Blood-Based Biomarkers

A blood screening test for Alzheimer's disease

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Abstract

Introduction: This study combined data across four independent cohorts to examine the positive and negative predictive values of an Alzheimer's disease (AD) blood test if implemented in primary care.

Methods: Blood samples from 1329 subjects from multiple independent, multiethnic, community-based, and clinic-based cohorts were analyzed. A "locked-down" referent group of 1128 samples was generated with 201 samples randomly selected for validation purposes. Random forest analyses were used to create the AD blood screen. Positive (PPV) and negative (NPV) predictive values were calculated.

Results: In detecting AD, PPV was 0.81, and NPV was 0.95 while using the full AD blood test. When detecting mild cognitive impairment, PPV and NPV were 0.74 and 0.93, respectively. Preliminary analyses were conducted to detect any "neurodegenerative disease". The full 21-protein AD blood test yielded a PPV of 0.85 and NPV of 0.94.

Discussion: The present study creates the first-ever multiethnic referent sample that spans community-based and clinic-based populations for implementation of an AD blood screen.

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1. Introduction

Alzheimer's disease (AD) is the most common dementia and is the fifth leading cause of death for those over 65 years [1]. Currently, over 5 million Americans suffer from AD [2], and it is estimated that those numbers will grow exponentially by 2050. AD has an annual health care cost similar to that of cardiovascular disease and more than cancer [3]. As a result of these rapidly increasing numbers, there is a growing need for the identification of a time-effective and cost-effective screening tool for use in primary care settings.

The Centers for Medicare and Medicaid Services recently implemented the annual wellness visit (AWV) that includes a cognitive examination (CMS.gov); however, the 2015 American Gerontological Society working group reported that "older adults are inadequately assessed for cognitive impairment during routine visits with their primary care providers" [4]. This limited access to early diagnostics has been associated with delayed treatment initiation, delays in provision of services to family members, overall decreased quality of life, and increased family burden [5]. Given the limited time available in primary care visits (average of 18 minutes), primary care providers are left with a significant dilemma of how to meet the AWV requirements.

In our prior work, we have proposed that an AD blood test could serve as the first stage in a multi-stage diagnostic

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workup [6] as is the case in infectious disease, cancer, and cardiovascular disease. A blood test can fit into the current infrastructure and be used to rule out patients who do not need further workup. We hypothesize that a blood-based screening tool for AD [7–10] can serve as the first step in a multistage detection process [11] within community-based clinics. Obtaining an early diagnosis within primary care settings can increase access to current therapies, reduce overall health care costs [12], delay nursing home placement [13], facilitate a connection with community resources, and reduce caregiver stress [13] as well as assist in future planning [13]. This model follows the evolution of breast cancer screening in primary care [13].

When designing a biomarker (blood based or otherwise), it is crucial to first define the context of use and outline the methods for development per that fit-for-purpose [14–16] as well as outline the minimum performance requirements of the biomarker itself. In this case, what is the overall purpose of the AD blood screener when applied to a primary care setting? Is it to "diagnose" AD or to determine who needs follow-up examination? In primary care settings (and other settings), a key context of use for nearly all screening tests is to rule out those who do not have the disease to decrease the numbers of patients that undergo more invasive and costly procedures. For example, mammography does not rule in breast cancer as the positive predictive values (PPV) are below 30% [17,18]. Additionally, screening of depression in primary care has low PPVs (e.g., 0.15–0.27) [19], but negative predictive power is excellent (>0.96) [19]. In both cases, the screening test ensures that only those who need the follow-up examination (biopsy, psychiatric referral) undergo such procedures, which serves as cost containment and reduces unnecessary medical services to patients. This strategy also provides a streamlined, step-wise process for physicians to make decisions regarding which tests are used in what order.

