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A Novel Panel of Plasma Proteins Predicts Progression in Prodromal Alzheimer's Disease

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Abstract.
Background: A cheap and minimum-invasive method for early identification of Alzheimer's disease (AD) pathogenesis is key to disease management and the success of emerging treatments targeting the prodromal phases of the disease.
Objective: To develop a machine learning-based blood panel to predict the progression from mild cognitive impairment (MCI) to dementia due to AD within a four-year time-to-conversion horizon.
Methods: We created over one billion models to predict the probability of conversion from MCI to dementia due to AD and chose the best-performing one. We used Alzheimer's Disease Neuroimaging Initiative (ADNI) data of 379 MCI individuals in the baseline visit, from which 176 converted to AD dementia.
Results: We developed a machine learning-based panel composed of 12 plasma proteins (ApoB, Calcitonin, C-peptide, CRP, IGFBP-2, Interleukin-3, Interleukin-8, PARC, Serotransferrin, THP, TLSP 1-309, and TN-C), and which yielded an AUC of 0.91, accuracy of 0.91, sensitivity of 0.84, and specificity of 0.98 for predicting the risk of MCI patients converting to dementia due to AD in a horizon of up to four years.
Conclusion: The proposed machine learning model was able to accurately predict the risk of MCI patients converting to dementia due to AD in a horizon of up to four years, suggesting that this model could be used as a minimum-invasive tool for clinical decision support. Further studies are needed to better clarify the possible pathophysiological links with the reported proteins.

Keywords: Alzheimer's disease, artificial intelligence, biomarkers, machine learning, proteomics

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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INTRODUCTION

The current diagnosis of Alzheimer's disease (AD) is challenging, especially at the early stages, due to the lack of specific clinical and biochemical models. While there is mounting evidence indicating that the disease starts many years before symptoms occur and dementia becomes apparent [1], it is still a considerable challenge to distinguish which blood biomarkers reflect signals of increased risk of neurodegeneration. Blood biomarkers may be any substance that can be measured in the blood that influences or predicts the incidence of outcome or disease [2]. Early biological processes may trigger multiple concomitant events such as axonal disintegration [3], synaptic dysfunction, and degeneration [4], innate immune response and neuroinflammation [5, 6], vascular and cell membrane dysregulation [7], and brain metabolic dysfunction [8]. These triggers may be associated with specific blood biomarkers, which in turn may serve as components of an effective risk model.

The initial pathological changes associated with preclinical and prodromal stages of the disease may be a better target for more efficacious treatments, avoiding the progression of the neurodegenerative process [9]. Thus, identifying biological processes possibly related to early AD pathogenesis is key to disease management and the success of emerging treatments targeting the asymptomatic or prodromal phases of the disease.

Mild cognitive impairment (MCI) individuals display cognitive impairment with no significant effect on functional activities, but they have an increased risk of developing dementia [10]. Recent neuroimaging techniques have been successfully used for predicting MCI-to-AD dementia conversion using the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [11]. Despite these developments, prediction of disease progression through minimally invasive and cheaper methods such as plasma biomarkers has been less explored and requires further investigation [12].

There are many studies analyzing significant biomarkers associated with MCI-to-AD dementia conversion, but most of them require either invasive methods or expensive resources, such as imaging biomarkers and cerebrospinal fluid (CSF) measurements of amyloid and tau [13–15]. Besides being expensive, positron emission tomography (PET) imaging is only accessible in specialized centers, and although CSF collection is cheaper, it requires a lumbar puncture, which is invasive and time-consuming [16]. Thus, measurements of blood biomarkers would be a more practical approach for the assessment of a considerable number of patients seeking medical advice for cognitive symptoms [16]. Mapstone et al. have published a study that uses blood markers to predict AD. Using a panel of 10 lipids, they were able to predict if an individual is going to develop MCI or AD within a 2–3-year timeframe with over 90% accuracy [17].

