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Plasma Total-Tau and Neurofilament Light Chain as Diagnostic Biomarkers of Alzheimer's Disease Dementia and Mild Cognitive Impairment in Adults with Down Syndrome

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Abstract.
Background: The need for diagnostic biomarkers of cognitive decline is particularly important among aging adults with Down syndrome (DS). Growing empirical support has identified the utility of plasma derived biomarkers among neurotypical adults with mild cognitive impairment (MCI) and Alzheimer's disease (AD); however, the application of such biomarkers has been limited among the DS population.
Objective: This study aimed to investigate the cross-sectional diagnostic performance of plasma neurofilament light chain (Nf-L) and total-tau, individually and in combination among a cohort of DS adults.
Methods: Plasma samples were analyzed from $n = 305$ ($n = 225$ cognitively stable (CS); $n = 44$ MCI-DS; $n = 36$ DS-AD) participants enrolled in the Alzheimer's Biomarker Consortium – Down Syndrome.
Results: In distinguishing DS-AD participants from CS, Nf-L alone produced an AUC of 90%, total-tau alone reached 74%, and combined reached an AUC of 86%. When age and gender were included, AUC increased to 93%. Higher values of Nf-L, total-tau, and age were all shown to be associated with increased risk for DS-AD. When distinguishing MCI-DS participants from CS, Nf-L alone produced an AUC of 65%, while total-tau alone reached 56%. A combined model with Nf-L, total-tau, age, and gender produced an AUC of 87%. Both higher values in age and total-tau were found to increase risk for MCI-DS; Nf-L levels were not associated with increased risk for MCI-DS.
Conclusion: Advanced assay techniques make total-tau and particularly Nf-L useful biomarkers of both AD pathology and clinical status in DS and have the potential to serve as outcome measures in clinical trials for future disease-modifying drugs.

Keywords: Neurofilament light chain, proteomics, sensitivity, specificity, total-tau, trisomy 21

INTRODUCTION

Diagnosing Alzheimer's disease dementia in individuals with Down syndrome (DS-AD) can be challenging based upon cognitive testing outcomes or neurological assessment, particularly in the clinic but also in a research setting. The ability to add fluid biomarker measures to enhance/improve diagnostic precision for AD dementia in individuals with DS would allow clinicians and researchers the opportunity to implement interventions as they become available in close proximity to the preclinical stage of disease. Plasma biomarkers, in particular, are easier to acquire than cerebrospinal fluid (CSF) in individuals with DS. Two biomarkers that have yielded exciting outcomes for late onset AD (LOAD) and autosomal dominant AD (ADAD) include plasma tau and neurofilament light chain (Nf-L).

Tau is the major microtubule-associated protein in neurons that interacts with tubulin to stabilize its assembly into microtubules, permitting axonal transport of intracellular vesicles in the cytoskeletal scaffold [1]. In people with AD, tau no longer associates with microtubules due to its hyperphosphorylated state, and as a result, leads to the development of neurofibrillary tangles (NFTs). NFTs disrupt the cytoskeletal scaffolding and impair axoplasmic flow leading to neuronal death. As in sporadic AD, postmortem studies of brains from individuals with DS, NFT counts were found to correlate more closely with cognitive status as compared to amyloid-β plaque counts in a prospectively followed clinical cohort [2].

In the elderly with LOAD, the ADAD population, as well as in individuals with DS, tau pathology appears to be a later and thus a more proximal marker for cognitive decline than the presence of amyloid pathology based upon neuroimaging using PET [3, 4]. With advances in bioassays, CSF and plasma tau levels have emerged as potential surrogates for the presence of brain NFTs [5]. Plasma total-tau as well

as levels of phosphorylated tau in individuals with DS demonstrate an age-related increase and a relative increase in levels as compared to individuals who do not have DS [6–8]. Because of this, biomarkers such as total-tau have been suggested as a potential biomarker for those with DS to detect AD pathology [8].

Nf-L is another important cytoskeletal scaffolding protein [9, 10] and can now be reliably measured in blood using the ultrasensitive single-molecule array (Simoa) technology. Recent work on Nf-L demonstrates its potential utility in diagnosing neurodegeneration in adults with ADAD [11–14] as well as in adults with DS-AD [15, 16]. Fortea and colleagues (2018) found that plasma Nf-L was better able to identify prodromal AD (i.e., mild cognitive impairment in adults with DS [MCI-DS]) as well as DS-AD among individuals with DS compared with other plasma biomarkers including amyloid-β peptides. Overall, the relationship between plasma Nf-L levels and AD biomarkers such as amyloid PET, tau PET, hippocampal volume, and cognitive and functional measures in individuals with DS demonstrates remarkable similarities with LOAD [17]. Due to this prior work supporting utility of both Nf-L and total-tau as biomarkers of AD pathology, in this study, we hypothesized that a cross-sectional analysis of baseline levels of plasma total-tau and Nf-L could serve (alone and in combination) as biomarkers for distinguishing cognitively stable individuals from MCI-DS and DS-AD.