Therefore, it is our proposal that a primary care AD blood screen can be used to rule out 85% or more of elderly patients seen in primary care who do not need to undergo more expensive procedures. Therefore, a screen positive on the AD blood test would trigger a multistage neurodiagnostic process of (1) neurology specialty exam for differential purposes, (2) cognitive testing, and finally, (3) cerebrospinal fluid analysis and/or PET amyloid imaging.

When moving from discovery to clinical consideration of biomarkers, there are a series of steps for validation purposes [20]. Once the biomarker has been identified and initial validation studies have been conducted (independent of the discovery set), the methods must be "locked down" for additional prospective studies (e.g., clinical trials) [20]. This "lock-down" procedure is where all steps in the process are solidified and no longer available for further manipulation. With regard to multimarker algorithm applications, such as our AD blood test, this includes the generation of a locked-down referent sample to which all future blood samples are compared. To date, no work globally has created

Table 1
Demographic characteristics across cohorts

Characteristic	Total Mean (SD) Range			HABLE Mean (SD) Range			UTSW-ADC Mean (SD) Range			PARI Mean (SD) Range			Mayo Mean (SD) Range		
	AD	MCI	NC	AD	MCI	NC	AD	MCI	NC	AD	MCI	NC	AD	MCI	NC
Age (y)	75.8 (8.6) 51–103	69.4 (8.6) 50–102	65.8 (9.8) 50–95	74.2 (9.0) 58–91	66.3 (8.0) 50–86	59.2 (6.7) 50–85	72.6 (8.1) 51–103	69.0 (7.2) 52–94	67.6 (7.3) 50–84	82.3 (9.1) 66–97	81.7 (7.8) 69–102	76.9 (6.7) 65–95	76.8 (5.7) 64–88		76.7 (5.7) 56–88
Gender female %	52.6	54.4	65.7	60.7	63.9	80.6	37.1	47.5	37.6	82.1	64.5	64.9	64.1		72.9
Education	13.1 (4.3) 0–22	11.4 (5.2) 0–22	11.8 (4.9) 0–23	5.6 (4.6) 0–16	6.9 (4.6) 0–20	8.8 (4.6) 0–20	14.6 (2.8) 8–20	14.6 (2.9) 7–22	15.5 (2.4) 10–22	6.8 (3.4) 0–14	6.8 (2.9) 1–12	9.2 (3.9) 1–16	13.7 (3.0) 6–20		14.6 (2.9) 7–20
Ethnicity %															
Non-Hispanic White	61.8	41.7	31.9		3.1	6.5	66.8	69.8	49.6			100.0			
Hispanic/Latino	14.4	41.7	49.5	100.0	96.9	93.3	0.5	1.7	0.4	100.0	100.0				
African American	3.1	11.4	3.1				6.8	19.6	11.9						
Choctaw	0.4	3.9	2.8				1.0	6.7	10.6						
Other		1.3						2.2							

such a locked-down referent sample, which is required for the next step of a clinical trial of the AD blood screen specifically within primary care settings. In this study, by leveraging samples and data across numerous clinic-based and community-based cohorts, we generated the first-ever locked-down referent sample for an AD blood screen and conducted a preliminary validation of this referent sample. Additionally, estimates of positive and negative predictive values were calculated simulating primary care setting base rates. To consider cost containment, analyses were conducted for a 10-protein version of the algorithm in addition to the full 21-protein algorithm that has been established in our prior work [6].

2. Methods

2.1. Participants

Blood proteomic data were analyzed from 1329 individuals across multiple community-based and clinic-based cohorts outlined below. Table 1 contains the demographic characteristics of each cohort.