In the current study, we present a novel machine-learned-based panel of plasma proteins for early detection of AD conversion in MCI individuals. In more detail, we aim to predict whether an MCI patient will convert to AD within a four-year period (i.e., MCI-to-AD converter) or not (i.e., stable MCI). The data used in this work were obtained from the ADNI database (http://www.loni.usc.edu/ADNI) and include 146 plasma proteins collected at the study baseline. We investigated over one billion candidate risk models, combining different proteins in the plasma, so that heterogeneous conversion mechanisms, acting at different time periods, may be taken into consideration, where each model considers a specific panel of biomarkers [18, 19]. The final model was composed of 12 proteins, and we provided high-level explanations to show how each protein contributed to the model's decision. Such explanations may enable the identification of possible mechanisms and biological processes that are strongly associated with conversion from MCI to AD dementia, as well as suggest new protein biomarkers for early AD diagnosis. The unprecedented modeling scale enabled us to find complex panels to estimate the conversion of MCI individuals, thus distinguishing the prodromal stages of AD from the expected cognitive decline associated with the normal aging process.

MATERIALS AND METHODS

Data

We used the ADNI database to investigate plasma proteins in prodromal AD [20]. ADNI (http://adni.loni.usc.edu) is a longitudinal, multicenter study that was launched with the primary goal of testing whether magnetic resonance imaging, PET, biological markers, and clinical and neuropsychological assessment can estimate the progression of MCI to dementia due to AD. Definitions of the participant classifications by ADNI are presented below.

MCI

Memory complaints, Mini Mental State Examination (MMSE) scores between 24 and 30 (inclusive), objective memory loss measured by education-adjusted scores on the Wechsler Memory Scale Logical Memory II, Clinical Dementia Rating (CDR) score of 0.5, absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living and absence of dementia.

AD

MMSE scores between 20 and 26 (inclusive), CDR of 0.5 or 1.0, and diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.

Participants undergo annual examinations, as well as clinical and neuropsychological assessments. CSF was collected from just those that volunteered and consented to have a lumbar puncture. Blood was collected from all participants. Additional details are available at https://adni-info.org.

We analyzed the concentration of 146 plasma proteins of MCI individuals from the ADNI cohort [21], which were measured through the Luminex xMAP technology, which uses a flow-based laser apparatus to detect fluorescent polystyrene microspheres which are loaded with different ratios of two spectrally distinct fluorochromes [22]. In total, we examined data from 379 individuals who were classified as MCI during the baseline visit. From these individuals, there were 176 MCI-to-AD dementia conversions within four years. All individuals had measures for the 146 plasma proteins.

Models

Complex biological processes associated with AD progression are mediated by interactions between diverse pathways [7]. Since these non-obvious interactions are not fully understood, we developed a large-scale exploration of risk models, each one exploiting different interactions between the 146 plasma proteins. We sampled over one billion models to predict if each MCI individual would or not go to convert to dementia within a four-year horizon. For each model, we randomly selected up to 30 proteins, thus resulting in models with diverse predictive performance.

The models were built using a fast implementation of the LightGBM algorithm [23], which produces a complex model composed of hundreds of simple decision trees that are finally combined into a single model by a process known as boosting [24]. The predictive performance of each LightGBM model is assessed in terms of the standard area under the ROC curve (AUROC) measurement [25].

For all models, we conducted five rounds of 120-fold cross-validation, thus improving the robustness and stability of the results. The data were arranged into 120 folds. At each run, 119 folds were used as a training set and the 120th fold was used as a test set, using early stopping with 30 iterations. The whole 120-fold procedure was repeated five times, and for each model, we estimated its AUROC means and standard errors. Figure 1 shows a diagram presenting the methodological steps.

The protein-protein interaction was evaluated using STRING software [26] and the pathway analysis was performed according to Kegg. p-value < 0.05 was considered significant.

Data availability

All data used in this study is available at the ADNI database (https://adni.loni.usc.edu).

RESULTS

We examined data from 379 MCI individuals, from which 176 converted to AD dementia and 203 remained as MCI within a four-year horizon. Table 1 shows the baseline characteristics of stable MCI and MCI-to-AD converter sub-populations.

After following the methodology represented in Fig. 1, we chose the best-performing model with regards to AUC measurement. AUC performance was obtained by plotting the rate of correctly classified positives among all positive predictions (i.e., the true-positive rate) as a function of incorrect positives among all negatives (i.e., the false-positive rate), at varying thresholds. Because the output of the model is a probability (i.e., the risk of each patient converting from MCI to dementia within four years), each threshold is a value ranging from 0 to 1.