METHODS

Participants

The study sample is comprised of $n = 305$ ($n = 225$ CS; $n = 44$ MCI-DS; $n = 36$ DS-AD) adult participants with DS enrolled in the Alzheimer's Biomarker Consortium – Down Syndrome (ABC-DS; https://www.nia.nih.gov/research/abc-ds). The ABC-DS is a prospective cohort study of biomarkers associated with AD among adults with DS ages 25 years and older. Proteomic analyses were restricted to those in the study age 35 years and older for purposes of examining biofluid changes associated with AD disease emergence and progression. Study visits include a baseline assessment, followed by repeated testing at 16 and 32 months, respectively. Data for the present study comes from blood collected at the ABC-DS baseline visit. Demographic characteristics of the cohort are presented in Table 1. All ABC-DS sites operate under IRB approved protocols and informed consent and/or assent was obtained for all participants.

Table 1
Demographic characteristics

	CS $N = 225$ Mean (SD) Range	MCI-DS $N = 44$ Mean (SD) Range	DS-AD $N = 36$ Mean (SD) Range	*p*
Gender N (%)				
Male	88 (51.5)	30 (71.4)	17 (48.6)	0.958
Female	83 (48.5)	12 (28.6)	18 (51.4)	
Age	45.7 (7.1) 35.1–72.0	52.1 (6.9) 40.0–81.0	54.0 (5.5) 44.0–65.0	<0.001
Nf-L pg/mL	15.6 (10.8) 3.9–90.5	30.2 (33.3) 5.5–233.0	46.2 (24.9) 10.6–122.0	<0.001
Total-tau pg/mL	2.4 (1.8) 0.0–18.4	2.7 (1.3) 0.0–8.6	3.6 (1.6) 0.0–7.3	<0.001

CS, cognitively stable; MCI-DS, mild cognitive impairment in Down syndrome; DS-AD, Alzheimer's disease dementia in Down syndrome.

Clinical assessment

Assessments included evaluations of cognition and functional abilities, behavioral and/or psychiatric conditions, and health status. Cognitive function was evaluated with a test battery designed for use with individuals with DS varying widely in their premorbid levels of intellectual functioning. Structured interviews were conducted with caregivers to collect information on changes in cognition, day-to-day functioning, adaptive behavior, neuropsychiatric conditions, and medical status. The interviews included the following measures: Vineland Adaptive Behavior Scale 3, The Dementia Questionnaire for People with Learning Disabilities, Reiss Screen for Maladaptive Behavior, and the National Task Group Early Detection Screen for Dementia (NTG-EDSD) [18, 19].

Classification of dementia

The classification of dementia status and age at onset were determined during clinical consensus conferences involving at least three research members with expertise in AD dementia in DS where information from available sources including medical, clinical, and cognitive testing were reviewed and considered in reference to baseline IQ and any recent major life transitions or events. Data on neuroimaging and blood biomarkers were not considered during the clinical consensus conference when determining diagnostic classification. Temporal evidence

of changes in cognitive, behavioral, and functional status at the baseline visit was derived from historical records as well as caregiver/informant interview. Participants were classified into 1 of 3 groups, generally consistent with the recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability. Participants were classified as *cognitively stable (CS)* if they were without cognitive or functional decline, beyond what would be expected with adult aging, *per se*. Participants were classified as having *mild cognitive impairment (MCI-DS)* if they demonstrated some cognitive and/or functional decline over and above what would be expected with aging *per se*, but not severe enough to indicate the presence of dementia. Participants were categorized as having *Alzheimer's disease dementia* (*DS-AD)* if there was evidence of substantial progressive declines in cognitive functioning and daily living skills. An "*unable to determine*" category was utilized to indicate that declines were observed but could be caused by significant life circumstance (e.g., staff changes, family death) or conditions unrelated to AD (e.g., severe sensory loss, poorly resolved hip fracture, psychiatric diagnosis primarily depression).

Assays

Plasma total-tau and Nf-L assays were analyzed at the Institute for Translational Research (ITR) Biomarker Core at the University of North Texas Health Sciences Center using Single Molecule Array (Simoa) technology (Simoa; Quanterix, Lexington, MA, USA). Tests were performed to optimize dilution factors and centrifugation. After thawing, the samples were vortexed and spun at 10,000 g for 5 min; the supernatant was directly transferred to a 96 well plate (90 μL for singlet).