2.1.1. Health & Aging Brain among Latino Elders

Samples were analyzed from the Health & Aging Brain among Latino Elders (HABLE) study, an ongoing epidemiologic study of cognitive aging among community-dwelling Mexican Americans and non-Hispanic whites [21,22]. The HABLE study uses a community-based participatory research approach, which involves partnering communities to conduct studies of human disease. This research was conducted under an institutional review board approved protocol with each participant (and/or informants for cognitively impaired persons) providing written informed consent. Each participant underwent an interview (i.e., medical history, medications, and health behaviors), detailed neuropsychological testing, blood draw, and medical examination (review of systems, Hachinski Ischemic Index scale, brief neurological screen). Testing was completed in English or Spanish depending on the participant's preference. Consensus diagnoses were assigned according to published criteria [23,24].

2.1.2. UTSW—Alzheimer's Disease Center

Samples from the NIA-funded UTSW-ADC biorepository were analyzed. Each participant underwent an interview, neuropsychological testing, blood draw, and medical examination per the NACC protocol. Consensus diagnosis was assigned based on published criteria [23–27]. Samples were drawn from both clinic-based and community-based subjects from prior ADC work.

2.1.3. Mayo Clinic—Jacksonville Alzheimer's Disease Center

Clinic-based samples were assayed from the NIA-funded Mayo Clinic Jacksonville ADC biorepository. Each participant underwent an interview, neuropsychological testing, blood draw, and medical examination per the NACC protocol. Consensus diagnosis was assigned based on published criteria [23–27].

2.1.4. Panama Aging Research Initiative study [28]

Samples were assayed from community-based participants from the Panama Aging Research Initiative (PARI) cohort, the first-ever study of Panamanian aging. PARI participants were recruited from the outpatient geriatric services from the largest public hospital of the social security located in Panama. Each participant underwent an interview, neuropsychological testing, and blood draw. All participants (or their proxies) signed informed consent forms, and patient confidentiality was not breached in accordance with the Declaration of Helsinki (1964). Consensus diagnosis was assigned according to published criteria [23,24].

2.2. Sample collection

UTSW-ADC, Mayo, and PARI samples were collected nonfasting, whereas HABLE samples were collected fasting. Serum—(1) 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 $1300\times$ g at room temperature within 1 hour of collection; (4) 1.0-mL aliquots were transferred into cryovial tubes; and (5) samples were placed into $-80°$ C freezers for storage until use. Plasma—(1) blood was collected into 10-mL lavender-top tubes and gently inverted 10–12 times; (2) tubes were centrifuged at $1300\times$ g at room temperature for 10 minutes within 1 hour of collection; (3) 1-mL aliquots were transferred to cryovial tubes; and (4) tubes were placed in $-80°C$ freezers for storage. Table 2 provides the breakdown of blood samples by diagnosis. Table 3 provides descriptive statistics for the individual proteomic markers assayed.

2.3. Proteomic assays

Proteomic data were obtained in duplicate via a multiplex biomarker assay platform using electrochemiluminescence 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. In our prior work, we conducted discovery and validation studies to identify and refine a putative AD blood profile. In our most recent work [6], we

Table 2
Breakdown of final "locked-down" referent sample by diagnosis

Diagnosis	Sample size
Normal cognition	722
Mild cognitive impairment	307
Alzheimer's disease	300
Total sample	1329

