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Blood-Based Biomarkers

Comparing biological markers of Alzheimer's disease across blood fraction and platforms: Comparing apples to oranges

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Abstract

Introduction: This study investigated the comparability of potential Alzheimer's disease (AD) biomarkers across blood fractions and assay platforms.

Methods: Nonfasting serum and plasma samples from 300 participants (150 AD patients and 150 controls) were analyzed. Proteomic markers were obtained via electrochemiluminescence or Luminex technology. Comparisons were conducted via Pearson correlations. The relative importance of proteins within an AD diagnostic profile was examined using random forest importance plots.

Results: On the Meso Scale Discovery multiplex platform, 10 of the 21 markers shared >50% of the variance across blood fractions (serum amyloid A $R^2 = 0.99$, interleukin (IL)10 $R^2 = 0.95$, fatty acid-binding protein (FABP) $R^2 = 0.94$, I309 $R^2 = 0.94$, IL-5 $R^2 = 0.94$, IL-6 $R^2 = 0.94$, eotaxin3 $R^2 = 0.91$, IL-18 $R^2 = 0.87$, soluble tumor necrosis factor receptor 1 $R^2 = 0.85$, and pancreatic polypeptide $R^2 = 0.81$). When examining protein concentrations across platforms, only five markers

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shared >50% of the variance (beta 2 microglobulin $R^2 = 0.92$, IL-18 $R^2 = 0.80$, factor VII $R^2 = 0.78$, CRP $R^2 = 0.74$, and FABP $R^2 = 0.70$).

Discussion: The current findings highlight the importance of considering blood fractions and assay platforms when searching for AD relevant biomarkers.

Keywords: Alzheimer's disease; Blood; Serum; Plasma; Biomarker discovery; Multiplex assay platform; Meso Scale Discovery; Rules Based Medicine; Proteins; Preanalytic processing; Standardization; Diagnostics

1. Introduction

Despite tremendous scientific advancements, there remains a significant concern regarding the lack of reproducibility of research findings [1–4] with most believing that "at least 50%" of academic findings will not be replicable within industry laboratories [4]. In fact, the National Institutes of Health recently highlighted this problem and outlined a plan to address the issue [2]. In recent years, there has been an explosion in the search for blood-based biomarkers related to Alzheimer's disease (AD) for a variety of functions, such as detection, diagnosis, risk estimation, as well as clinical trial enrichment, stratification, and treatment response. However, this work has not been immune to the problem of replicability as conflicting findings are commonplace in the field. In an effort to generate consistent methods and protocols to increase replicability and move the field of blood-based biomarkers for AD forward, the international collaboration of the blood-based biomarker professional interest area (BBB-PIA) of the Alzheimer's Association's International Society to Advance Alzheimer's Research and Treatment was formed, which has published consensus statements regarding the current state of the field along with most of the immediate research needs [5,6]. More recently, the BBB-PIA published the first ever consensus-based guidelines for preanalytic processing for blood-based AD biomarker research [7]. The purpose of the present study was to examine two potential sources contributing to failures to replicate in the blood-based biomarker field of AD, (1) blood fraction (i.e., serum vs. plasma) and (2) analytic platform. These initiatives have been of paramount importance and additional topics require careful consideration.

