. Briefly, 25  $\mu\text{L}$  of diluted plasma and 25  $\mu\text{L}$  of biotinylated complement C3 were added to wells coated with

a complement C3-specific antibody and incubated for 2 hours at room temperature. After washing six times with wash buffer, 50  $\mu$ L of streptavidin-peroxidase conjugate was applied, and additional incubation was performed at room temperature for 30 minutes. The signal of tetramethylbenzidine (TMB) solution, which turned blue color by the catalysis of streptavidin-peroxidase, was measured in a microplate reader (Victor3, PerkinElmer) after adding the stop solution.

A $\beta$  oligomerization tendency in plasma was measured using the Oligomerized Amyloid- $\beta$  Measurement Kit (MDS-OA $\beta$  Test; PeopleBio Inc.) following the manufacturer's protocol.<sup>33,34</sup> Briefly, 25  $\mu$ L of plasma sample was added to the mixed buffer in the kit and incubated at 37°C for 1 hour. Then, 100  $\mu$ L of prepared samples were added to the 96-well plate and incubated for 1 hour at room temperature. After washing the plate three times with wash buffer, 100  $\mu$ L of detection solution with provided antibody was added to each well and incubated for 1 hour at room temperature. Next, 100  $\mu$ L of TMB was added to each well after washing it in the same way. After incubating for 15 minutes at room temperature, 50  $\mu$ L of stop solution was added, and the absorbance signal was read at 450 nm wavelength using a microplate reader (Victor3, PerkinElmer).

## 2.5 | Statistical analysis

All statistical analyses and visualizations were performed using the commercial software GraphPad Prism 8 software (GraphPad). *t* tests and Mann-Whitney *U* tests were used to evaluate statistical differences between two groups with normal and non-normal distributions, respectively. A chi-square test was conducted to assess statistical differences in sex and visual amyloid PET readings. Spearman correlation was applied to investigate the relationship between biomarkers and clinical parameters. A statistically significant value was considered when the *P* value was less than 0.05. In the receiver operator characteristic (ROC) analysis, the area under the curve (AUC) was examined to assess the accuracy of the biomarker diagnostic value. Cut-offs were determined based on the point where sensitivity and specificity were maximized.

## 3 | RESULTS

### 3.1 | Demographics and levels of CSF and plasma biomarkers

Demographics and clinical features of the individuals who participated in this study are listed in Table 1. The AD and HC groups showed no statistically significant differences in age and sex distribution. As expected, MMSE and CSF biomarker results, including A $\beta$ <sub>42</sub>, t-tau, and p-tau<sub>181</sub>, were different between the AD and HC groups. Amyloid PET was carried out on a subset of participants, revealing a high proportion of positive individuals in the AD group.

### 3.2 | Plasma complement C3 and A $\beta$ Ot in the classified groups

In the preliminary study, a limited number of samples were used to measure levels of complement components such as C3, C3b, C8, and C9. The analysis revealed that only C3 showed a significant difference between the AD and HC groups (Figure S1 in supporting information). While larger sample sizes are necessary for accurate conclusions, C3b, C8, and C9 were excluded from further analysis in this study. Plasma complement C3 and A $\beta$ Ot were examined in the AD and HC groups. In the clinically diagnosed group, both plasma complement C3 and A $\beta$ Ot were significantly higher in patients with AD compared to HC (Table 1 and Figure 1A). Using ROC curve analysis to confirm the diagnostic accuracy of biomarkers, plasma complement C3 and A $\beta$ Ot had an AUC of 0.761 (sensitivity = 95.2%; specificity = 40.5%) and 0.710 (sensitivity = 61.3%; specificity = 76.2%), respectively. Combining the two biomarkers slightly increased the AUC value to 0.773 (sensitivity = 74.2%; specificity = 74.3%). In machine learning, support vector machines exhibited a mean ROC of 0.68  $\pm$  0.29 for C3 and 0.71  $\pm$  0.18 for A $\beta$ Ot. The combined biomarker demonstrated a mean ROC value of 0.75  $\pm$  0.19 (Figure S2 in supporting information). After that, complement C3, A $\beta$ Ot, and combined biomarkers were also found to be significantly higher in the amyloid PET-positive group compared to the negative group (Figure 1B and Table S1 in supporting information). In the final classification method, the groups were divided according to the CSF biomarkers. Patients with AD who were positive for CSF A $\beta$ <sub>42</sub> and non-AD who were negative for CSF A $\beta$ <sub>42</sub> but positive for t-tau or p-tau<sub>181</sub> were compared to the HC group in which all CSF biomarkers were negative. Interestingly, plasma complement C3 was elevated only in the AD group compared to non-AD and HC (Figure 1C and Table S2 in supporting information). Plasma A $\beta$ Ot also revealed higher values in AD, and the combined biomarkers also had statistically significant differences in the AD group compared to non-AD and HC.