The best-performing model can distinguish between MCI-to-AD converters from individuals with stable MCI with an AUC of 0.91 ± 0.01, with accuracy of 0.91, sensitivity of 0.84 and specificity of 0.98. Figure 2 shows the AUC performance of this model. The red dot shows the point where the sensitiv-

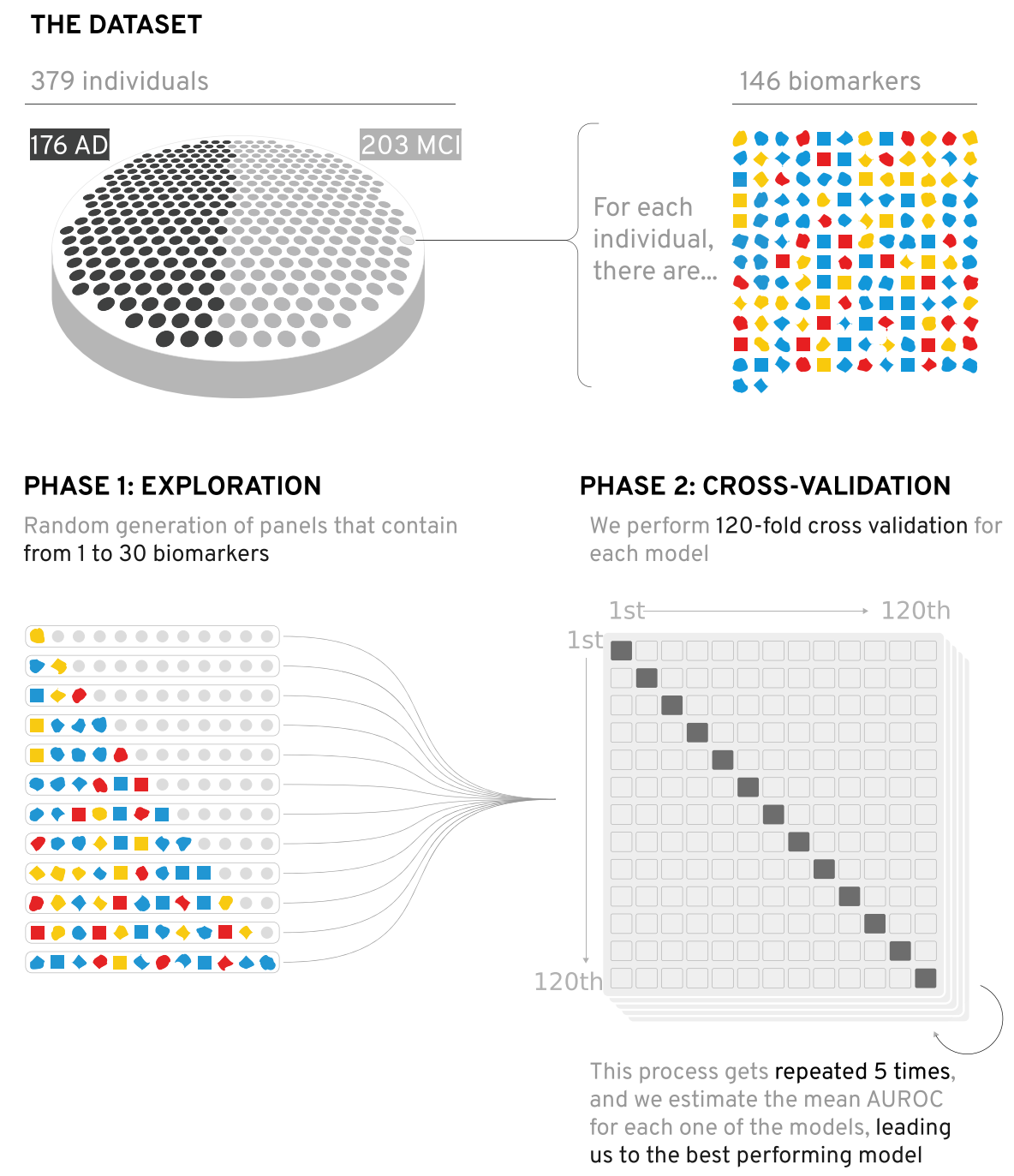

Fig. 1. Diagram presenting the dataset and the steps of the proposed methodology. First, we went into a large exploration, creating models from the random combination of up to 30 proteins (out of the 146 possible proteins). Then, for each one of these models, we performed a 120-fold cross-validation. At each iteration, the data (from the 379 patients) were divided by 120; 119 folds were used for training and the last fold was used for testing. This whole process of cross-validation was repeated five times. All the metrics are reported as averages of these test measures.

ity is 0.84 and the specificity is 0.98. Covariates, such as age, sex, and *APOE ε4* carriers, were also included to assess the dependence of the composite panel on these risk factors. The predictive performance varied slightly and a decrease in the AUROC was observed with the inclusion of covariates.

Table 1
Descriptive statistics of Stable MCI versus MCI-to-AD converters in the baseline

Variable	Stable MCI Average ± SD (min-max)	MCI-to-AD converters Average ± SD (min-max)	*p*
No. of subjects	203	176	–
Age, y	74.9 ± 7.7 (54–89)	74.6 ± 7 (55–88)	0.700
Education, y	15.5 ± 3.2 (4–20)	15.8 ± 2.8 (6–20)	0.430
Sex, % female	34%	38%	0.410
APOE genotype (%, ε4+)	54%	80%	< 0.001