A major concern for blood-based AD biomarker studies is the selection of the most suitable blood fraction. The type of blood fraction is important not only for the abundance of specific analytes but also for the role of additives such as heparin, citrate, or ethylenediaminetetraacetic acid (EDTA), which can significantly impact both stability and detectability of biomarkers [8,9]. However, to date, there remains little consistency in the type of blood fraction assayed across studies. One of the most extensively studied plasma-based biomarkers is amyloid β (Aβ), which is one of the hallmarks of AD pathology investigated at autopsy and is a well-validated marker of AD in cerebrospinal fluid samples. Work by Watt et al. [10], however, highlights many of the issues regarding plasma Aβ studies. Although some markers appear to be robust in both serum and plasma (e.g., C-reactive protein), other markers appear to be more robust in one fraction over the other. For example, EDTA inhibits many proteases, which may preserve many proteins better than serum; however, EDTA can interfere with some mass spectrometry assays. Recent reviews on the topic highlight the variability in blood-fraction selection as a major contributor to inconsistent findings in blood-based biomarker studies [11,12]. On the one hand, several markers have been found to be significant across multiple studies and cohorts, despite different blood fractions used (e.g., pancreatic polypeptide [PPY] and C-reactive protein [CRP]) [13–16]. Few studies, however, have directly compared plasma to serum-based findings in AD. When examining the association between serum- and plasma-based proteomics in the Texas Alzheimer's Research & Care Consortium (TARCC; available at http://www.txalzresearch.org/), a total of 40 proteins (from >100 candidate proteins) were highly correlated across blood fractions ($R^2 \geq 0.75$; ≥56% shared variance of proteins) [17]. In another study using the TARCC and Alzheimer's Disease Neuroimaging Initiative (ADNI) data, only 11 proteins (from >100) were highly correlated across serum and plasma ($R^2 \geq 0.75$) and significantly associated ($P < .05$) with AD status (CRP, adiponectin, PPY, fatty acid-binding protein [FABP], interleukin 18 [IL-18], beta 2 microglobulin [β2M], tenascin C [TNC], I309, factor VII [FVII], soluble vascular cell adhesion molecule-1 [sVCAM-1], and monocyte chemoattractant protein-1). The serum-plasma biomarker algorithm yielded an area under the curve (AUC) = 0.88 across cohorts [18]. These data suggest that some markers are consistent across blood fraction and may be useful for diagnostic purposes; however, others are likely less comparable despite statistically significant correlations.

Another key issue for blood-based AD biomarker studies is the selection of the most appropriate assay platform. Many cohorts have used the Myriad Rules Based Medicine (Myriad RBM) platform (e.g., ADNI, TARCC, and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Aging) [13,14,16,18]; however, many other approaches have been used, including the Meso Scale Discovery

(MSD; available at http://www.mesoscale.com) [19] and SOMAscan [20] multiplexed protein technologies. Recently, several investigations have focused on identifying and validating biomarkers or biomarker algorithms across platforms [14,19–21]; however, most studies have not attempted cross-platform validation and others have failed to cross-validate across platforms [22]. The use of different assay methodologies likely has substantially contributed to the inconsistencies within the blood-based AD biomarker field.

The present study was undertaken to directly compare serum- and plasma-based protein concentrations for putative AD biomarkers as well as data obtained from the same participants at the same blood draw using Myriad RBM versus MSD.

2. Methods

2.1. Participants

2.1.1. Texas Alzheimer's Research & Care Consortium

Nonfasting serum and plasma samples from the same blood draw in 300 participants (150 with AD and 150 controls) enrolled in the TARCC study were analyzed. Serum samples were assayed using the Myriad RBM and MSD platforms. Of the 300 samples, specimens from 144 participants (79 with AD and 65 controls) were assayed from both serum and plasma using the MSD platform (as described in the following). The methodology of the TARCC protocol has been described elsewhere [14]. Briefly, each participant completed an annual assessment at one of the five participating sites that included a medical evaluation, neuropsychological testing, a clinical interview, and a blood draw. Diagnosis of AD dementia was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [23]; controls performed within normal limits on psychometric testing (mild cognitive impairment was not included in this study). Institutional review board approval was obtained at each site, and written informed consent was obtained for all participants.

2.2. Human serum sample collection

TARCC samples were collected as follows: Serum—(1) nonfasting serum samples were collected into 10-mL tiger-top tubes; (2) samples were allowed to clot for 30 minutes at room temperature in a vertical position; (3) samples were centrifuged for 10 minutes at 1300 × *g* at room temperature within 1 hour of collection; (4) 1.0-mL aliquots were transferred into cryovial tubes; (5) Freezerworks barcode labels were affixed to each aliquot; and (6) samples were placed into −80°C freezers for storage until use. Plasma—(1) nonfasting blood was collected into 10-mL lavender-top (EDTA) tubes and gently inverted 10–12 times; (2) tubes were centrifuged at 1300 × *g* at room temperature for 10 minutes within 1 hour of collection; (3) 1-mL aliquots were transferred to cryovial tubes; (4) Freezerworks barcode labels were affixed; and (5) tubes were placed in −80°C freezers for storage.