### 3.3 | Correlations of plasma complement C3 with other biomarkers and cognitive performance

Plasma complement C3 and A $\beta$ Ot revealed a positive correlation trend without statistical significance (Spearman rho [ $\rho$ ] = 0.181, *P* = 0.076; Figure 2A), and the trend slightly improved in the AD group ( $\rho$  = 0.296, *P* = 0.084; Figure S3A in supporting information). Interestingly, both plasma complement C3 ( $\rho$  = -0.358, *P* = 0.006) and A $\beta$ Ot ( $\rho$  = -0.355, *P* = 0.006) similarly had a negative correlation with CSF A $\beta$ <sub>42</sub>, but not when the AD or HC group was examined separately (Figure S3A). MMSE and Clinical Dementia Rating (CDR), which indicates cognitive performance, appeared to have a strong negative correlation with plasma biomarkers (Figure 2B). Compared to MMSE, plasma complement C3 and A $\beta$ Ot had correlation coefficients of -0.398 (*P* < 0.0001) and -0.284 (*P* = 0.005), respectively. The combined biomarker showed the strongest negative correlation with MMSE, with a correlation coefficient of -0.418 (*P* < 0.0001). Plasma complement C3 and A $\beta$ Ot

**TABLE 1** Demographic, clinical, and biomarker data summarized by diagnostic group.

	AD <sup>a</sup> (n = 35)	HC <sup>a</sup> (n = 62)	P
Age	62.0 (59.0–74.0)	63.0 (58.8–67.3)	0.784 <sup>b</sup>
Sex (male/female)	15/20	22/40	0.518 <sup>c</sup>
MMSE	19.0 (13.0–22.0)	28.0 (27.8–30.0)	< 0.0001 <sup>b</sup>
CSF A $\beta$ <sub>42</sub> (pg/mL)	360 (229–689)	1028 (893–1228)	< 0.0001 <sup>b</sup>
CSF t-tau (pg/mL)	571 $\pm$ 285	246 $\pm$ 122	< 0.0001 <sup>d</sup>
CSF p-tau <sub>181</sub> (pg/mL)	76.3 $\pm$ 33.2	47.0 $\pm$ 19.9	< 0.0001 <sup>d</sup>
Plasma complement C3 (mg/mL)	0.777 $\pm$ 0.231	0.593 $\pm$ 0.110	< 0.0001 <sup>d</sup>
Plasma A $\beta$ Ot (ng/mL)	0.960 (0.880–1.120)	0.860 (0.738–0.950)	0.001 <sup>b</sup>
Complement C3 x A $\beta$ Ot	0.743 $\pm$ 0.334	0.481 $\pm$ 0.138	< 0.0001 <sup>d</sup>
Amyloid PET (+/-) <sup>e</sup>	23/7	1/27	< 0.0001 <sup>c</sup>

Abbreviations: A $\beta$ <sub>42</sub>, amyloid beta 1-42; A $\beta$ Ot, amyloid beta oligomerization tendency; AD, Alzheimer's disease; amyloid-PET, 18F-florbetaben positron emission tomography; CSF, cerebrospinal fluid; HC, healthy control; MMSE, Mini-Mental State Examination; p-tau<sub>181</sub>, phosphorylated tau 181; t-tau, total tau.

