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Assessing Candidate Serum Biomarkers for Alzheimer's Disease: A Longitudinal Study

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Abstract

Because of the growing impact of late onset cognitive loss, considerable effort has been directed toward the development of improved diagnostic techniques for Alzheimer's disease (AD) that may pave the way for earlier (and more effective) therapeutic efforts. Serum-based biomarkers are the least expensive and invasive modality for screening and routine monitoring. We systematically reviewed the literature to assemble a list of serum biomarkers relevant to AD. In parallel, we conducted a proteomic LC-MS/MS analysis of serum collected from neurologically normal subjects and subjects with mild cognitive impairment (MCI) and early AD ($n = 6$ in all). Complement C3 and alpha-2-macroglobulin were identified from both the literature review and our proteomic screen for further validation. For these two candidates, ELISA was performed on serum collected from a small independent cohort of subjects for longitudinal analysis. Serum was serially collected from neurologically normal subjects ($n = 5$) and subjects with MCI who were subsequently followed for a period of two years ($n = 5$) and regrouped into stable MCI and progressive MCI or AD ($n = 6$). The ability of each marker to predict which subjects with MCI would progress to dementia and which would remain cognitively stable was assessed. Patients with probable cerebral amyloid angiopathy were also identified ($n = 3$). This preliminary analysis tested the most-promising serum protein biomarkers for AD and we concluded that none are yet ready for use in the clinical diagnosis and management of dementia. However, a more thorough assessment in longitudinal studies with higher statistical power is warranted.

Keywords

Alpha₁-antichymotrypsin; C1 inhibitor; cerebral amyloid angiopathy; complement C4; plasma; prospective

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INTRODUCTION

A key challenge in the current clinical management of Alzheimer's disease (AD) is the lack of an accurate biomarker to reliably diagnose the disease. The clinical features of AD overlap with a number of other dementing pathologies and conclusive diagnosis is only achieved at autopsy (or in the rare case of a brain biopsy). In practice, the clinical diagnosis of AD is accurate in 65–70% of cases [1]. Additionally, clinical diagnosis is based upon decline in cognitive and executive function—at this stage, the pathology may be irreversible. For these reasons, considerable effort has been targeted toward identifying a clinically useful biomarker, and numerous candidates have been suggested. Biomarkers based on neuroimaging findings and biochemical analysis of blood and cerebrospinal fluid (CSF) have been studied in some depth and offer considerable promise for improving the diagnostic criteria for AD [2]. Of these, serum biomarkers have a number of ideal properties, including minimally invasive collection and lower expense compared to both CSF and neuroimaging studies, which make them an important continuing research focus.

A number of candidate proteins in plasma have been identified as potential biomarkers, but none has yet achieved clinical usefulness. The ability to analyze hundreds and even thousands of proteins simultaneously through proteomic techniques has greatly accelerated the search for biomarkers, but thorough validation of candidates remains a key challenge. The characteristics of an ideal serum biomarker are: 1) clear separation (either increase or decrease) between AD cases and age-matched neurologic controls; 2) the change in biomarker level occurs at an early stage of AD (preferably pre-clinically); 3) the change is relatively selective for AD; and 4) results are reproducible across multiple methods and in numerous cohorts of AD patients (Fig. 1) [1, 3, 4]. Finally, an ideal biomarker should be directly connected to some relevant central nervous system (CNS) pathology [5].

The objective of the current study is two-fold: to assess the reproducibility of proteomic-based candidate biomarkers previously reported in the literature and to determine if any of these candidates are able to accurately predict which subjects with mild cognitive impairment (MCI) are most at-risk of subsequent cognitive decline to dementia over the following two years. Currently, the field of AD biomarker discovery lacks a molecular marker that accurately reflects the longitudinal changes in disease progression [1]. Several candidates were identified in the current study, which reliably distinguished between serum from AD subjects (at the time of progression) and neurologically normal cases with the unbiased proteomics approach using LC-MS/MS. We further assessed the degree to which these promising candidate biomarkers could distinguish between patients with MCI who would progress to AD and those who would remain cognitively normal over a minimum of two years, adding an important prospective component to the field of serum biomarker research for AD.