Nf-L

A recombinant Nf-L calibration curve was constructed and transferred to the 96 well plate (334 μL). Calibration range was 0–500 pg/mL with the dynamic range of 0–2000 pg/mL. Analog 200 pg/mL and digital controls 10 pg/mL were transferred to the 96 well plate (90 μL for singlet).

Tau

Total-tau detection was accomplished using a multiplex 3-plex assay. The 3-plex assay plate included total-tau, amyloid-β 40 and 42; however, due to the aim of the current study, only the assay for total-tau was included in the analyses and is further reported on. Beads were labeled with dyes with various wavelengths absorptions and concentrations creating distinct sub-populations. Antibodies for total-tau were immobilized to a single color-encoded bead. A recombinant 3-Plex calibration curve was constructed and transferred to the 96 well plate (334 μl). Calibration range for total-tau was 0–100 pg/mL with the dynamic range of 0–400 pg/mL. Analog 99.5 pg/mL and digital controls 2.24 pg/mL were transferred to the 96 well plate (90 μL for singlet).

All determinations were accompanied with pooled plasma control samples (derived from $n = 40$ sibling control cases of the participants with DS), alongside inter-assay controls. The loaded 96 well plate was placed onboard and the desired dilution factors for the samples were created by the Simoa HD-1 analyzer.

Utilizing a 2-step procedure in a reaction cuvette, samples were incubated with antibody coated paramagnetic beads and biotinylated antibody detector simultaneously. After a wash, streptavidin-conjugated β-galactosidase (SBG) reagent was added binding the biotinylated antibodies leading to SBG enzyme labeling of the captured total-tau and Nf-L. After a second wash, the beads were re-suspended in resorufin β–D-galactopyranoside (RGP) reagent, transferred to a Simoa disc array and sealed. Total-tau and Nf-L captured by the antibody coated paramagnetic beads and labeled with the SBG reagent hydrolyze the RGP substrate to produce a fluorescence signal. The fluorescent signal values generated from the calibration curve of known concentrations were fit using a 4-parameter logistic (PL) curve and $1/y^2$ weighting. The lower limit of detection (LLOD) was determined at the concentration 2.5 standard deviations above the background measurement. The coefficient of variation (CV) was determined by (standard deviation/mean)*100 for the each plasma biomarker. For Nf-L, the LLOD was 0.038 pg/mL and the CV was 0.038. For total-tau, the LLOD was 0.019 pg/mL and the CV was 0.061.

Statistical analyses

Statistical analyses were conducted using the R (V 3.3.3) statistical software (R Development Core Team, 2009). Differences in demographic characteristics between diagnostic groups were determined by Fisher's exact test and Mann Whitney U test for categorical variables (gender) and continuous variables (age, blood biomarkers). Because participants with

a diagnosis of unable to determine ($n = 17$) reflected those with cognitive decline unlikely to be related to AD, all of those who met diagnostic criteria for this classification were initially removed thereby leaving only those participants with a consensus diagnosis of cognitively stable, MCI-DS, or DS-AD for analysis. Support vector machine (SVM) analysis was utilized with blood-biomarker prediction models. SVM is a classification method that performs classification tasks by constructing hyperplanes in a multidimensional space that separates cases of different class labels. Diagnostic accuracy was calculated using blood-based biomarkers alone and in combination with demographic characteristics (i.e., age and gender) using receiver operating characteristic (ROC) curves. SVM analyses were run with a 5-fold internal cross-validation. Blood biomarkers were kept in their raw value as machine learning methods do not require normalization of data. Cross sectional analyses were conducted in a series of steps starting with demographic (age, gender) variables alone to examine what value (if any) was added by including additional biomarkers of interest (Nf-L and total-tau) in detecting cognitive status at baseline. Logistic regression analyses were also conducted with select biomarkers (Nf-L, total-tau) as the predictors variables with consensus diagnosis as the dependent variable. Covariates included age and gender. Significance was set at $p < 0.05$.

RESULTS

Analyses were conducted on adults with DS who were age 35 years and older. From the combined cohorts, a total of 225 participants were determined to be CS; $n = 44$ were classified as having MCI-DS; $n = 36$ were classified as having DS-AD. Table 1 presents the demographic characteristics, along with summary statistics for the biomarker findings. Adults with DS-AD were found to be significantly older with higher levels of both Nf-L and total-tau as compared with those with MCI-DS and those who were CS. There was no difference in gender distribution across groups. Figure 1 presents box-plot distribution for Nf-L and total-tau across diagnostic groups. For both Nf-L and total-tau, a stepwise increase was shown with higher levels found in the DS-AD group as compared to the MCI-DS or CS group.