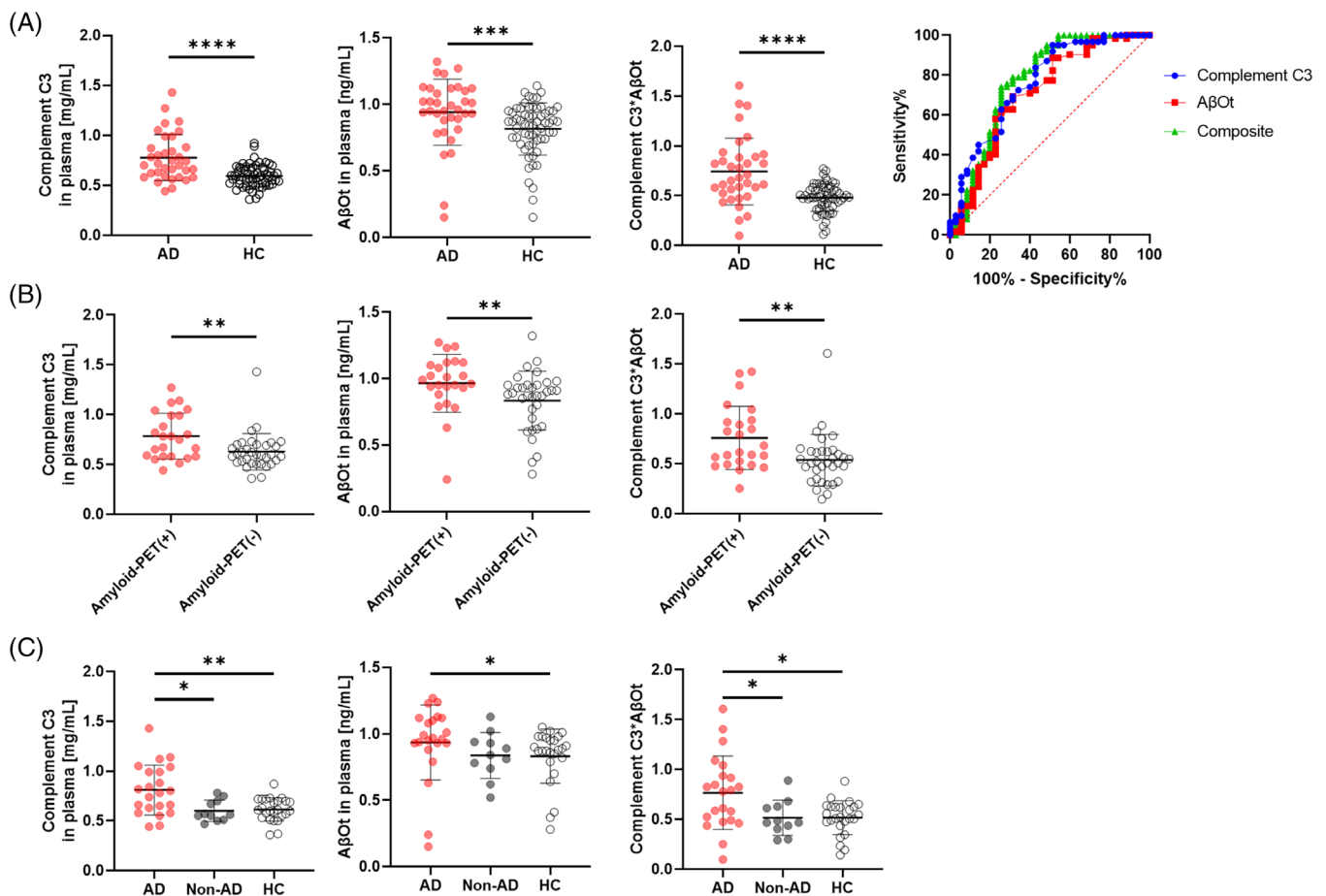
<sup>a</sup>Data are shown as median (interquartile) or mean  $\pm$  standard deviations.

<sup>b</sup>Mann-Whitney *U* test.