MATERIALS AND METHODS

Literature search and assembly of candidate plasma biomarkers

A systematic review of the literature was conducted by searching PubMed-Medline and ISI Web of Science databases using combinations of the following keywords: Alzheimer's or Mild cognitive impairment; plasma or serum; proteomics or the name of a specific protein of interest. To assemble a list of candidate plasma biomarkers that were well documented in AD pathophysiology, we focused on references from primary proteomics studies in plasma or serum and serum protein analyses; we did not include neuroimaging studies or studies in CSF or other bodily fluids/tissues. Additionally, we reviewed the citation lists from each paper to supplement the literature search. Because the identification of an ideal biomarker should be consistent across multiple methods, we included all studies analyzing plasma or

serum proteins by any appropriate method. In addition, we also searched the literature for proteomic studies in plasma or serum in other neurological diseases to assess the specificity of candidate biomarkers (CBMs) to discriminate Alzheimer's-type dementia from other neuropathologies.

Patient recruitment and plasma collection

To assess the reproducibility of the CBMs, serum was collected from patients enrolled in the study from community-based recruitment as previously described [6, 7]. Briefly, individuals with subjective memory complaints were screened and those classified as MCI were enrolled in the study. The diagnosis of MCI was established according to Mayo Clinic criteria including objective confirmation of an isolated memory complaint (Clinical Dementia Rating = 0.5) in a patient with otherwise normal cognition and capable of performing normal activities of daily living [8].

Additionally, a group of aged neurologic controls were enrolled. Neurologic controls were without subjective memory complaints and within normal limits on neuropsychological testing consisting of a Global Clinical Dementia Rating score of 0. All participants consented to detailed neuropsychological testing, blood donation, and neuroimaging as part of a previously published observational/longitudinal study [6]. Serum collection and cognitive evaluations were performed at approximately six month intervals and neuroimaging was conducted approximately yearly over the five year span of the study. From this cohort, 24 subjects were selected at random from the following groups: neurologic controls ($n = 6$), MCI patients whose cognitive status remains unchanged for the duration of the study (stable MCI; $n = 6$), patients who progressed to clinical dementia without radiographical evidence of microbleeds (AD only; $n = 6$), and patients who progressed to clinical dementia with radiographical evidence of cerebral amyloid angiopathy (AD with CAA; $n = 6$).

Proteomic LC-MS/MS

Blood specimens were stored at 4°C overnight to allow clotting, followed by a centrifugation at 1800 g, 4°C for 10 min to remove cellular elements and the majority of clotting factors. 1 ml serum sample from each patient was resuspended in 20 µL of 8 M urea, reduced by 10 mM dithiothreitol (DTT) for 30 min at 37°C, and then alkylated by 50 mM iodoacetamide for 20 min at room temperature. The concentrated urea in the sample was diluted to a final concentration of 2 M, and the proteins were digested by trypsin at 37°C for 6 h in a buffer containing ammonium bicarbonate (50 mM, pH 9). The digestion mixture was then acidified by adding glacial acetic acid to a final concentration of 2% and desalted by ZipTip (Millipore). The peptides were analyzed by high sensitive reversed-phase liquid chromatography coupled nanospray tandem mass spectrometry (LC-MS/MS) using an LTQ-XL mass spectrometer (Thermo Fisher). The reversed-phase LC column was slurry-packed in-house with 5 µm, 200 Å pore size C₁₈ resin (Michrom BioResources, CA) in a 100 µm i.d.×10 cm long piece of fused silica capillary (Polymicro Technologies, Phoenix, AZ) with a laser-pulled tip. After sample injection, the column was washed for 5 min with mobile phase A (0.1% formic acid), and peptides were eluted using a linear gradient of 0% mobile phase B (0.1% formic acid, 80% acetonitrile) to 50% B in 120 min at 200 nL/min, then to 100% B in an additional 10 min for the proteomics analysis. The LTQ-XL mass spectrometer was operated in a data-dependent mode in which each full MS scan was followed by five MS/MS scans where the five most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation using a normalized collision energy of 35%. The Dynamic Exclusion Time was 60 s, and the Dynamic Exclusion Size was 200. Tandem mass spectra collected by Xcalibur were searched against the NCBI human protein database using SEQUEST (Bioworks software from ThermoFisher,