In distinguishing MCI-DS participants from CS participants (see Table 2), age and gender alone produced a detection accuracy (area under the curve [AUC]) of 82% (sensitivity [SN] = 0.00; specificity [SP] = 1.00). Nf-L alone produced an AUC of 65% (SN = 0.02; SP = 1.00); use of an optimized cut-off score did not improve the detection model. When combined, Nf-L along with age and gender increased the AUC to 82% (SN = 0.02; SP = 1.00); mirroring the detection accuracy of age and gender alone. An optimized cut-off score of –0.725 increased sensitivity to 0.14 while AUC and specificity remained unchanged. In a logistic regression that included age and gender, Nf-L was not shown to be significantly associated with increased risk for MCI-DS; however, age was significantly associated with increased risk (odds ratio [OR] 95% confidence interval [CI] = 1.1 [1.05–1.17], $p < 0.001$).

When total-tau was examined alone in distinguishing MCI-DS from CS, it produced an AUC of 56% (SN = 0.00; SP = 1.00), which was lower than the AUC obtained from Nf-L alone. Use of an optimized cut-off score of –0.788 did not greatly impact the model as sensitivity remained minimal (0.05) while specificity and AUC remained the same. When total-tau was combined with age and gender, AUC again increased to 83% (SN = 0.02; SP = 1.00). Optimizing the model with a cut-off score of –0.933 provided a slight increase to sensitivity (SN = 0.13) while specificity and AUC remained unchanged. Total-tau was found to offer the least predictive value to the algorithm as compared with age and gender. In a logistic regression including age and gender, higher values in both total-tau (OR [95%CI] = 1.4 [1.03–1.91], $p = 0.030$) and age (OR [95%CI] = 1.1 [1.08–1.19], $p < 0.001$) were shown to be associated with increased risk for MCI-DS diagnosis.

When Nf-L and total-tau were included as the only two biomarkers in the model to distinguish MCI-DS from CS, the selected biomarkers produced an AUC of 66% (SN = 0.14; SP = 1.00). When the optimized cut-off score of –0.876 was applied, it increased the sensitivity to 0.41 while specificity and AUC remained unchanged. When age and gender were added into a combined model with Nf-L and total-tau, the AUC increased to 87% (SN = 0.40; SP = 1.00). Among the four biomarkers, Nf-L again was shown to be the strongest predictor followed by age. When the optimized cut-off score of –0.999 was applied to the model, sensitivity increased to 0.86 but specificity decreased to 0.85 while AUC remained unchanged. Thus, the optimal model for distinguishing CS from MCI-DS was identified as a single combined model, which included total-tau, Nf-L, age, and gender (Fig. 2).