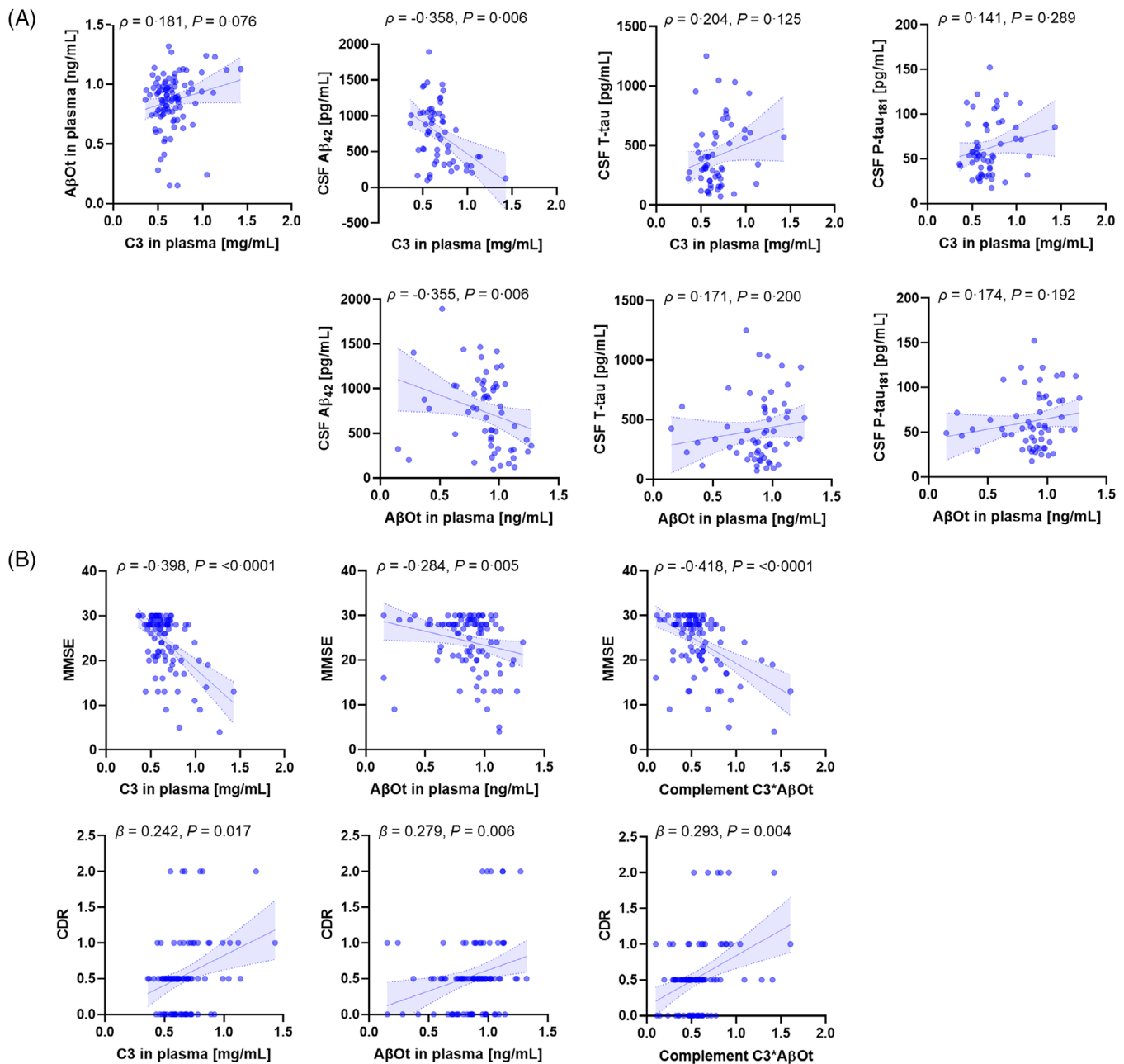
<sup>c</sup>Chi-square test.

<sup>d</sup>t test.

<sup>e</sup>A limited number of participants underwent for amyloid PET scanning.



**FIGURE 1** Plasma biomarker levels in each group classified by clinical, imaging, and CSF biomarker data. A, Scatter plots and ROC curves of plasma complement C3, A $\beta$ Ot, and combined biomarkers in the clinically diagnosed groups. Individual levels of biomarkers in groups classified according to the amyloid PET (B) or CSF biomarkers (C). All data were given as means  $\pm$  SD \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001. A $\beta$ Ot, amyloid beta oligomerization tendency; AD, Alzheimer's disease; amyloid PET, 18F-florbetaben positron emission tomography; C3, component 3; CSF, cerebrospinal fluid; HC, healthy control; non-AD, non-Alzheimer's disease; ROC, receiver operating characteristic; SD, standard deviation