version 3.3.1) with full tryptic cleavage constraints, static cysteine alkylation by iodoacetamide, and variable methionine oxidation. Mass tolerance for precursor ions was 0.5 Da and mass tolerance for fragment ions was 0.25 Da. The SEQUEST search results of proteomics data were filtered by the criteria “Xcorr versus charge 1.9, 2.2, 3.0 for 1+, 2+, 3+ ions; DCn >0.1; probability of randomized identification of peptide <0.01”. Confident peptide identifications were determined using these stringent filter criteria for database match scoring followed by manual evaluation of the results. The “false discovery rate (FDR)” was 1% estimated by searching a combined forward-reversed database as described by Elias [9]. The SEQUEST search results were exported to Excel files and spectral counts compared as estimates of protein abundance.

Longitudinal analysis with competitive ELISA

ELISA analysis was conducted on serum from a separate longitudinal cohort selected with the same diagnostic testing described above; the four groups similarly consisted of neurologic controls ($n = 5$), MCI patients whose cognitive status remains unchanged for the duration of the study (stable MCI; $n = 5$), patients who progressed to clinical dementia without radiographical evidence of microbleeds (AD only; $n = 6$) and patients who progressed to clinical dementia with radiographical evidence of CAA ($n = 3$). For each case, serum samples were subjected to ELISA at two distinct time points; the initial point was collected at the time of enrollment in the study (all subjects except controls were diagnosed as MCI), the second time point was collected approximately two-years later thus providing before and after cognitive decline samples for each MCI subject whose cognitive function declined. This cohort of subjects was chosen from a larger cohort previously described [6] based upon the availability of the serum samples described; there is 21% overlap in the subjects in this prospective verification stage compared to the subjects in the initial proteomic screen. A commercially available C3 and alpha-2-Macroglobulin (a2M) competitive enzyme linked immunoassays were used for these experiments (Abnova, Taiwan). Briefly, a polyclonal antibody specific for human C3 and a2M was pre-coated onto a 96-well plate. 25 μ l of standard or samples was added per well and 25 μ l of biotinylated C3 or a2M was added to each well (on top of the sample or standard), gently mixed and incubated for 2 h. Plates were washed with 200 μ l of wash buffer five times. 50 μ l of streptavidin-peroxidase conjugate was added to each well and incubated for 30 min. Plates were washed and incubated with 50 μ l of chromagen substrate per well for 10 min. 50 μ l of stop solution was added and plates were immediately read on a microplate reader at 450 nm.

Statistical analysis

Data are reported as mean $\pm$ standard error. Differences between groups in the cross-sectional data were assessed by one way ANOVA followed by Fisher LSD post-hoc test. Longitudinal data were analyzed using a paired t test to measure within group time effects with respect to changes in the levels of C3 and a2M. All statistical analysis was done on SPSS 20 software. An $\alpha = 0.05$ was considered significant.

RESULTS

Literature review of CBMs

The first and second authors conducted a literature search. PubMed and ISI Web of Science databases were systematically queried, resulting in the retrieval of 25 cross-sectional, proteomic serum and plasma-based biomarker studies. Candidate biomarkers were chosen from these studies by identifying proteins, which were significantly altered between control and AD groups and demonstrated relatively reproducible changes across multiple studies. This resulted in the identification of 18 candidate protein biomarkers (Table 1). Non-

proteomic studies which describe the serum or plasma levels of candidate protein biomarkers, either in AD or other disease states were also included in Table 1.