ADAS-Cog-11	10.5 ± 4.3 (2–25)	13.0 ± 4.1 (4–28)	< 0.001
MMSE Score	27.3 ± 1.8 (24–30)	26.7 ± 1.7 (24–30)	< 0.001

Student's *t*-test was used to compare the groups. *APOE* genotype, Carriers of ε4 alleles; ADAS-Cog-11, Alzheimer's Disease Assessment Scale-Cognitive Subscale; MMSE Score, Mini-Mental State Examination.

Table 2
Descriptive statistics of the 12 plasma proteins that compose the selected model.

Variable	Stable MCI Average ± SD (min-max)	MCI-to-AD converters Average ± SD (min-max)	*p*
ApoB (μg/ml)	24.69 ± 2.47 (18.3–31.8)	24.60 ± 2.57 (18.4–31.8)	0.737
C-peptide (ng/ml)	1.54 ± 0.35 (0.9–3.1)	1.50 ± 0.33 (0.4–3)	0.177
Calcitonin (pg/ml)	2.82 ± 0.52 (1.6–4.5)	2.80 ± 0.72 (1.4–9.3)	0.863
CRP (μg/ml)	1.39 ± 0.85 (0.2–5.6)	1.12 ± 0.64 (0.4–4.9)	**< 0.001**
IGFBP-2 (ng/ml)	7.49 ± 1.77 (4.8–15)	7.76 ± 2.08 (4.8–17.1)	0.187
IL-3 (ng/ml)	0.20 ± 0.06 (0.1–0.4)	0.21 ± 0.06 (0.1–0.4)	0.149
IL-8 (pg/ml)	2.89 ± 0.63 (1.3–6.8)	2.71 ± 0.52 (1.3–4.1)	**0.002**
PARC (ng/ml)	8.00 ± 1.41 (5.1–15.3)	7.66 ± 1.22 (5.1–10.8)	0.015
Serotransferrin (mg/dl)	31.36 ± 2.37 (25–38.9)	31.84 ± 2.29 (25.3–42.0)	0.040
THP (μg/ml)	0.26 ± 0.05 (0.1–0.4)	0.25 ± 0.04 (0.1–0.4)	0.477
TLSP I-309 (pg/ml)	1.02 ± 0.01 (1.01–1.04)	1.02 ± 0 (1.02–1.0.5)	0.643
TN-C (ng/ml)	16.32 ± 3.21 (6.8–29.8)	16.10 ± 2.76 (8.7–25.3)	0.490

Student's *t*-test was used to compare the groups. ApoB, Apolipoprotein B; CRP, C-Reactive Protein; IGFBP-2, Insulin-like Growth Factor-Binding Protein 2; IL-3, Interleukin 3; IL-8, Interleukin 8; PARC, Pulmonary and Activation-Regulated Chemokine; THP, Tamm–Horsfall protein; TLSP 1-309, T-Lymphocyte Secreted Protein; TNC, Tenascin-C.