2.3. Human assays

2.3.1. Electrochemiluminescence

Plasma and serum samples were assayed in duplicate via a multiplex biomarker assay platform using electrochemiluminescence (ECL) on the SECTOR Imager 2400A from MSD (available at http://www.mesoscale.com). The MSD platform has been used extensively to assay biomarkers associated with a range of human diseases including AD [24,25]. The markers assayed included FABP, β2M, PPY, soluble tumor necrosis factor receptor 1 (sTNFR1), CRP, VCAM-1, thrombopoietin, α2 macroglobulin, eotaxin3, tumor necrosis factor-alpha (TNF-α), tenascin C (TNC), IL-5, IL-6, IL-7, IL-10, IL-18, I309, FVII, thymus and activation-regulated chemokine (TARC), serum amyloid A (SAA), and intercellular cell-adhesion molecule-1. (Information regarding assay performance, least detectable dose (LDD), and coefficient of variation (CV) can be obtained on request.)

2.3.2. Myriad RBM

Serum samples were shipped to Myriad RBM for assay on the Luminex-based HumanMAP 1.0 platform. Over 100 proteins were quantified using fluorescent microspheres with protein-specific antibodies. (Information regarding LDD, inter-run CV, dynamic range, and overall spiked standard recovery as well as cross-reactivity with other HumanMAP analytes are available through Myriad-RBM directly.)

2.4. Other relevant measures

Other information extracted from the database included *APOE* ε4 genotype, age, gender, education, clinical dementia rating scale, and mini-mental state examination (MMSE) for demographic characterization of the sample. Variable importance plots from random forest (RF)-generated algorithms using these data in prior publications were compared to determine the overlap of the top 10 biomarkers across blood fraction and platforms.

2.5. Statistical analyses

Analyses were performed using IBM SPSS21. χ^2 and *t* tests were used to compare case versus controls for categorical (*APOE* ε4 allele frequency sex, race, dyslipidemia, diabetes, hypertension, and obesity) and continuous variables (age, education, MMSE, and clinical dementia rating sum of boxes scores [CDR-SB]), respectively. In our prior work, we demonstrated that the serum-based proteomic profile was more robust in detecting AD when compared with plasma in this cohort using the MSD platform [19]. Here, we compared the top 10 biomarker importance rankings

across serum and plasma within the same cohort. Correlations across serum and plasma were conducted using Pearson correlations. Analyses were conducted from proteomic data taken from the same participant at the same blood draw only.

3. Results

Compared with normal controls (NC), the AD group was significantly older ($P < .001$), had fewer years of formal education ($P < .001$), and scored lower on the MMSE ($P < .001$) and higher on the CDR-SB ($P < .001$). There were no significant differences between groups with regard to sex or presence of dyslipidemia, diabetes, or hypertension. The AD group included significantly more *APOE* ε4 carriers (Table 1). Table 2 lists means and standard deviations of protein levels across blood fraction and assay platforms (RBM plasma data for NCs were not available).

As listed in Table 3, nearly all the markers were statistically significantly correlated across blood fraction, only sTNFR1, FABP, I309, IL-18, IL-10, IL-6, IL-5, PPY, eotaxin3, and SAA were correlated substantially high to share at least 50% of the shared variance. However, although the correlations were statistically significant for others, the amount of variance shared was less than 50% for thrombopietin (THPO), IL-7, TARC, TNF-α, alpha-2-macroglobulin, β2M, FVII, CRP, TNC, soluble intercellular adhesion molecule 1 (sICAM-1), and sVCAM-1. As an example, this implies that approximately 44% of what was measured as CRP in serum was similarly measured in plasma, whereas 66% of the measurement was error or something else.