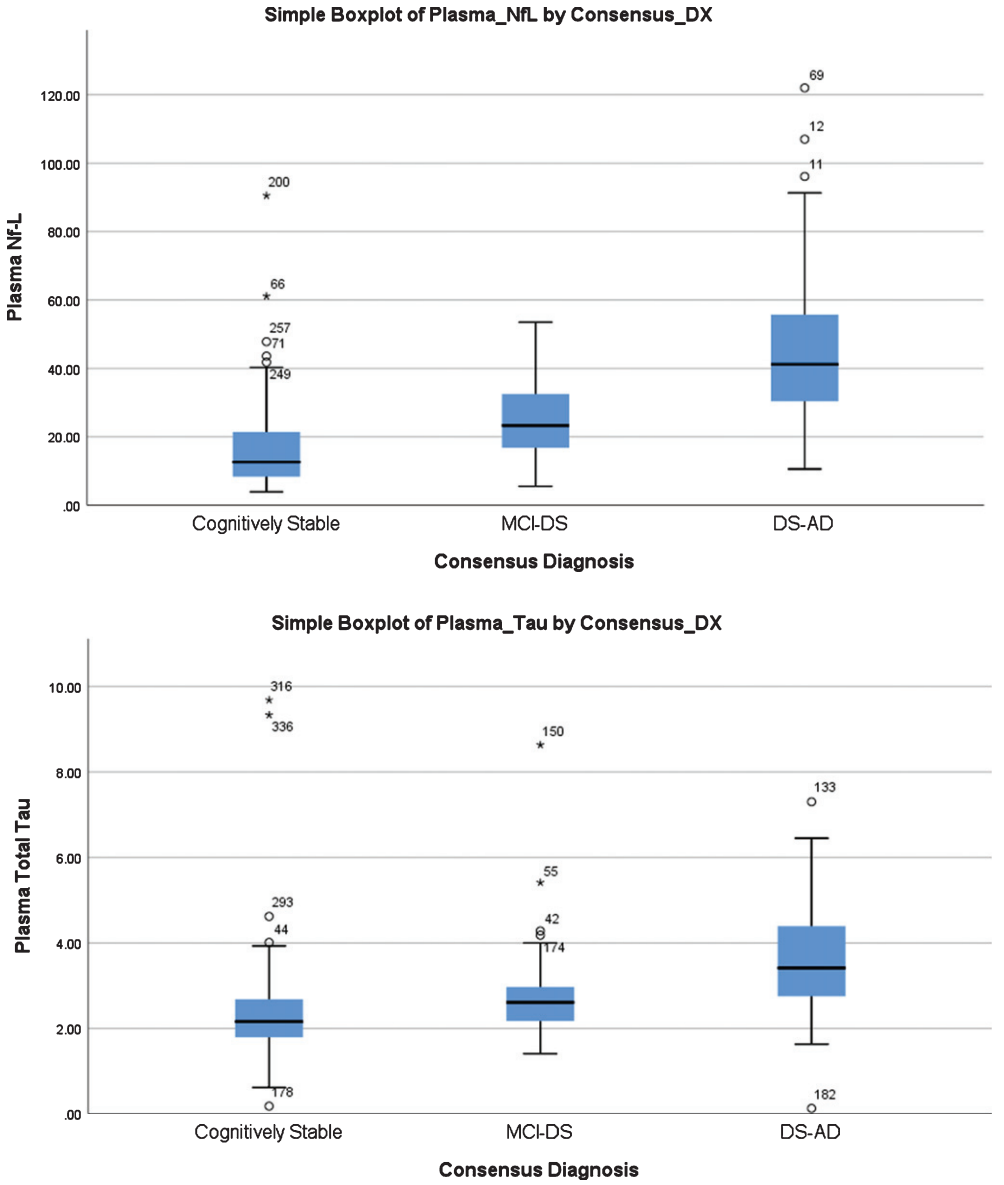

Fig. 1. Box-plot of Nf-L and Total-tau Split by Diagnostic Category of CS, MCI-DS, and DS-AD.

In distinguishing DS-AD participants from CS participants (Table 2), age and gender alone produced an AUC of 82% (SN = 0.00; SP = 0.86); comparable to MCI-DS. Age alone was shown to be the strongest predictor while gender produced only limited benefit to the detection model. When Nf-L was the only biomarker included into the detection model, it alone produced an AUC of 90% (SN = 0.50; SP = 0.97); however, with an optimized cut-off score of –0.982, sensitivity increased to 0.81 and specificity decreased to 0.92 while AUC remained unchanged. When Nf-L was combined with age and gender, it produced an AUC of 89% (SN = 0.50; SP = 0.98), which remained comparable to Nf-L alone. An optimized cut-off score of –0.888 increased sensitivity to 0.83 while lowering specificity to 0.92. AUC again remained unchanged. Nf-L was found to be a stronger predictor as compared to either age or gender. In a combined logistic regression that included age and gender, higher values in both Nf-L (OR [95% CI] = 1.1 [1.05–1.13], $p < 0.001$) and age (OR [95%CI] = 1.1 [1.04–1.19], $p = 0.002$) were associated with an increased risk for DS-AD.

Table 2
Support vector machine (SVM) models for detecting MCI-DS and DS-AD with and without the inclusion of age and gender