LC-MS/MS serum proteomics

Serum was collected from subjects in four groups (Table 2): neurologic controls ($n = 6$), stable MCI ($n = 6$), early AD without evidence of CAA ($n = 6$), and early AD with MR evidence of CAA ($n = 6$) [7]. No subjects were enrolled with AD at baseline; serum for AD cohorts was collected at the first visit when a subject with MCI was identified as meeting the clinical criteria for a diagnosis of AD. This represented the earliest definable clinical time point of probable AD per current diagnostic criteria. There was no statistically significant difference between groups in either C3 ($F(3,20) = 2.1$, $p > 0.1$; Fig. 2A). There was only a marginally significant effect between groups for a2M ($F(3,20) = 2.8$, $p < 0.06$; Fig. 2B), where a2M was significantly increased in both AD-only and AD with CAA compared to control ($p < 0.05$ for both). There was, however, a very significant difference between groups for C4 ($F(3,20) = 4.7$, $p < 0.01$; Fig. 2C). C4 was significantly increased in the AD-only group compared to controls, MCI, and AD with CAA cases ($p < 0.01$ for all). C1 inhibitor, however, showed no significant difference between groups ($F(3,20) = 1.9$, $p > 0.1$; Fig. 2D). Descriptive statistics for all CBMs are described in supplementary Table 1 (available online: <http://www.j-alz.com/issues/30/vol30-2.html#supplementarydata02>).

Longitudinal ELISA analysis

Longitudinal analysis of these two proteins with competitive ELISA yielded non-significant time effect results over an approximately two-year follow-up period for each group (paired t test). Interestingly, both C3 (Fig. 3A) and a2M (Fig. 3B) were increased in the MCI groups compared to the other groups at baseline, which is in conflict with the LC-MS/MS data and could be due to the sensitivity differences between the two methods. The estimated marginal means of the time effect for each protein at baseline to follow up is diagrammed in Fig. 4A and B for easier visual interpretation. Unexpectedly, there is a much greater change (decrease) in both proteins with time in the MCI group than in either of the two groups that progress to dementia (Fig. 4A, B). Descriptive statistics for the longitudinal data is summarized in supplementary Table 2.

DISCUSSION

Accurately diagnosing a prodromal state of AD may allow for the application of more effective treatments, which could slow or reverse disease progression [5]. This will rely heavily on the ability to enroll patients early enough in clinical trials so as to assess the efficacy of therapeutics before noticeable signs of cognitive loss; in other words, at a stage before detrimental and irreversible neuron loss. The acquisition of serum biomarkers is less expensive and less invasive than alternative methods like neuroimaging and lumbar puncture and which would be ideal for a screening diagnostic test. However identifying and validating a molecular marker that meets the requirements for a biomarker has proven more difficult than anticipated, even with modern proteomic tools. This study was designed to aggregate information on serum biomarkers, to assess the reproducibility of previous findings (both by comparison to other literature sources and comparison to a *de novo* proteomic study) and finally to perform a preliminary prospective validation of the identified markers to assess their predictive value for the development of AD. Systematic review of the literature yielded approximately 18 proteins reported to change in AD serum, which we compared to our unbiased LC-MS/MS spectral data on control versus AD serum allowing us to assess the reproducibility of these CBMs. Finally we chose to longitudinally validate C3 and a2M based on their significance in the literature and the cross-sectional cohort.

The many previously published studies offer a wealth of data comparing various protein levels in normal cases and MCI and AD cases. These studies in aggregate offer considerable power for evaluating candidate biomarkers; multiple small cohort studies have the advantage of illustrating the reproducibility of a finding in multiple clinical groups and with multiple analytical techniques. However, the majority of these studies are cross-sectional, offering only one time point in the disease process. Longitudinal studies for biomarker characterization enable temporal correlation of the changes in a candidate protein with the progression to clinical dementia. We collected serum samples from participants before and at the time of clinical progression to dementia providing a valuable prospective analysis to evaluate the levels of complement C3 and a2M as clinical cognitive function is acutely declining. It should be realized, however, that the most powerful diagnostic tool in the area of AD will be a high throughput, multi-parameter combination of modalities including clinical assessment, objective fluid biomarkers, and sophisticated imaging techniques [10].