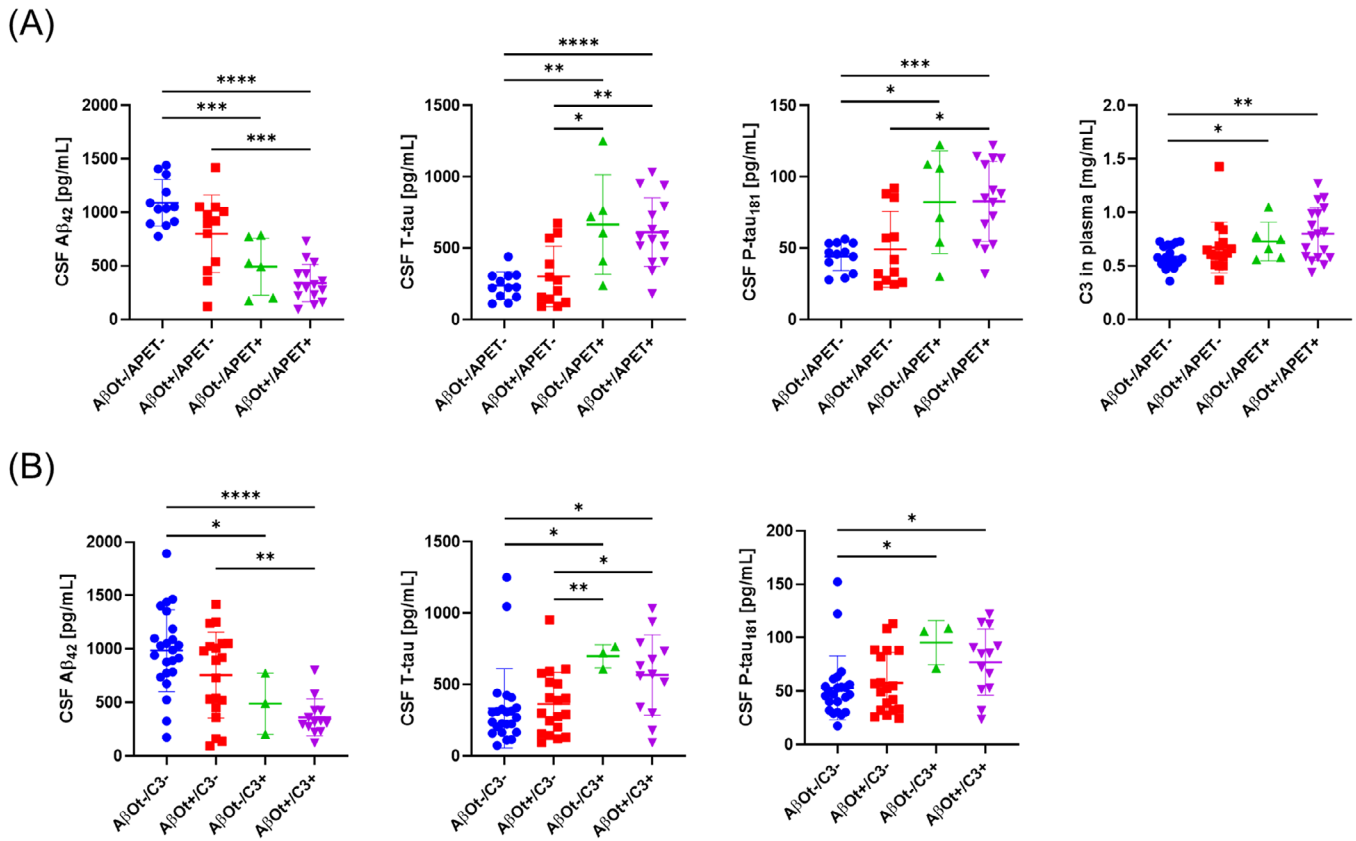
**FIGURE 2** Association of plasma complement C3 with other biomarkers and cognitive performance. A, Scatter plots of plasma and CSF biomarkers. B, Correlation of plasma biomarkers with MMSE and CDR. The dashed line means the 95% prediction interval of the regression line. Aβ<sub>42</sub>, amyloid beta 1-42; AβOT, amyloid beta oligomerization tendency; C3, component 3; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; p-tau<sub>181</sub>, phosphorylated tau 181; t-tau, total tau

showed significant correlations with CDR (C3,  $\beta = 0.242, P = 0.017$ ; AβOT,  $\beta = 0.279, P = 0.006$ ), with the combined biomarker exhibiting a stronger association ( $\beta = 0.293, P = 0.004$ ).

### 3.4 | Differences in biomarkers in groups divided by the concordance of the two different biomarkers

Changes in biomarkers were observed by categorizing the groups according to the concordance of the two different biomarkers. Individ-

uals were divided into positive (+) and negative (-) categories based on whether the two biomarker levels were higher or lower than the cut-offs (Table S3 in supporting information), respectively. According to the diagnostic agreement between plasma AβOT and amyloid PET, they were further divided into four groups. In the group with both plasma AβOT and amyloid PET negative, all biomarkers were within the normal range (Figure 3A and Table S4 in supporting information). On the other hand, the AβOT (+)/amyloid PET(-) group revealed decreased CSF Aβ<sub>42</sub> and increased t-tau, p-tau<sub>181</sub>, and plasma complement C3. In the AβOT(-)/amyloid PET(+) and AβOT(+)/amyloid PET(+)



**FIGURE 3** Changes of biomarkers in groups. A, CSF biomarkers and plasma complement C3 levels in groups classified by plasma AβOt and amyloid PET. B, CSF biomarkers according to the concordance between plasma AβOt and complement C3. All data were shown as means ± SD \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001. Aβ<sub>42</sub>, amyloid beta 1-42; AβOt, amyloid-beta oligomerization tendency; APET, 18F-florbetaben positron emission tomography; C3, component 3; CSF, cerebrospinal fluid; p-tau<sub>181</sub>, phosphorylated tau 181; SD, standard deviation; t-tau, total tau

groups, all biomarker results were concordant with AD status and were statistically different from the AβOt(-)/amyloid PET(-) group.