MCI-DS				DS-AD			
SVM Model for Age + Gender				SVM Model for Age + Gender			
AUC	82%			AUC	82%		
SN	0.00%			SN	0.00%		
SP	100%			SP	86%		
SVM Model for Biomarkers				SVM Model for Biomarkers			
	Total-tau	Nf-L	Total-tau + Nf-L		Total-tau	Nf-L	Total-tau + Nf-L
AUC	56%	65%	66%	AUC	74%	90%	86%
SN	0.00%	0.20%	14%	SN	31%	50%	47%
SP	100%	100%	100%	SP	99%	97%	99%
SVM Model for Biomarkers with Age + Gender				SVM Model for Biomarkers with Age + Gender			
	Total-tau	Nf-L	Total-tau + Nf-L		Total-tau	Nf-L	Total-tau + Nf-L
AUC	83%	82%	87%	AUC	94%	89%	93%
SN	2.70%	0.20%	0.40%	SN	44%	50%	53%
SP	100%	100%	100%	SP	99%	99%	100%
Optimized SVM Model for Biomarkers				Optimized SVM Model for Biomarkers			
	Total-tau	Nf-L	Total-tau + Nf-L		Total-tau	Nf-L	Total-tau + Nf-L
AUC	56%	65%	66%	AUC	74%	90%	86%
SN	4.50%	0.20%	41%	SN	47%	81%	72%
SP	100%	100%	100%	SP	96%	92%	96%
Optimized SVM Model for Biomarkers with Age + Gender				Optimized SVM Model for Biomarkers with Age + Gender			
	Total-tau	Nf-L	Total-tau + Nf-L		Total-tau	Nf-L	Total-tau + Nf-L
AUC	83%	82%	87%	AUC	94%	89%	93%
SN	13%	14%	86%	SN	86%	83%	81%
SP	100%	100%	85%	SP	89%	92%	95%

When total-tau was examined alone in distinguishing DS-AD from CS participants, it produced an AUC of 74% (SN = 0.31; SP = 0.99). Use of an optimized cut-off of –0.819 resulted in a relative increase in sensitivity (SN = 0.47) while specificity decreased to 0.96 and AUC remained stable. When total-tau was combined with age and gender it increased the AUC to 94% (SN = 0.44; SP = 0.99). Use of an optimized cut-off score of –0.986 increased sensitivity to 0.86 while decreased specificity to 0.89; however, AUC remained unchanged. Age was found to be the strongest predictor; however, total-tau was shown to add some significant value in distinguishing diagnostic categories. In a combined logistic regression that included age and gender, higher values in both total-tau (OR [95%CI] = 2.3 [1.62–3.37], $p < 0.001$) and age (OR [95%CI] = 1.2 [1.12–1.29], $p < 0.001$) were linked to increased risk for DS-AD.

When Nf-L and total-tau were included as the only two biomarkers into the model to distinguish DS-AD from CS, the selected biomarkers produced an AUC of 86% (SN = 0.47; SP = 0.99). When the optimized cut-off score of –0.838 was applied, it increased the sensitivity to 0.72 while specificity decreased to 0.96 and AUC remained unchanged. When age and gender were added to a combined model with Nf-L and total-tau, the AUC increased to 93% (SN = 0.53; SP = 1.00). When the optimized cut-off score of –0.858 was applied, it increased sensitivity to 0.81 but decreased specificity to 0.95 while AUC remained unchanged. Among the four biomarkers, Nf-L again was shown to be the strongest predictor followed by age then total-tau. The optimal model for distinguishing CS from DS-AD was found to be either Nf-L alone or in a fully combined model with total-tau, gender, and age (Fig. 3).

DISCUSSION

The findings from this study reveal that both plasma Nf-L alone and in combination with total-tau correctly distinguished between DS-AD cases and those who were CS in the DS population, with AUCs ranging between 86–90% prior to optimization of the SVM model. Despite the group with DS-AD being significantly older, the inclusion of demographic variables (age and gender) did not significantly impact the predictive accuracy for either Nf-L alone or in