Several of the thirty proteins involved in the complement cascade were found in the serum of AD patients; however, C4 was the only of these that increased significantly in the proteomic screen. The complement system, an important pathway in both the innate and adaptive immune system, has been associated with the pathology of AD and other neurodegenerative diseases as neurons express the mRNA and proteins of classical complement pathway, as do the supporting cells of the CNS and vascular smooth muscle of cerebral blood vessels [11–14]. C3 is the central component of all three complement pathways, where most of the effectors of the complement cascade occur [11]. Two previous studies have found C3 elevated; Giometto and colleagues found C3 significantly increased in AD ($p < 0.005$), and Zhang et al. also found an increase, but studied pooled samples so variance could not be assessed [15, 16]. In a third study, Cutler and coworkers [17] found no significant change in serum C3 in AD patients. We did not find a significant change in C3 in the cross-sectional cohort. On the other hand, C4 was increased in the AD-only group compared to the other three groups. Additionally, C3 failed to demonstrate a longitudinal change in our prospective cohort, although interestingly, the MCI group showed higher levels at baseline and follow up compared to the other groups. Numerous additional members of the complement family of proteins (such as C1 inhibitor, which did not show significant changes in our cohort) have been shown to be changed in AD serum in this and various other studies, which may suggest that the status of the complement cascade is broadly altered in this disease (in both the CNS and the periphery). Complement component C3 remains potentially useful as a biomarker, but perhaps its utility can be bolstered by incorporation into a broader analysis of the complement system (such as C4) in serum; this will require additional study in a larger, longitudinal cohort.

a2M also has shown some potential as a biomarker, both in the literature and in our initial proteomics screen. a2M is a protease inhibitor that uses a trapping method to capture and block the function of a wide variety of proteases [18]. In addition, a2M has been shown to complex with the amyloid- β ($A\beta$)₄₂ and $A\beta$ ₄₀ peptides, resulting in their clearance via the lipoprotein receptor-related protein, suggesting a function in the pathology of AD [19]. In a study by Hye et al. [23], a very significant increase in a2M ($p < 0.001$ and $p < 0.01$) was found in the serum of two separate cohorts. An additional study by the same group confirmed this increase ($p < 0.05$) in patients with mild to moderate AD compared to those diagnosed with MCI [20]. Zhang and collaborators [13] also found an increase in AD serum a2M compared to control cases in their cohort, although their serum samples were pooled from a cohort of five AD patients and five controls, which limits statistical analysis. Two studies by Licastro and colleagues in consecutive years using a radial immunodiffusion assay and immunonephelometric assay, however, found a2M was not increased in the serum of AD patients compared to neurological controls [21, 22]. The same group found no change between neurologic controls and patients with vascular dementia [21, 22]. Finally, the latest

proteomics study using LC-MS/MS reported no change in the AD group compared to controls [17]. While a2M was a promising biomarker from our proteomic screen in the cross-sectional cohort, the literature review did not consistently defend the ability of this protein to separate AD from control subjects and this heterogeneity in findings was a considerable weakness of this CBM. This may be due to this study's small statistical power, but also emphasizes the weakness of a predictive value of a2M. Our study found this protease inhibitor was increased in the AD-only cohort ($p < 0.05$) compared to non-demented controls and also increased in the AD with CAA cohort ($p < 0.05$). However, we found no significant changes in a2M levels in any groups in the longitudinal cohort.

Clearly, the major caveat of this study is its weak statistical power due to the small number of subjects, thus resulting in a higher possibility of Type II error. It should, however, be recognized that this preliminary work will lead to further studies in much larger, longitudinal cohorts and will add value to any meta-analysis performed in the future. The major strength of our study is that we assessed serum from patients who were diagnosed with isolated AD or AD with CAA both before and after progression from MCI and compared to similar time points from stable MCI. The two time points for prospective study were approximately two years apart, providing adequate time for progression from MCI to AD since approximately 12% of MCI patients per year face further cognitive decline [23]. Using early time points for serum collection is important in biomarker research to discern alterations in protein concentrations predictive of disease progression. However, the subjects in this study did not have definitive diagnoses, because the clinical diagnosis of AD is considerably less accurate than autopsy diagnosis [2]. One serum biomarker study collected serum from AD and neurologically control patients immediately postmortem via cardiac puncture enabling concrete confirmation of subject diagnoses [24]. While this study analyzed very advanced AD and therefore cannot describe the predictive value of changes in the CBMs identified, it ensured a very accurate diagnosis of AD and an exact picture of the serum protein profile at a specific time point in the disease process. Neither study is perfect. It will be difficult to achieve higher serum biomarker performance without long-term follow-up to subject autopsy, which will enable reliable patient diagnosis, as will the most-valuable early time points. This type of study is currently lacking in literature. Nevertheless, a second important feature of this study somewhat mitigates the previous weakness—that findings from our experiments were analyzed in the context of the rigorously aggregated and critically assessed data previously reported in this field. The reproducibility of the multitude of CBMs reported in the literature, including the two measured in the prospective validation cohort in this study, is an important feature of any biomarker that will ultimately achieve clinical usefulness.