Subsequently, groups were compared to the CSF biomarkers based on the concordance of plasma AβOt and complement C3. All CSF biomarkers were distributed at normal levels when both biomarkers were negative (Figure 3B and Table S5 in supporting information). In the plasma AβOt(+)/C3(-) group, CSF biomarkers tended to observe decreased Aβ<sub>42</sub> and increased t-tau and p-tau<sub>181</sub>. In the plasma AβOt(-)/C3(+) and Plasma AβOt(+)/C3(+) groups, most CSF biomarkers showed AD-like results, the prevalence of which was statistically different from the group in which both plasma AβOt and complement C3 were negative.

## 4 | DISCUSSION

Reliable blood-based biomarkers, such as Aβ, tau, NFL, and GFAP, have been reported to aid in the early diagnosis of AD.<sup>42</sup> Still, no meaningful biomarker has been established from complement components, despite the significant role of the complement system in the pathophysiology of AD. For the first time, to our knowledge, the associations of plasma complement C3 with plasma AβOt and CSF biomarkers

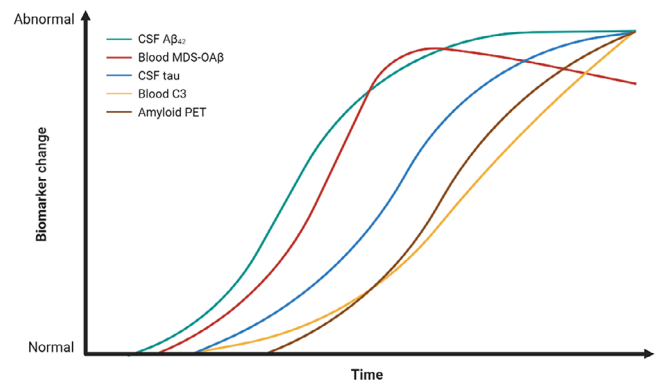
were investigated in this study. Increased levels of plasma complement C3 and AβOt were observed in patients with AD compared to HC, with a positive correlation between the two biomarkers and a negative correlation with CSF Aβ<sub>42</sub> and cognitive performance. Although improvement in diagnostic accuracy through biomarker integrations was limited, it could be noted that complement C3 was closely related to Aβ pathology in blood.

Previous studies have typically revealed higher levels of CSF complement C3 in patients with AD. Complement C3 is elevated in the brains of AD patients, and the intact complement C3 and processed complement C3 (C3b, iC3b, and C3c) are higher in the CSF of AD patients than in cognitively normal individuals.<sup>43</sup> Furthermore, increased complement C3 in the CSF correlates well with the severity of cognitive status in autopsy-confirmed AD patients.<sup>16</sup> On the other hand, plasma complement C3 is also increased and correlates well with atrophy of hippocampal volume in AD patients.<sup>26</sup> Comparison between individuals with high and low brain amyloid burden has revealed that plasma complement C3 is associated with brain amyloid deposition.<sup>44,45</sup> In this study, plasma complement C3 was increased in all groups with clinically diagnosed AD, amyloid PET positivity, and CSF Aβ<sub>42</sub> positivity, supporting that elevated plasma complement C3 concentration was closely associated with amyloid

pathology. The correlation of plasma complement C3 with A $\beta$ Ot and CSF A $\beta$ <sub>42</sub> strengthened the connections between complement C3 and A $\beta$ . Although the sample size was limited, the plasma complement C3 showed a different aspect from the CSF biomarker when analyzed by grouping into AD and HC (Figure S3). Plasma complement C3 tended to be positive with CSF A $\beta$ <sub>42</sub> and to be negative with CSF tau in HC, but these correlations were altered in AD. Perhaps plasma complement C3 plays different roles in maintaining brain homeostasis at the physiological and pathological stages. In HC, complement activation may play a protective role in controlling neuroinflammation and preventing protein accumulation. However, in AD, chronic inflammation and proteinopathies may disrupt the normal function of complement activation, resulting in a dysregulation or overactivation of the complement system that could contribute to the pathogenesis of AD. Given that the correlations of CSF complement C3 with CSF A $\beta$ <sub>42</sub> and p-tau<sub>181</sub> differ according to apolipoprotein E (APOE) genotype,<sup>21</sup> it could be speculated that additional accurate analyses of plasma complement C3 would be possible if APOE genotypes were obtained and applied as a covariate, which was not possible in the current study.