Next, the variable importance plots from our previously generated RF analyses [19] were examined (Table 4). We previously demonstrated that the overall accuracy of the algorithm using our specific profile was superior when using serum (AUC = 0.96) versus plasma (AUC = 0.76) [19]. When examining the protein importance plots across serum versus plasma, there was minimal overlap across blood fractions in ranking among the top 10 biomarkers (of our 21-protein profile). In fact, only IL-5, IL-6, and IL-7 were consistently ranked among the top 10 biomarkers across serum and plasma.

Next, data from 17 common markers assayed using the MSD and RBM platforms were compared. As listed in Table 5, 14 of the 17 correlation coefficients are statistically significant ($P < .05$); however, the amount of shared variance in protein concentrations was <50% for 12 of the 17 markers and >50% only for FABP, CRP, FVII, IL-18, and β2M. Additionally, as listed in Table 4, only two of the top 10 markers (IL7 and TNF-α) were common among the top 10 biomarkers across the MSD and RBM platforms.

Table 1
Demographic characteristics of cohort

	AD (n = 79)	Normal controls (n = 65)	
Characteristics	Mean (SD)	Mean (SD)	*P* value
Age (y)	76.1 (8.6)	71.2 (9.2)	.002
Education (y)	14.7 (3.0)	15.5 (2.6)	.02
Sex (male), %	30	32	.76
APOE ε4 presence (yes/no), %	60	23	<.001
Hispanic ethnicity, %	3	7	.33
Race (non-Hispanic white), %	96	90	.04
MMSE	19.1 (6.4)	29.6 (0.7)	<.001
CDR-SB	7.8 (4.1)	0.0 (0.1)	<.001
Hypertension (% yes), %	54	55	.86
Dyslipidemia (% yes), %	51	40	.31
Diabetes (% yes), %	10	11	.59
Obese (% yes), %	15	14	.53

Abbreviations: AD, Alzheimer's disease; SD, standard deviation; MMSE, mini-mental state examination; CDR-SB, clinical dementia rating sum of boxes scores.

4. Discussion

The current findings clearly illustrate the importance of blood fraction and assay platform on obtained results. In fact, our findings highlight that a blood-based algorithm that is highly accurate in detecting AD could (and likely would) be very different if it was conducted in serum versus plasma or on an ECL versus a Luminex-based platform. Therefore, as the science currently stands, accurate blood-based algorithms for detecting AD likely have internal consistency only when performed on a specific blood fraction and by a specific laboratory. Therefore, if transition to clinical practice was the goal, the laboratory developed test (LDT) would be the only viable option. The international working group recently published guidelines for processing of blood samples when conducting work in the area of AD biomarkers [7]. The present study builds on this prior work and points to the urgent need for greater standardization if a blood-based biomarker test is to be reliable and clinically applicable for the detection of AD.

First, the selection of blood fraction is a nontrivial choice. Although there have been many blood-based biomarkers of AD identified, studies have frequently used different blood fractions. A blood-based algorithm for detecting AD in serum will likely not be the same as one in plasma. In fact, only a single study to date has published a proteomic profile that was accurate in detecting AD in both serum and plasma [18]. Importantly, blood fraction must be taken into consideration in studies examining or reviewing the state of the science. A review (or meta-analysis) on specific biomarkers that does not consider blood fraction will likely be highly uninterpretable. It is likely that an approach that takes into account both serum and plasma markers will be the most robust and reliable and should be investigated further.

When looking at platforms, the current results demonstrate that protein concentrations are not consistently comparable across platforms. This variability emphasizes