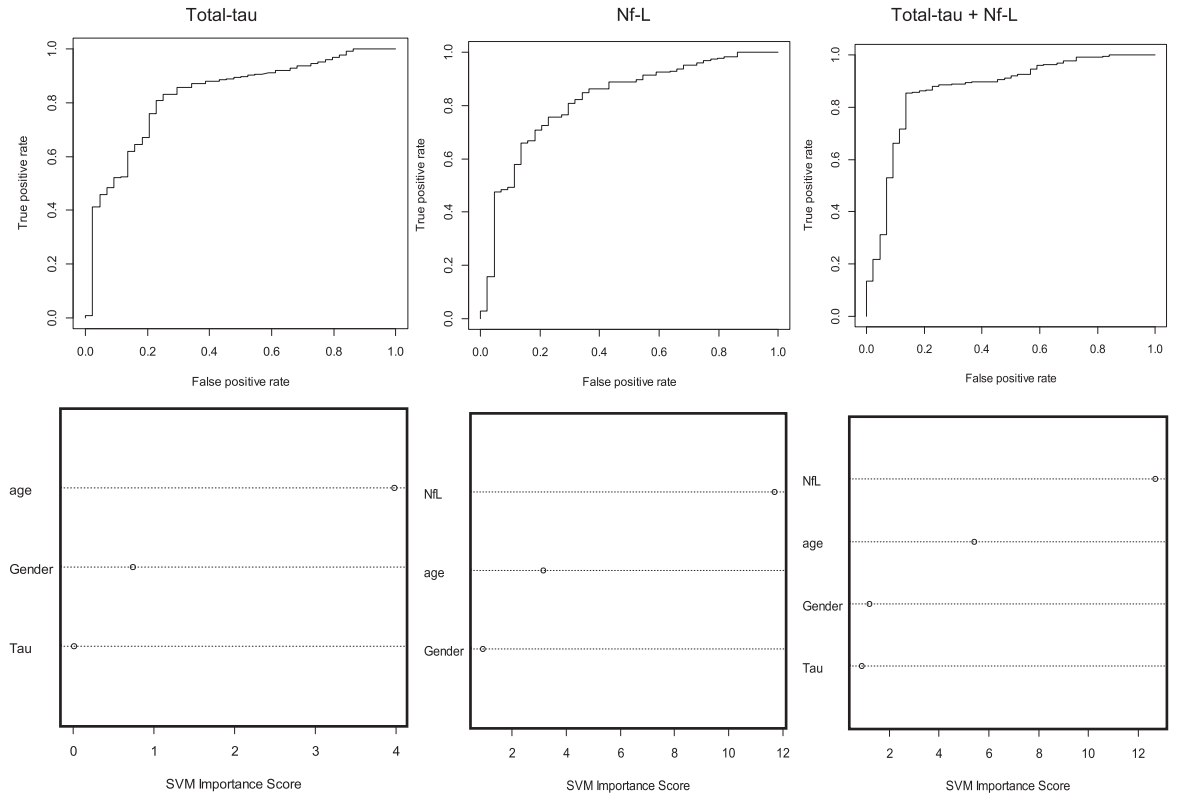

Fig. 2. ROC Curves and Variable Importance Plots for Plasma Proteomic Profile for Detecting MCI-DS with the inclusion of age and gender.

combination with total-tau; however, predictive models utilizing only total-tau revealed a 20% increase in detection accuracy (AUCs) when demographic variables were included. Utility of Nf-L and total-tau either alone or in combination when distinguishing MCI-DS from CS was significantly lower than that found for DS-AD cases with AUCs ranging anywhere from 56–66% for models without demographic variables to upwards of 83–87% with demographics of age and gender included. The highest AUC (87%; SN = 0.86; SP = 0.85) was found for the combined (Nf-L and total-tau) model that also included age and gender. These results reflect similar findings from a recent analysis of metabolomics that found no differentially expressed metabolites when comparing MCI-DS versus CS but strong AUCs when comparing CS versus DS-AD and MCI-DS versus DS-AD [20].

The excellent diagnostic performance of plasma Nf-L in individuals with DS for the cognitively stable versus DS-AD group has been previously reported, with an AUC of 0.95 (95% CI 0.92–0.98), and a sensitivity of 90% and specificity of 92% [16]. In that study, however, although they found higher values in plasma total-tau in the DS-AD group compared with the cognitively stable DS group, there was a significant overlap across the diagnostic groups, which restricted the diagnostic performance. In the present study, we combined the plasma total-tau and plasma Nf-L and found AUC of 0.93, which is comparable.

In our study, the optimized SVM model, which relies on a five-fold internal cross validation, revealed that both total-tau and Nf-L alone as well as in combination produced comparable levels of detection accuracy for cases with DS-AD (AUCs ranging from 89–94%) thus revealing limited benefit for combining such biomarkers. Recent findings from the same ABC-DS cohort that utilized a combined targeted proteomic panel derived from plasma revealed comparable results with an AUC of 95% (SN = 0.86; SP = 1.00) for the optimized SVM model [21]. The study, however, examined a combination of 20 proteins, previously validated in the general AD population [22, 23] and did not include biomarkers of tau or neurodegeneration (Nf-L).

Plasma biomarkers remain appealing in this population as they are less expensive and better tolerated than MRI, PET, or CSF biomarkers. Studies continue to support their use both in the detection of disease