Table 3
Descriptive statistics of the proteomic markers assayed

	Total Mean (SD) Range	AD Mean (SD) Range	MCI Mean (SD) Range	Normal control Mean (SD) Range	LLOD range
A2M pg/mL	2,207,345,434 (726,174,209.6) 122,991.2– 7,206,174,358	2,259,607,984 (604,654,494.3) 146,894.0– 4,542,176,487	2,229,140,420 (831,608,551.4) 280,762.9– 7,206,174,358	2,119,302,013 (637,895,357.6) 853,628,046.9– 5,511,893,404	0.908–0.958
B2M pg/mL	2,390,617.9 (1,272,067) 0–18,045,940.0	2,460,678.7 (1,123,022.5) 105–9,799,050.5	2,348,623.8 (1,211,847.8) 0–14,355,126.7	2,234,756.8 (1,063,833.6) 792,827.1–17,914,318.7	6.1
CRP pg/mL	846,864.7 (2,857,127.7) 0–34,466,996.7	742,972.9 (3,144,226.5) 73.1–33,929,332.0	1,173,191.1 (2,997,816.7) 40.9–22,954,496.7	602,395.9 (2,272,422.7) 36.9–24,661,007.0	0.69–19.8
Eotaxin 3 pg/mL	14.9 (174.6) 0–6117.0	19.6 (292.0) 0–6117.0	23.8 (184.2) 0–3055.8	6.1 (22.9) 0–407.0	1.29–4.13
FABP ng/mL	66,067.2 (61,188.4) 0–669,094.2	52,850.6 (59,117.6) 679.4–346,447.1	105,976.6 (63,600.9) 13,985.0–408,764.7	61,480.8 (54,688.9) 1339.9–669,094.2	0.0952–0.107
FVII pg/mL	1,057,670.6 (390,333.5) 0–3,079,368.0	948,151.9 (325,428.8) 3580.9–2,535,543.1	1,112,188.9 (409,878.2) 0–2,500,304.8	1,079,483.0 (365,270.0) 121,001.3–2,901,171.2	3.44–4.32
I309 pg/mL	4.9 (5.3) 0–85.4	4.4 (5.3) 0–85.4	6.0 (5.0) 0.5–36.1	5.0 (5.5) 0.1–48.4	0.408–0.46
IL10 pg/mL	8.7 (54.9) 0–933.3	5.1 (29.0) 0.1–528.1	9.6 (53.2) 0–690.0	10.7 (67.2) 0.1–933.3	0.01–0.15
IL18 pg/mL	264.4 (168.0) 0–1996.2	224.6 (124.6) 33.1–965.1	296.8 (192.4) 47.1–1366.9	271.6 (173.2) 12.6–1996.2	0.71
IL5 pg/mL	1.6 (10.4) 0–229.7	1.6 (11.7) 0–229.7	0.9 (1.1) 0.1–10.5	1.8 (11.2) 0–200.4	0.05–0.56
IL6 pg/mL	3.9 (32.8) 0–1296.1	7.1 (63.1) 0.2–1296.1	3.5 (11.4) 0.2–173.8	2.5 (3.3) 0.1–56.5	0.01–0.11
IL7 pg/mL	10.1 (8.7) 0–70.6	9.8 (6.2) 0–40.6	13.9 (11.9) 0.1–70.6	9.9 (8.7) 0–63.7	0.11–0.57
MIP1-α pg/mL	288.6 (1663.0) 0–43,165.7	267.4 (1057.0) 0–11,224.8	277.8 (1320.5) 12.3–20,077.5	357.6 (2170.3) 0–43,165.7	2.28–4.01
PPY pg/mL	922.4 (718.6) 0–5948.5	769.0 (630.1) 0–5948.5	1258.0 (763.7) 0–3767.7	921.8 (707.4) 0–3889.6	68
SAA pg/mL	2,008,947.1 (11,394,948.1) 0–268,920,684.1	1,570,719.8 (7,092,494.0) 217.2–76,765,892.5	1,389,405.3 (3,822,218.7) 263.2–39,870,868.6	1,489,218.3 (9,981,282.1) 3.7–168,188,978.4	1.07–35.5
sICAM1 pg/mL	93,923.5 (207,907.0) 0–2,028,774.2	72,355.4 (176,827.9) 128.1–1,053,408.2	140,460.6 (265,191.1) 136.7–1,953,128.9	68,741.3 (175,030.4) 104.1–962,580.7	0.46–14.4
sVCAM1 pg/mL	139,452.9 (301,075.4) 0–2,263,171.5	109,219.4 (271,034.9) 227.3–2,149,384.1	187,033.9 (326,444.2) 149.4–1,711,980.5	102,407.2 (264,527.0) 62.8–2,263,171.5	0.93–35.8
TARC pg/mL	441.4 (545.5) 12.8–7944.6	527.2 (549.4) 18.7–3783.5	415.1 (408.3) 28.4–2507.7	392.2 (479.9) 15.9–6867.9	0.17–0.54
Thrombopoietin pg/mL	756.1 (429.4) 0–3906	657.8 (306.6) 147.7–3822.7	921.2 (507.8) 174.3–2546.7	738.8 (420.3) 96.9–3906.9	19
TNC pg/mL	43,394.1 (18,901.8) 0–187,278.8	44,560.2 (16,727.2) 1822.4–128,344.8	46,704.2 (23,407.6) 0–187,278.8	40,826.4 (16,991.4) 11,475.1–1,583,594.5	0.44–0.47
TNFα pg/mL	3.1 (2.4) 0–37.9	3.4 (3.2) 0.5–37.9	4.3 (2.1) 0.4–21.1	2.7 (1.8) 0–14.3	0.01–0.13