Table 2
Mean protein values across blood fraction and assay platform

	MSD				RBM		
	AD		Normal control		AD		Normal control
	Serum	Plasma	Serum	Plasma	Serum	Plasma	Serum
Marker	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
A2M (pg/mL)	2180,273,262 (488,669,567.0)	2492,412,927 (1281,547,552)	2072,211,091 (592,581,531.2)	2993,631,363 (1715,510,790)	2.2 (4.0)	0.9 (0.2)	1.2 (0.3)
β2M (pg/mL)	2528,759.6 (1061,896.0)	3006,474.7 (1532,558.3)	2313,211.85 (1019,598.5)	3503,494.1 (2082,171.5)	2.4 (0.9)	2.4 (1.0)	2.3 (1.0)
Eotaxin3 (pg/mL)	3.0 (14.7)	1.4 (1.5)	1.9 (3.6)	1.8 (1.6)	128.5 (140.0)	278.7 (219.2)	89.8 (350.5)
FABP (pg/mL)	8401.3 (4402.2)	7757.3 (4809.8)	7751.8 (3296.3)	7480.3 (4514.0)	3.2 (3.8)	5.5 (5.7)	3.2 (4.1)
THPO (pg/mL)	616.4 (205.6)	488.5 (191.4)	564.0 (163.6)	418.2 (163.7)	7.3 (1.5)	2.3 (1.0)	6.0 (1.8)
PPY (pg/mL)	435.0 (539.9)	946.3 (853.7)	302.9 (225.5)	719.6 (664.5)	147.8 (139.6)	265.0 (201.5)	198.3 (196.9)
CRP (pg/mL)	3787.3 (6154.3)	3928.1 (6242.8)	8044.2 (13,846.6)	4326.4 (7052.6)	3.9 (6.3)	3.7 (4.6)	3.3 (4.4)
sTNFR1 (pg/mL)	4239.4 (2291.2)	3466.3 (1357.4)	3807.4 (1270.2)	3262.6 (1248.7)			
IL5 (pg/mL)	3.1 (19.6)	12.6 (83.9)	3.8 (18.7)	3.0 (11.4)	6.3 (5.0)	6.4 (2.8)	7.2 (4.7)
IL6 (pg/mL)	13.6 (105.5)	4.8 (5.9)	2.1 (2.1)	4.7 (5.6)		4.2 (3.0)	
IL7 (pg/mL)	10.4 (4.3)	4.4 (4.3)	4.9 (2.5)	3.5 (3.5)	80.8 (53.2)	49.2 (36.3)	108.9 (61.7)
IL10 (pg/mL)	8.2 (46.2)	208.1 (1985.9)	29.2 (119.5)	11.4 (41.9)	9.5 (8.2)		10.1 (5.8)
IL18 (pg/mL)	227.8 (109.2)	252.5 (139.6)	242.48 (112.9)	271.3 (166.2)	278.5 (132.6)	243.3 (93.6)	296.4 (164.3)
I309 (pg/mL)	3.4 (2.5)	2.5 (1.5)	2.8 (2.2)	2.2 (1.5)	265.5 (508.6)	766.0 (1890.0)	585.7 (2241.8)
Factor VII (pg/mL)	898,400.6 (253,545.6)	1282,175.0 (866,370.5)	832,189.1 (221,072.9)	1710,329.8 (1237,574.5)	565.2 (198.5)	591.2 (164.4)	625.4 (226.1)
TARC (pg/mL)	894.3 (608.0)	419.9 (388.2)	761.3 (498.0)	311.2 (468.2)			
TNC (pg/mL)	44,085.9 (13,140.6)	56,351.8 (34,425.1)	37,734.3 (10,342.9)	67,010.0 (46,125.5)			
TNF-α (pg/mL)	3.4 (3.6)	2.7 (1.0)	1.3 (0.8)	2.8 (1.0)	4.3 (1.7)	9.4 (4.7)	5.2 (4.7)
SAA (pg/mL)	9379.4 (18,741.4)	9351.4 (15,380.3)	7232.6 (21,202.0)	7458.3 (24,674.1)			
ICAM1 (pg/mL)	280.7 (64.5)	313.8 (83.5)	321.7 (121.5)	312.4 (67.3)	134.0 (40.4)	107.6 (23.1)	132.8 (33.5)
VCAM1 (pg/mL)	520.7 (121.5)	582.6 (189.3)	482.5 (130.8)	567.3 (132.1)	831.3 (212.6)	772.2 (173.6)	769.9 (209.8)

Abbreviations: MSD, Meso Scale Discovery; RBM, Rules Based Medicine; AD, Alzheimer's disease; SD, standard deviation; β2M, beta 2 microglobulin; FABP, fatty acid-binding protein; PPY, pancreatic polypeptide; sTNFR1, soluble tumor necrosis factor receptor 1; IL, interleukin; TARC, thymus and activation-regulated chemokine; TNC, tenascin C; TNF-α, tumor necrosis factor-alpha; SAA, serum amyloid A; ICAM1, intercellular cell-adhesion molecule-1; VCAM1, vascular cell adhesion molecule-1.