The complement system could sequester A $\beta$  through innate immune clearance mechanisms such as microglia phagocytosis in the brain.<sup>46,47</sup> Although microglial activation could exacerbate AD pathology, these sequestering proteins in the blood may have neuroprotective capabilities and help mitigate the pathological progression of AD through A $\beta$  clearance, especially in the peripheral system. Besides, it is possible that the complement system mainly contributes to A $\beta$  elimination from the blood. A study reported an A $\beta$  clearance mechanism of the complement system in blood when complement components are abundant.<sup>48</sup> A $\beta$  was captured by complement receptor 1 in erythrocytes after being opsonized by complement components. Then, hepatic Kupffer cells would recognize and degrade erythrocytes bound with A $\beta$ . Consequently, because blood complement C3 was primarily produced in the liver, it was anticipated that activation of Kupffer cells by this mechanism may result in complement C3 over-production. Still, as complement C3 is also generated in other tissues or cell types, further investigation should be undertaken to address the main reason for elevated plasma complement C3 levels in patients with AD.

The change point of plasma complement C3 was shown to start similarly to tau pathology and after CSF A $\beta$ <sub>42</sub>. In Figure 3A, plasma complement C3 tended to be marginally elevated in the A $\beta$ Ot(+)/amyloid PET(-) group where CSF A $\beta$ <sub>42</sub> was slightly decreased, similar to CSF t-tau or p-tau<sub>181</sub>. Moreover, significant increases in CSF t-tau or p-tau<sub>181</sub> appeared only in plasma complement C3-positive groups in Figure 3B, demonstrating that plasma complement C3 and CSF tau pathologies occurred at similar time periods. The absence of elevated plasma complement C3 levels in the non-AD group, in which only CSF t-tau or p-tau<sub>181</sub> increased (Figure 1C), indicates that the change in complement C3 levels needed to be preceded by amyloid rather than tau pathology. Meanwhile, a study suggested a model in which A $\beta$  mediates the effect of complement C3 on tau pathology, supporting the concept that the amyloid cascade modulates inflammatory processes, particularly downstream pathomechanisms via activation of the complement cascade.<sup>21</sup> Comprehensively, we depicted the predicted temporal pat-



**FIGURE 4** The temporal staging of biomarker abnormalities during AD progression. Combining our findings and previous reports,<sup>49–51</sup> the graph demonstrates an approximate pattern of how plasma A $\beta$ Ot and complement C3 change with other AD-related core biomarkers over time. Change in CSF A $\beta$ <sub>42</sub> start first the earliest detectable biomarker change mirroring AD pathology, followed by an abnormality in plasma A $\beta$ Ot. Shortly prior to the alteration of amyloid PET, CSF t-tau and p-tau<sub>181</sub> may turn positive. Simultaneously or shortly thereafter, plasma complement C3 increase at a low rate with CSF tau. A $\beta$ <sub>42</sub>, amyloid beta 1-42; A $\beta$ Ot, amyloid-beta oligomerization tendency; AD, Alzheimer's disease; amyloid PET, 18F-florbetaben positron emission tomography; C3, component 3; CSF, cerebrospinal fluid; p-tau<sub>181</sub>, phosphorylated tau 181; t-tau, total tau

tern model of AD-related biofluid biomarkers in Figure 4. CSF A $\beta$ <sub>42</sub> was the earliest biomarker to change, corroborating previously reported hypothetical models and the amyloid cascade theory.<sup>49,52</sup> Considering the decrease in CSF A $\beta$ <sub>42</sub> in the A $\beta$ Ot(+)/amyloid PET(-) group, it was inferred that plasma A $\beta$ Ot changed at a similar time to CSF A $\beta$ <sub>42</sub>. In addition, we observed the tendency of plasma A $\beta$ Ot to decrease as the severity of the disease increased in a previous study.<sup>35</sup> The change point of plasma complement C3 was at a time similar to CSF tau, but the slope of plasma complement C3 was relatively lower due to the limited increases in level width from amyloid PET(-) to amyloid PET(+) and the substantial overlaps between the two groups. Even though other studies showed inconsistent results when amyloid PET was compared to CSF tau pathology,<sup>50,53</sup> we assumed that amyloid PET abnormalities began last because CSF t-tau and p-tau<sub>181</sub> and plasma complement C3 tended to increase in the A $\beta$ Ot(+)/amyloid PET(-) group. However, the presented model should be verified in large cohorts or longitudinal studies.