Here we provide a systematic literature review on previously identified serum protein biomarkers plus validation of two candidates that showed significant changes in our own cross-sectional cohort, C3 and a2M, in a separate prospective cohort, where C3 showed a decrease over time in the clinically stable MCI group. Although these two CBMs looked promising in our cross-sectional group, based on the literature and our longitudinal analysis, each candidate biomarker identified in this study (including C4 and C1 inhibitor) will need further validation in larger longitudinal cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Characteristics of an ideal biomarker

1. **Biomarker must reliably discriminate pathological from normal cases at an early stage of disease progression.**
2. **Reasonable expense and minimal patient discomfort should be associated with collection to enable serial studies to monitor disease progression.**
3. **The biomarker must be consistently validated in multiple populations of Alzheimer's disease patients.**
4. **The biomarker should predict the onset of cognitive symptoms, and therefore should be validated in prospective clinical trials (ideally with follow-up to autopsy to enable conclusive diagnosis)**

Fig. 1.
Characteristics of an ideal biomarker.

Fig. 2.

Cross-sectional LC-MS/MS analysis on human serum of normal controls, clinically diagnosed MCI cases, AD cases that do not manifest with CAA, and AD cases that do manifest with radiological evidence of CAA. A) Complement C3 was not statistically significant between groups ($F(3,20) = 2.1$, $p > 0.1$). B) a2M was only marginally statistically significant between groups ($F(3,20) = 3.0$, $p < 0.05$). C) Complement C4 was significantly increased in AD-only patients compared to controls, MCI, and AD with CAA ($F(3,20) = 4.7$, $p < 0.01$). D) C1 inhibitor was not statistically significant between groups ($F(3,20) = 1.9$, $p > 0.1$). Data analyzed with one way ANOVA followed by Fisher LSD post-hoc test. * $p < 0.05$, ** $p < 0.01$. Error bars represent SEM.

Fig. 3.

Longitudinal analysis of human serum with competitive ELISA. The two time points used for every case were 1) baseline or before progression to AD (dark grey) and 2) end point of the study for those who do not progress or within a year of clinically diagnosed progression for those who do progress to AD (light grey). No significant differences in between subjects time effect were observed for either protein (A) Complement C3. (B) a2M. Data analyzed with paired samples *t* test. Error bars represent SEM.

Fig. 4.

Estimated marginal means of time for longitudinal analysis of select CBMs. A, B) Graphical representation of change in the levels of C3 and a2M, respectively, from baseline (first blood draw) to approximately 2 years follow up (second blood draw) in control, MCI, AD-onl, and AD with CAA cases. No significant change over time was found for either CBM.