Table 3
Correlations between serum and plasma markers

Marker	R^2	*P* value
SAA	0.99	<.001
IL10	0.95	<.001
FABP	0.94	<.001
I309	0.94	<.001
IL5	0.94	<.001
IL6	0.94	<.001
Eotaxin3	0.91	<.001
IL18	0.87	<.001
sTNFR1	0.85	<.001
PPY	0.81	<.001
CRP	0.66	<.001
THPO	0.66	<.001
sVCAM1	0.65	<.001
β2M	0.56	<.001
TARC	0.53	<.001
A2M	0.45	<.001
TNF-α	0.44	<.001
sICAM	0.43	<.001
IL7	0.36	<.001
FVII	0.35	<.001
TNC	0.08	>.05

Abbreviations: SAA, serum amyloid A; IL, interleukin; FABP, fatty acid-binding protein; sTNFR1, soluble tumor necrosis factor receptor 1; PPY, pancreatic polypeptide; β2M, beta 2 microglobulin; TARC, thymus and activation-regulated chemokine; TNF-α, tumor necrosis factor-alpha; FVII, factor VII; TNC, tenascin C.

Table 4
Random forest variable importance and diagnostic accuracy for detecting AD with proteomic profile

MSD Serum [19]		MSD Plasma [19]		RBM Serum [14]	
AUC	0.96	AUC	0.76	AUC	0.91
SN/SP	0.91/0.86	SN/SP	0.65/0.79	SN/SP	0.80/0.90
Rank	Marker	Rank	Marker	Rank	Marker
1	**IL7***	1	Eotaxin3	1	Thrombopoietin
2	TNF-α*	2	PPY	2	MIP1α
3	**IL5**	3	**IL7**	3	Eotaxin3
4	**IL6**	4	**IL6**	4	TNF-α*
5	CRP	5	TPHO	5	Creatine kinase MB
6	IL10	6	β2M	6	FAS ligand
7	TNC	7	sTNFR1	7	Fibrinogen
8	sICAM1	8	FABP	8	IL10
9	FVII	9	TARC	9	IL7*
10	I309	10	**IL5**	10	CA19-9

Abbreviations: AD, Alzheimer's disease; MSD, Meso Scale Discovery; RBM, Rules Based Medicine; AUC, area under the receiver operating characteristic curve; SN, sensitivity; SP, specificity; IL, interleukin; TNF-α, tumor necrosis factor-alpha; PPY, pancreatic polypeptide; MIP1a, macrophage inflammatory protein 1 alpha; THPO, thrombopoietin; β2M, beta 2 microglobulin; TNC, tenascin C; sTNFR1, soluble tumor necrosis factor receptor 1; FABP, fatty acid-binding protein; FVII, factor VII; TARC, thymus and activation-regulated chemokine CA 19-9, cancer antigen 19-9.

NOTE. The AUC was calculated using the full 21-protein model [19]; three bolded markers overlap on the MSD platform from serum to plasma.

*Indicates serum markers common across MSD and RBM platforms.