The discordance issues between fluid and imaging biomarkers have been often raised. A longitudinal study suggested that biomarker disagreement was a general phase in the natural A $\beta$  accumulation and that two distinct pathways toward A $\beta$  pathology (CSF-first or PET-first) could be mentioned.<sup>54</sup> Changes in plasma biomarker abnormalities, on the other hand, preceded amyloid PET in studies on plasma biomarker trajectories.<sup>50</sup> However, several reports revealed that amyloid PET first turned to positive deposition, despite no change in plasma biomarkers.<sup>55–58</sup> The reasons for this discordance remained obscure. In the present study, there were also individuals with positive amyloid PET with negative plasma A $\beta$ Ot. It is noteworthy that



most of the CSF biomarker levels in this group belonged to the AD continuum in Figure 3A. Similarly, a study also revealed a significant decrease in CSF A $\beta$ <sub>42</sub> in the group in which plasma p-tau<sub>181</sub> was negative with positive in tau PET.<sup>55</sup> This evidence suggested that the plasma biomarker(-)/amyloid PET(+) group may include patients with sufficiently advanced AD progression and that plasma biomarkers were limited in reflecting brain abnormalities. These biomarker disagreements, which could be regarded as misdiagnosis of plasma biomarkers, may have resulted from the pathological or physiological responses of our body rather than from diagnostic technology. The first hypothesis was the difference in the transfer of A $\beta$  or tau from the brain to the blood, which could be influenced by variables such as the integrity of the blood-brain barrier, the clearance system in the brain, or the extent of protein deposits in the brain. The second hypothesis was the presence of interfering factors in the blood. Additionally, interacting proteins with A $\beta$ , such as C3 and other members of the complement protein family,<sup>59</sup> may eliminate blood A $\beta$  or interfere with the measurement process. Therefore, research on A $\beta$ -bound proteins, including complement C3, would be necessary, and surrogate biomarkers should be introduced in the future to improve the accuracy of blood-based AD diagnosis.

There are several limitations in our study. First, the number of participants in the cohort was small due to the high resistance to CSF tapping and the cost of amyloid PET. Correlation analyses using numerous biomarkers strengthened the reliability of the cohort and measurement data to compensate for this. Still, verification research in a large cohort should be carried out in the future. Next, although APOE genotype is an important factor for both A $\beta$  and complement C3, sufficient data could not be obtained due to the low rate of consent from the participants. The associations between complement C3 and A $\beta$  in blood were expected to be understood by comparing the classification according to APOE genotype in a large cohort. Another limitation was that the measured plasma samples by A $\beta$ Ot were in storage for more than 5 years from collection. According to a previous study, the value of plasma A $\beta$ Ot could be affected after 4 years of storage.<sup>60</sup> This could explain why the diagnostic performance and associations with CSF A $\beta$ <sub>42</sub> were lower in this study compared to previous studies. Finally, the limited clinical data may not fully present any influence on plasma complement C3 levels, such as inflammation or infections. In the future, accurate criteria for enrolling participants should be established.

## 5 | CONCLUSION

Plasma complement C3 seems to increase after CSF A $\beta$ <sub>42</sub> or plasma A $\beta$ Ot changes in AD patients. The increased plasma complement C3 may be a protective consequence of the clearance system after increased A $\beta$  in the periphery system. To understand the changes in the blood system caused by abnormalities of A $\beta$ , studies on proteins that directly interact with A $\beta$ , such as complement C3, would be needed. In particular, during the analysis process, these A $\beta$ -binding proteins may cause significant interference with plasma A $\beta$  measurement. Profiling of A $\beta$  binding proteins, including complement C3, would be needed to

accurately interpret the diagnostic results. For instance, the diagnosis discordance, in which amyloid -PET was positive with plasma A $\beta$ Ot in the normal range, could help accurately understand the current condition by considering the increased plasma complement C3. Additional evidence for proteins interacting with AD-related core biomarkers in blood will increase the interpretability of AD biomarker results and may also lead to novel biomarkers and potentially even therapeutic targets.

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## CONFLICT OF INTEREST STATEMENT

H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). All other authors declare no conflicts of interest. Author disclosures are available in the [supporting information](#).

## CONSENT STATEMENT

This study obtained ethical approval from the ethics committees of all participating institutions, and informed consent was obtained from all participants for the use of their clinical data for research purposes.

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