Table 1

Systematic review of candidate biomarkers for Alzheimer's disease (AD) in serum

Identified protein	Function	Reports on AD serum	Reported change in AD	Method, # subjects Cont/AD	Findings in other diseases	Reported change	Current result LC/MS/MS	p
Alpha ₂ -macroglobulin	Protease inhibitor	Giometto [16]	No change	6, 18/20	Licastro [21], VaD	No change	Increased	$p = 0.02$
		Licastro [22]	No change	5, 13/30				
		Licastro [21]	No change	6, 60/7				
		Zhang [15] *	↑ NEV	2, 5/5				
		Hye [25] *	↑ $p = 0.006$	1, 50/50				
		Hye [25] *	↑ $p = 0.001$	1, 50/50				
		Cutler [17] *	No change	1, 47/47				
Alpha ₁ -antichymotrypsin	Protease inhibitor	Matsubara [26]	↑ $p < 0.001$	5, 76/20	Matsubara [26],		Trend toward increase	$p = 0.14$
		Matsubara [27]	↑ $p < 0.001$	5, 89/38	VaD	No change		
		Brugge [28]	↑ $p < 0.05$	5, 17/26	PD	No change		
		Hinds [29]	↑ $p < 0.05$	5, 36/36	CVD	No change		
		Licastro [22]	↑ $p < 0.001$	4, 24/30	Brugge [28], DS	No change		
		Licastro [21]	↑ $p < 0.01$	4, 11/36	Hinds [29], DS	No change		
		Lieberman [30]	↑ $p < 0.00001$	5, 86/57	Pirttila [39], PD	No change		
		Altstiel [31]	↑ $p < 0.001$	4, 48/77	Licastro [21], VaD	No change		
		Licastro [32]	↑ $p < 0.0001$	↑ 5, 51/145	Licastro [40], VaD	No change		
		Licastro [33]	↑ $p < 0.001$	5, 201/281	Nielsen [36], DLB	No change		
		McIlroy [34]	↑ $p < 0.001$	5, 105/106				
		DeKosky [35]	↑ $p = 0.01$	5, 113/359				
		Nielsen [36]	↑ $p < 0.005$	7, 37/258				
		Porcellini [37]	↑ $p < 0.0001$	4, 830/26				
		Furby [38]	No change	5, 25/24				
		Pirttila [39]	No change	4, 42/40				
		Zhang [15] *	No change	2, 5/5				
Alpha ₁ -antitrypsin	Protease inhibitor	Cutler [17] *	No change	1, 47/47				$p = 0.65$
		Giometto [16]	↑ $p < 0.001$	6, 18/20	Nielsen [36], DLB	No change	No change	

Identified protein	Function	Reports on AD serum	Reported change in AD	Method, # subjects Cont/AD	Findings in other diseases	Reported change	Current result LC/MS/MS	p
Complement C3	Inflammation	Matsubara [26]	No change	6, 10/10				
		Licastro [22]	No change	5, 13/30				
		Licastro [21]	No change	6, 60/7				
		Nielsen [36]	No change	7, 37/258				
		Cutler [17]	No change	1, 47/47				
		Liao [24]	$\uparrow p = 0.0003$	4, 9/9				
Complement C4	Inflammation	Giometto [16]	$\uparrow p < 0.005$	6, 18/20			Increased	$p = 0.06$
		Zhang [15] *	$\uparrow$ NEV	2, 5/5				
		Cutler [17] *	No change	1, 47/47				
		Giometto [16]	$\uparrow p < 0.005$	6, 18/20	Mehra [41], AIS	$\uparrow p < 0.0001$	Increased	$p = 0.004$
C1 inhibitor	Complement regulation	Zhang [15] *	$\uparrow$ NEV	2, 5/5				
		Hye [25] *	$\downarrow p = 0.021$	1, 50/50				
		Cutler [17] *	No change	1, 47/47				
		Zhang [15] *	$\uparrow$ NEV	2, 5/5			Trend toward increase	$p = 0.035$
		Cutler [17] *	$\uparrow p = 0.017$	1, 47/47				
		Cutler [17] *	$\uparrow p = 0.029$	1, 100/100				
Complement factor H	Complement regulation	Zhang [15] *	$\uparrow$ NEV	2, 5/5	Ingram [42], MS	$\uparrow p = 0.007$	No change	$p = 0.567$
		Hye [25] *	$\uparrow p = 0.001$	1, 50/50				
		Cutler [17] *	No change	1, 47/47				
Serum amyloid P	Immune regulation	Hye [25] *	$\uparrow p = 3E-04$	1, 50/50			No change	$p = 0.567$
		Verwey [43]	No change	4, 30/144				
CD5 antigen	Immune regulation	Hye [25] *	$\downarrow p = 0.029$	1, 50/50			No change	$p = 0.298$
		Giometto [16]	$\uparrow p < 0.001$	6, 18/20				
Ceruloplasmin	Copper chaperone	Licastro [22]	No change	4, 13/30				
		Kessler [44]	$\downarrow p = 0.015$	6, 13/16				
		Squitti [45]	No change	3, 25/25				
		Squitti [46]	No change	3, 53/51				
		Cutler [17] *	No change	4, 47/47			No change	$p = 0.717$