Table 5
Correlation of protein levels across assay platforms

Marker	R^2	*P* value
β2M	0.92	<.001
IL18	0.80	<.001
FVII	0.78	<.001
CRP	0.74	<.001
FABP	0.70	<.001
sVCAM1	0.69	<.001
A2M	0.59	<.001
TNC	0.53	<.001
sICAM	0.47	<.001
I309	0.38	<.001
TNF-α	0.19	.001
THPO	0.17	.004
PPY	0.15	.01
IL7	0.09	.12
IL10	0.01	.89
Eotaxin3	0.01	.89
IL5	−0.08	.17

Abbreviations: β2M, beta 2 microglobulin; IL, interleukin; FVII, factor VII; FABP, fatty acid-binding protein; TNC, tenascin C; TNF-α, tumor necrosis factor-alpha; PPY, pancreatic polypeptide.

the need to cross validate biomarker profiles across platforms in cross-sectional and longitudinal specimens, particularly those identified on large-scale discovery platforms. A seminal article in this field by Ray et al. [26] identified a proteomic signature that was highly accurate in detecting and predicting AD; however, the findings did not cross validate across platforms [22]. It is unlikely that a discovery-based platform will demonstrate the properties, precision, replicability, and accuracy necessary to become a LDT and, therefore, cross validation on platforms with greater precision is of paramount importance. One example of a putative biomarker that has been consistently measured across blood fractions and platforms is that of clusterin (ApoJ). Lovestone and colleagues have identified an association of clusterin with AD in genetic studies [27], using proteomics across multiple platforms [20,21], and within primary neurons [28]. These and other evolving validation studies can offer novel insights into the pathobiology of AD and new therapeutic options. Using a serum-based profile approach, O'Bryant et al. [14,29] identified an algorithm that was highly accurate in detecting AD on the Myriad RBM discovery platform. The algorithm was then cross validated to the MSD platform (also in serum), and across species (humans and mouse model) and tissues (serum and brain microvessels) [19]. Such steps are ultimately necessary to ensure the confidence in the biomarkers or biomarker profiles themselves.

There are limitations to the present study. First, the analyses are cross sectional in nature and, therefore, any links between blood biomarkers and disease incidence or progression cannot be assessed. Although the current sample reflects a sizable collection of serum- and plasma-based data from the same individuals at the same blood draw, larger

samples are needed to validate these findings as well as examine additional markers and sources of variability. A study simultaneously examining multiple markers across multiple assay platforms would be of tremendous value to the field (across multiple neurodegenerative diseases). Such a study would allow for the validation of approaches and markers when used in combination, allow researchers to optimize specific markers for fit-for-use purposes, as well as offer a unique opportunity to take a systems biology approach to understanding neurodegenerative disease-specific versus overlapping pathologies. Additionally, our recent work shows that the link between blood-based biomarkers and disease status (AD vs. controls) and disease outcomes (i.e. cognition) varies by ethnicity [15,30]. However, the current findings are from primarily non-Hispanic whites and may not generalize to other ethnic or racial groups. Despite these limitations, our findings strongly emphasize the need to consider blood fraction and assay platform when interpreting or comparing findings across studies to increase replicability of findings across laboratories and methodologies. Additional work is needed to directly compare biomarkers across cohorts, blood fractions, assay platforms, and stages of neurodegenerative disease to push this work closer to clinical utility.

5. Conclusion

The current findings not only point toward a significant potential source of variability across studies but they also provide further demonstration of measurement consistency in select putative AD biomarkers. CRP and PPY have been consistently touted as key biomarkers for multiple cohorts [13,14]. It is also important to note that these more robust markers could, in fact, be contributing to the statistical significance many of the significant algorithms generated to date. If the more robust markers can be identified and validated across blood fractions and assay platforms, these efforts will most certainly move the field forward.

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RESEARCH IN CONTEXT

1. Systematic review: A literature review was conducted to evaluate the current state of the artwork in blood-based biomarkers of Alzheimer's disease. Prior research looking at the accuracy and use of these markers was reviewed.
2. Interpretation: Potential blood-based biomarkers of Alzheimer's disease have received a great deal of attention in the recent literature. However, little attention has been focused specifically on factors limiting the reproducibility of this work.
3. Future directions: This work establishes a clear need to investigate the comparability of markers across platforms and blood fractions before comparisons across studies can be made. Additionally, if "fit-for-purpose" biomarkers are to be developed, greater attention must be paid to the preanalytic and analytic aspects of these studies before any marker will make it to clinic.

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