Abbreviations: LLOD, lowest level of detection; SAA, serum amyloid A; FABP, fatty acid binding protein; CRP, c-reactive protein; TNC, tenascin C; sVCAM1, soluble vascular cell-adhesion molecule-1; sICAM1, soluble intercellular cell-adhesion molecule-1; PPY, pancreatic polypeptide; TNF, tumor necrosis factor.

refined the AD algorithm to 21-proteins, validated the algorithm on an independent assay platform technology, and validated the algorithm across species and tissue type [6]. Additionally, this 21-protein AD algorithm retains excellent diagnostic accuracy across ethnic groups. Therefore, this study sought to create the locked-down referent sample for the full 21-protein AD blood screen [6]: fatty acid binding protein (FABP), beta 2 microglobulin, pancreatic polypeptide, macrophage inflammatory protein 1α (MIP1α), c-reactive protein (CRP), soluble vascular cell-adhesion molecule-1 (sVCAM-1), thrombopoietin, α2 macroglobulin, eotaxin 3, tumor necrosis factor-alpha (TNF-α), tenascin C, interleukin-5 (IL-5), IL-6, IL-7, IL-10, IL-18, I309, Factor VII, thymus and activation-regulated chemokine, serum amyloid A, and soluble intercellular cell-adhesion molecule-1.

2.4. Statistical analyses

Analyses were performed using IBM SPSS21 and R. Chi square and t tests were used to compare case versus controls for categorical variables (sex and race) and continuous variables (age and education), respectively. Per the Institute of

Medicine (IOM) guidelines [20], we created a "locked-down" referent sample of n = 1128 samples. To do this, a random sample of n = 201 samples was selected from the full sample of n = 1329 for initial validation of the referent cohort. The remaining sample of 1128 samples was combined into a single-referent sample. Once validation studies were completed, the full existing sample (n = 1329) becomes the complete "locked-down" referent sample for all future clinical trials and community-based projects looking at this AD blood screen. This locked-down referent sample is multi-ethnic, community-based and clinic-based and covers a broad age spectrum as is needed for implementation of a validated biomarker algorithm [14,15]. Sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were generated from the random forest analyses. It has been estimated previously that approximately 12% of individuals age 65 years and above suffer from AD [2] (i.e., the estimated population base rate). Therefore, approximately 12% of all older adults age 65 years and above being seen in primary care settings are suffering from AD. Therefore, PPV and NPV were calculated for AD using Bayesian statistics [29] using the estimated population base rate of 12% of AD among those age 65 years and above.