Identified protein	Function	Reports on AD serum	Reported change in AD	Method, # subjects Cont/AD	Findings in other diseases	Reported change	Current result LC/MS/MS	p
Transferrin	Thyroxine/retinol transport	Zhang [15] [*]	↑ NEV	2, 5/5			Trend toward increase	p = 0.182
Clusterin	Lipid transport	Cutler [17] [*]	No change	1, 47/47				
		Cutler [17] [*]	↑ p = 0.019	1, 47/47			No change	p = 0.243
		Liao [24]	No change	1, 100/100				
			↓ p = 0.0004	1, 10/10				
Apolipoprotein A-I	Lipid transport	Zhang [15] [*]	↓ p = 0.0061					
		Zhang [15] [*]	No change	2, 5/5			No change	p = 0.243
		Liu [47]	↑ p < .0002	2, 74/59				
Apolipoprotein B100	Lipid transport	Cutler [17] [*]	No change	1, 47/47				
		Zhang [15] [*]	↑ NEV	2, 5/5			No change	p = 0.616
Apolipoprotein E	Lipid transport	Scacchi [48]	No change	6, 156/85			No change	p = 0.545
		Zhang [15] [*]	↑ NEV	2, 5/5				
Gelsolin	Actin-binding protein	Cutler [17] [*]	No change	1, 47/47				
		Cutler [17] [*]	No change	1, 47/47			No change	p = 0.656
Vitamin D-binding protein	Vitamin D metabolite transport	Liao [24]	↑ p = 0.0015	1, 10/10			No change	p = 0.346
Inter-alpha-trypsin inhibitor	Acute Phase Protein	Matsubara [26]	No change	5, 10/10	Keyshap [49], AIS	↓ NEV	Increased ^{**}	p = 0.031
		Liao [24]	↑ p = 0.0016	1, 10/10				
			↑ p = 0.0045					
			↑ p = 0.0030					

* Serum Proteomics studies,

** Only when AD only and AD with CAA are combined, NEV = No estimate of variance, DS = Down Syndrome, MS = Multiple Sclerosis, DLB = Dementia with Lewy Bodies, VaD = Vascular Dementia, AIS = Acute Ischemic Stroke, CVD = Cardiovascular Disease, NPH = Normal Pressure Hydrocephalus.

Table 2

Demographic subject data for LC-MS/MS and ELISA methods

Method	Diagnosis	n	M : F	MMSE	Age at serum draw 1 (yr)	Age at serum draw 2/progression (yr)	Time difference (months)
LC-MS/MS	Control	6	2 : 4	29.3 ± 0.4	–	73.8 ± 4.5	–
	MCI	6	2 : 4	26 ± 0.7 ^b	–	75.3 ± 5.3	–
	AD only	6	1 : 5	26 ± 0.9 ^b	–	85.7 ± 0.9	–
	AD/CAA	6	3 : 3	27.2 ± 0.9 ^a	–	84 ± 1.5	–
ELISA	Control	5	2 : 3	29.2 ± 0.4	72.4 ± 2.4	74 ± 2.3	22.8 ± 1.8 ^b
	MCI	5	3 : 2	28.2 ± 0.6	73.4 ± 1.4	75.4 ± 1.4	25.2 ± 1.4
	AD only	6	4 : 1	27.2 ± 0.8 ^a	77 ± 3.1	78.2 ± 3.0	13.7 ± 3.4 ^b
	AD/CAA	3	1 : 2	27.7 ± 0.4	78.3 ± 2.9	80 ± 3.5	14.7 ± 4.4

* Subjects classified according the Clinical Dementia Rating (CDR) scale, as described in the “Methods” section; Data analyzed with one way ANOVA for the cross-sectional (LC-MS/MS) cohort and paired *t* test for the longitudinal (ELISA) cohort. Data expressed as mean ± SEM.

^a *p* < 0.05,

^b *p* < 0.01.