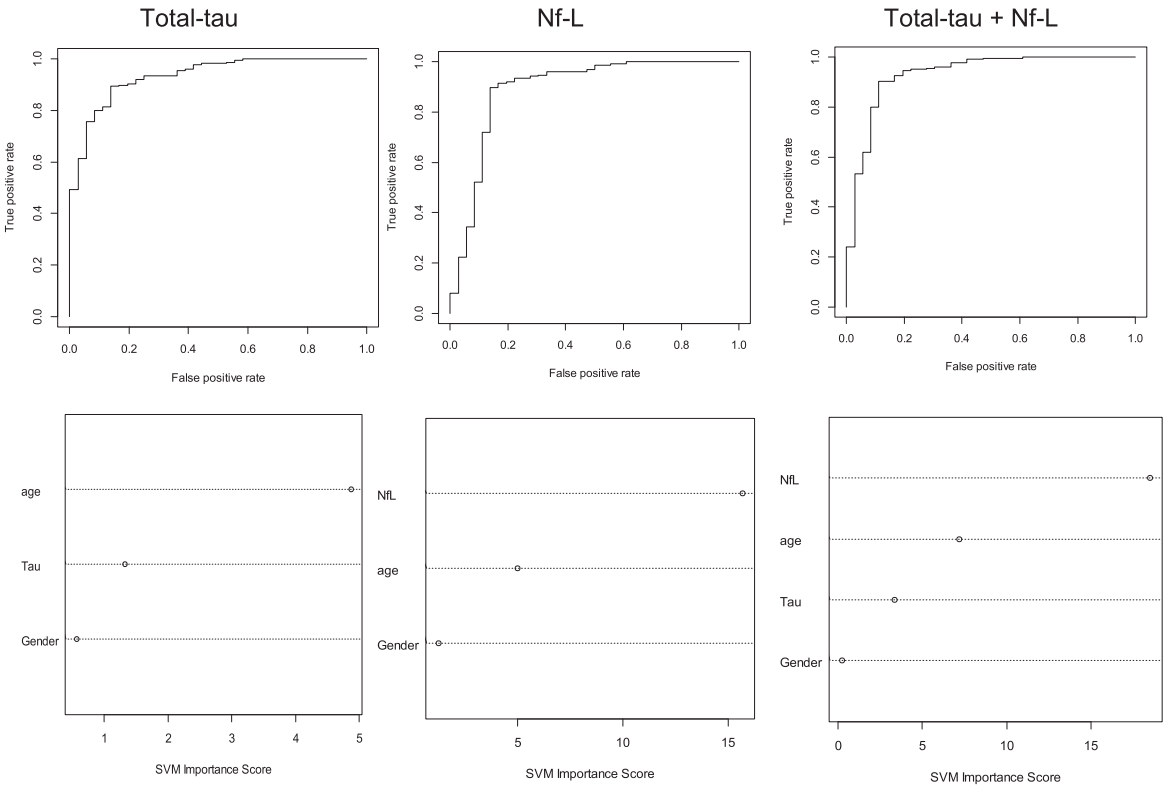

Fig. 3. ROC Curves and Variable Importance Plots for Plasma Proteomic Profile for Detecting DS-AD with the inclusion of age and gender.

presence (either alone or in combination) as well as in the potential application in a multi-tiered diagnostic screening process. This study serves to highlight the utility of plasma biomarkers of neurodegeneration such as total-tau and Nf-L in this population and cross-validates prior findings [16]. As noted above, the results of this study indicate that plasma Nf-L alone but not total-tau alone can produce an AUC for classifying DS-AD (from CS) with accuracy levels similar to that of proteomics models based on a combination of 20 proteins [21–23]. However, the same detection accuracy was not identified for MCI-DS suggesting that such markers might be limited in their detection to more advanced stages of AD as other studies have supported the utility of detecting DS-AD [6, 7, 24]. This may be due to the slow accumulation of amyloid and tau that occurs over several decades [6] and therefore, the threshold for utilizing total-tau as a biomarker of early disease detection might be lowered when applied alone as compared to when it is combined with additional biomarkers such as Nf-L, which is associated with more general neurodegeneration [13]. Another possible reason that total-tau was lower in its utility for detecting MCI-DS cases could be due the specific diagnostic category itself, as MCI is poorly defined particularly among adults with DS, which poses a limitation to this study. Another limitation of the study is the use of baseline proteomic data; however, future work is ongoing with the ABC-DS to be able to examine longitudinal proteomic data. Additionally, other forms of tau including phosphorylated at threonine 181 (p-tau 181) have been shown to be useful biomarkers of AD and early pilot work has shown its potential utility with DS [25], therefore, this should be further explored in future work possibly alone or in combination with total-tau and Nf-L.

In conclusion, we found excellent diagnostic performance of plasma Nf-L and total-tau separately and in combination as biomarkers of prevalent AD among adults with DS. Our findings show that plasma Nf-L and total-tau concentrations, measured in combination using an ultrasensitive assay, are less sensitive in detecting MCI-DS in individuals with DS, but may be more informative in longitudinal studies of incident MCI-DS or DS-AD. Plasma total-tau and Nf-L will be of value in clinical trials as an easily accessible biomarker of AD-related neurodegeneration.

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