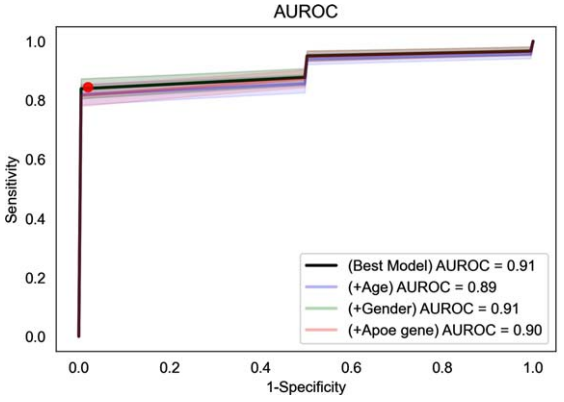

Fig. 2. AUROC curve of the model.

This model was composed of a panel containing 12 plasma proteins that are shown in Table 2. Although C-reactive protein (CRP) and Interleukin-8 (IL8) levels were significantly lower in MCI converters compared to non-converters ($p < 0.04$ for both after Bonferroni correction), any of these proteins performed well at differentiating the two groups individually.

In order to assess proteins' importance and thus extract intuitive insights from the prediction, we applied the SHapley Additive exPlanations (SHAP) algorithm [27] to the model. Briefly, SHAP calculates the importance of each protein by estimating the effect of its absence on the model's decision. We plot the importance of each protein for every individual, and these results are shown in the SHAP Summary Plot (Fig. 3), where proteins are depicted in the order of importance. Pink dots are associated with individuals for which the corresponding protein shows a relatively higher value. Blue dots, on the other hand, are associated with individuals for which the corresponding protein shows a relatively lower value. Further, there is a vertical line separating patients – dots located on the left side are those with

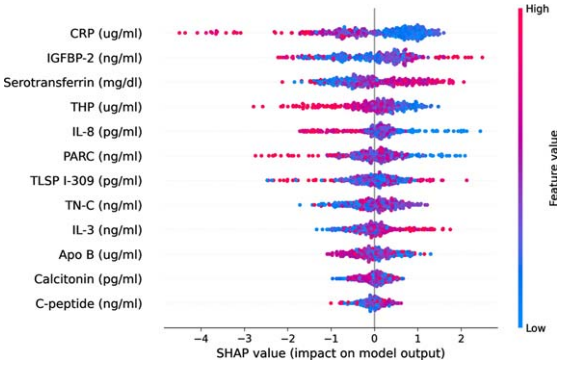

Fig. 3. SHAP Summary plot showing the effect each protein has on conversion from MCI to AD dementia within 4 years. CRP, C-Reactive Protein; IGFBP-2, Insulin-like Growth Factor-Binding Protein 2; THP, Tamm–Horsfall protein; IL-8, Interleukin 8; PARC, Pulmonary and Activation-Regulated Chemokine; TLSP 1-309, T-Lymphocyte Secreted Protein; ApoB, Apolipoprotein B; IL-3, Interleukin 3; TN-C, Tenascin C.

negative SHAP values, for which the model provided a lower risk of conversion, and on the right, those with positive SHAP values, related to a higher risk of conversion.

According to Fig. 3, among the 12 plasma proteins that compose the model, the most important one was CRP, followed by Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2), Serotransferrin, Tamm-Horsfall protein (THP), IL8, Activation-Regulated Chemokine (PARC), TLSP I-309, Tenascin C (TN-C), Interleukin 3 (IL3), Apolipoprotein B (ApoB), calcitonin, and C-peptide. Besides the order of importance of each protein, this summary plot enables us to grasp general patterns about AD pathogenesis, as in many cases one of the sides has significantly more blue or pink dots than the other side. These visual patterns are confirmed by Table 3, which shows the Pearson correlations and respective *p*-values for each protein and its SHAP values. For CRP, we can see clearly more blue dots on the right and pink dots on the left side, being moderately strongly [28] negatively correlated with its SHAP values, meaning that individuals with higher probabilities of converting to AD dementia usually have lower CRP levels than stable MCI ones. The same pattern happens to THP (very strong negative correlation) [28], IL8 (moderately strong negative correlation) [28], PARC (moderately strong negative correlation) [28]. In the cases of Serotransferrin (fair positive correlation) [28] and IL3 (moderately strong positive correlation) [28], we can see the inverse pattern: most of the pink dots are concentrated on the right, and the blue dots on the left side, meaning that