3. Results

Table 1 provides the demographic characteristics of the sample. The referent "locked-down" sample (n = 1128; control, n = 613; AD, n = 255; mild cognitive impairment [MCI], n = 260) was used to detect AD among the validation sample (n = 201; control, n = 109; AD, n = 45; MCI, n = 47). Applying the AD blood screen from the "locked-down" referent sample, the 21-protein algorithm yielded an AUC was of 0.87. The addition of age, gender, and education improved the AUC to 0.89. Therefore, PPV and NPV were calculated using the full algorithm of 21-proteins + demographics (age, gender, and education). Holding specificity (SP) at 0.98, sensitivity (SN) was 0.63 which resulted in a PPV of 0.81 and NPV of 0.95. In an effort to consider cost reduction and scalability, we restricted the AD blood test to only the top 10 proteomic markers + demographics. The overall AUC was 0.90. When holding SP = .98, SN fell to 0.58, which resulted in a PPV = 0.80 and NPV = 0.95.

Next, the referent "locked-down" sample was used to detect MCI. Using the full 21-protein algorithm + demographics, the AUC was 0.88. With SP set to 0.98, SN was 0.42 which yielded a PPV = 0.74 and NPV = 0.93. When restricted only to the 10-protein + demographics algorithm, the AUC was to 0.89. With SP set at 0.98, SN was 0.45, which resulted in a PPV = 0.75 and NPV = 0.93.

Finally, preliminary analyses were conducted to determine the accuracy of the AD blood screen at detecting any neurodegenerative disease (Parkinson's disease [PD, n=53], Lewy Body Dementia [LBD, n=53], Down syndrome [DS n=19], AD vs NC) using samples from the ADCs. Using the 21-protein + demographics AD blood test, the overall AUC was 0.92. Setting SP = 0.98, SN was 0.62. Using a 15% base rate of any neurodegenerative disease, PPV was 0.85 and NPV = 0.94 for detecting any neurodegenerative disease. Using the top 10 proteins + demographics, the AUC was 0.89. Holding SP = 0.95, SN was 0.40 which resulted in a PPV = 0.59 and NPV = 0.90.

4. Discussion

These results provide the first-ever locked-down referent sample and proof-of-concept support for the potential utility of our AD blood test as a primary care assessment tool to determine which patients warrant follow-up examination. As noted above, the context of use for this test is not diagnostic, but rather to provide a tool for assisting primary care physicians in making an empirically based judgment on who requires a referral for more costly and invasive procedures. The availability of such a tool for primary care providers would serve to increase access to specialty clinics, cerebrospinal fluid biomarker analysis and amyloid PET scans by reducing the numbers of inappropriate referrals.

The AD blood screen provided an excellent NPV (0.95) and PPV (0.80) for detecting AD. In fact, the AD blood test outperformed many screening instruments currently available for primary care. The AD blood screen was also excellent in ruling out MCI (NPV = 0.93), and PPV was also very good (0.75). Given that the AD blood test was built for the context of use (COU) as a primary care screening tool for AD, this lower PPV is not surprising. However, when applied to MCI, the AD blood screen still performed comparable to or better than many commonly used primary care screens.

Table 4 provides an overview of a broad range of screening tools for various conditions for comparison purposes. This table is intended to put the current work into context of existing tools and to set appropriate estimated performance parameters for this specific COU. For example, the 15-item Geriatric Depression Scale yields a PPV = 0.15 and NPV = 0.99 for screening depression in a primary care setting [19] when appropriate base rates are applied [38]. The CES-D provided a PPV = 0.27 and NPV = 1.0 for major depression and PPV = 0.10 and NPV = 0.96 for minor depression [19]. Urine dipstick in an emergency room screening setting for detecting diabetic ketoacidosis yields a PPV = 0.15 but NPV = 0.99. G-FOBT provides a PPV = 0.35 and NPV = 0.99 for detecting colorectal cancer [34]. Low-dose computed tomography for lung-cancer screening provides a PPV = 0.42 and NPV = 0.99. PSA has a poor PPV but excellent NPV [30]. Capillary blood glucose only has a PPV of 0.20 for detecting gestational diabetes but a NPV of 0.95 [31]. As seen in Table 4, a host of screening instruments provide excellent NPV and therefore these initial screening tests rule out a tremendous number of patients who do not need subsequent examinations that are more invasive and costly. Therefore, our AD blood

Table 4
Positive (PPV) and negative (NPV) predictive values of screening tests used in primary care and other settings

Screening test	PPV	NPV	Context of use
Breast cancer			
Mammography [18]	0.19–0.29	Not provided	Breast cancer screening in primary care
Digital mammography [17]	0.12	Not provided	Breast cancer screening in primary care
Geriatric depression			
GDS-15 [19]	0.15	0.99	Depression screening in primary care
CES-D major dep [19]	0.27	1.0	Depression screening in primary care
CES-D minor dep [19]	0.10	0.96	Depression screening in primary care
Prostate cancer			
PSA [30]	0.06	0.97	Prostate cancer screening and treatment monitoring
Gestational diabetes			
Capillary blood glucose [31]	0.20	0.95	Screening for gestational diabetes
Hypertension			
Blood pressure testing [32]	0.35–0.95		OBPM screening for hypertension as confirmed by ABPM or HBPM
Diabetic ketoacidosis			
Urine dipstick [33]	0.15	0.99	Emergency department screening of diabetic ketoacidosis in hyperglycemic patients
B-OHB [33]	0.35	0.99	
Colorectal cancer			
G-FOBT [34]	0.35	0.99	Colorectal Ca screening within hospital settings
FIT [34]	0.11	0.99	Colorectal Ca screening within hospital settings
CT colonography [35]	0.62–0.92	0.79–0.97	Follow-up colorectal cancer assessment with positive FOBT when colonoscopy is not available
HIV			
HIV screening [36]	0.10	0.99	Screening HIV in older children in primary care in high HIV prevalence settings
Lung cancer			
Low-dose CT [37]	0.42	0.99	Screening for lunch cancer with low-dose CT

screen (and when applied to MCI) performs within acceptable estimated parameters for the intended COU, and the next step in this work is to conduct prospective studies (e.g., clinical trials) leveraging the current locked-down referent sample.

In addition to serving as a means for primary care screening, the AD blood test also has a tremendous advantage for increasing access to disease-modifying drugs (clinical trials and medications when Food and Drug Administration [FDA] approved). Specifically, the AD blood screen can be used to rule out those who should not undergo PET amyloid imaging for inclusion into clinical trials or consideration for treatment once FDA approval is acquired for one of these drugs. PET amyloid scanning is expensive and, as with cancer, not a viable first line in determining drug intervention. If our AD blood screen provides a NPV = 0.90 with a PPV = 0.70, this would reduce the PET amyloid scanning needs significantly. For example, using the MCI results above with SP = 0.98 and SN = 0.42, PPV = 0.74 and NPV = 0.93. If a total of 10,000 patients were screened for eligibility to PET scans (for clinical trial entry or drug administration), PET amyloid screening costs would be approximately $50 million at $5000 per scan (less than the anticipated clinical cost of this scan). If the AD blood test were used as the first step, it could accurately rule out 8642 adults from receiving PET scans and reduce the PET scan screening cost by over $43 million. Again, the key purpose is to rule out those who do not need a PET scan.

Availability of this AD blood screen would result in a significant cost savings of the screening budget for clinical trials and a cost savings when considering incorporating disease-modifying drugs into clinical practice. Given that insurance companies do not pay for the FDA-approved amyloid scanning methods, the availability of this AD blood screen could also be used to build a successful reimbursement strategy for amyloid PET scans for those who screen positive on the blood test (i.e., cost containment). This model for seeking reimbursement can follow what has been successful in the cancer space. For example, more expensive imaging modalities such as PET scans for breast cancer only became reimbursed by CMS as an adjunct to other imaging modalities (that are less expensive) rather than the first-line or standalone procedure [39]. The comparative effectiveness research in the cancer space [40–42] could help outline a landscape for seeking approval and reimbursement for screening and diagnostic testing in AD in anticipation of the availability of disease-modifying agents in the near future. Therefore, the availability of the AD blood test could provide a cost-effective method for implementation of disease-modifying drugs into the current medical system.

Overall, the current findings are supportive of further investigation into the current AD blood screen as a tool for primary care physicians and a clinical trial should be conducted. This tool is intended to refine the diagnostic process such that those who screen positive undergo additional steps for the diagnosis and differential diagnosis. This process can also streamline and maximize cost-effectiveness of PET amyloid scans once disease-modifying drugs become FDA approved. Fig. 1 provides an example of an updated patient flow diagram for the multistage neurodiagnostic workup and
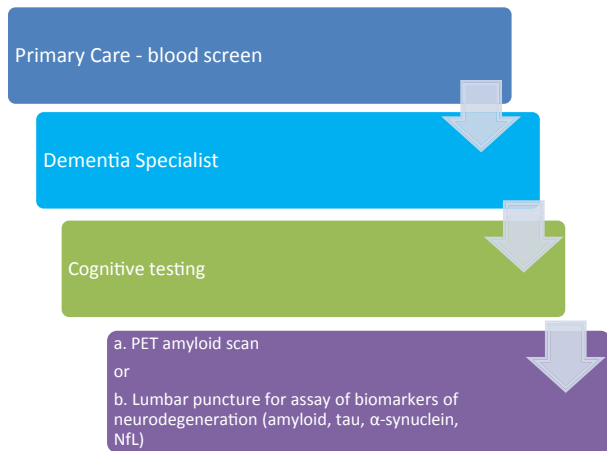
Fig. 1. Multistate diagnostic process for detecting Alzheimer's disease (AD) and discriminating AD from other dementias.

differential diagnosis for AD. This process could be used for AD and non-AD dementias.

When put into the context of the IOM guidelines for steps from discovery to clinical utility, the AD blood test is ready for a full-scale clinical trial within the context of use of primary care settings. Additional lock-down steps would be required if the AD blood screen is to be reduced in size (e.g., 5-proteins rather than 21 or even 10). Additional lock-down work should be conducted to maximize the blood collection protocols to ensure global scalability of the methods. The current work establishes the "locked-down" reference sample for the first-ever clinical trial of an AD blood test in primary care.

5. Conclusion

The current findings suggest that an AD blood test for primary care settings is a viable option for a cost-effective and time-effective means of making determinations as to which patients require follow-up examinations and procedures, and a clinical trial is required to demonstrate the prospective diagnostic accuracy. Provision of this AD blood screen could increase access to currently available medications and resources. Additionally, the availability of an AD primary care assessment tool would increase access to more advanced diagnostic procedures (CSF or imaging biomarkers) as well as disease-modifying drugs, once available. Our AD blood screen performs equivalent to or better than many primary care screening examinations. The current work is poised for the first-ever clinical trial of an AD blood test in primary care, which is required for validation of this work.

Acknowledgments

RESEARCH IN CONTEXT

1. Systematic review: To date, there is no blood screening tool for primary care providers to determine which patients should be referred for more expensive and invasive diagnostic procedures. Such a screening test would meet the annual wellness visit (AWV) requirements and increase appropriate access to advanced diagnostic procedures (imaging, cerebrospinal fluid, clinical) as well as disease-modifying medications, once FDA approval has been achieved.

2. Interpretation: Data were combined across multiple clinic-based and community-based cohorts to generate a multi-ethnic "locked-down" referent cohort for the AD blood screen. Diagnostic accuracy, positive and negative predictive power, was excellent for the AD blood screen.

3. Future directions: With the availability of the locked-down referent cohort, the first-ever clinical trial of an AD blood screen in primary care clinics can be conducted. Additionally, the utility of this blood-screen as the first step in the diagnostic process for PET scans should be examined as this would significantly decrease costs and increase access to disease-modifying agents once FDA approval is obtained.

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