Table 3
Pearson correlations and *p*-values for each protein and its respective SHAP values

Protein	Pearson Correlation	*p*	Strength of Linear relationship Chan 2003 [28]
ApoB (μg/ml)	–0.28	**0.000**	Poor
C-peptide (ng/ml)	0.14	0.007	Poor
Calcitonin (pg/ml)	0.13	0.014	Poor
CRP (μg/ml)	–0.64	**0.000**	Moderately Strong
IGFBP-2 (ng/ml)	0.24	**0.000**	Poor
IL-3 (ng/ml)	0.65	**0.000**	Moderately Strong
IL-8 (pg/ml)	–0.73	**0.000**	Moderately Strong
PARC (ng/ml)	–0.59	**0.000**	Moderately Strong
Serotransferrin (mg/dl)	0.48	**0.000**	Fair
THP (μg/ml)	–0.83	**0.000**	Very Strong
TLSP I-309 (pg/ml)	–0.25	**0.000**	Poor
TN-C (ng/ml)	0.12	0.017	Poor

See Table 2 for abbreviations.

individuals with higher probabilities of converting to AD dementia usually have higher levels of each protein than stable MCI ones. For IGFBP-2 (poor positive correlation), TLSP I-309 (poor negative correlation), TN-C (poor positive correlation), ApoB (poor negative correlation), calcitonin (poor positive correlation) [28], and C-Peptide (poor positive correlation), this pattern is not so clear. It is worth mentioning though, that while proteins are evaluated individually, their corresponding importance is estimated by taking into account non-obvious interactions among all markers within the model.

The protein-protein interactions showed that, among the 12 proteins selected, nine are within the same biological network [IGFBP-2, Serotransferrin, ApoB, C-peptide, CRP, IL8, IL3, TLSP-1, and PARC]. The significant pathways identified, which involve the inter-related proteins, are the chemokine signaling pathway (FDR = 0.0108) and the cytokine-cytokine receptor interaction (FDR = 0.0024) (Fig. 4).

According to the ADNI database, CSF was collected from 178 (47%) individuals out of the 379 evaluated at baseline, namely 90 (51%) MCI-to-AD converters and 88 (43%) stable MCI subjects. We compared the predictive power of the CSF biomarkers at baseline (i.e., $A\beta_{42} < 980$ pg/mL and p-tau > 21.8 pg/mL) [29] with our AI model, for these 178 individuals. The AI model showed superior results both in terms of sensitivity (0.88 versus 0.73) and specificity (0.97 versus 0.55).

Figure 5A and B shows a 2D representation of the two groups: red dots represent individuals who converted to AD dementia within four years, whereas

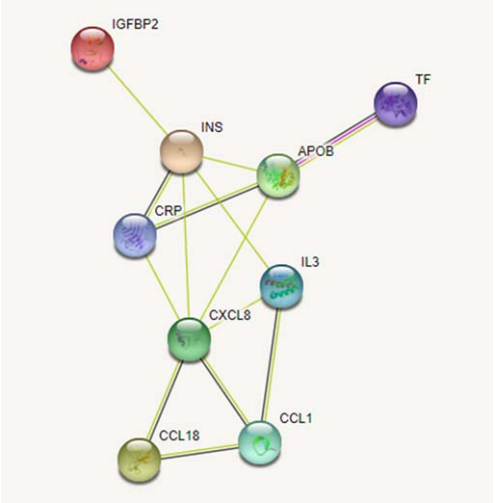

Fig. 4. Protein–protein interactions. Each node represents a protein and each line refers to an interaction. Enrichment *p*-value: 2.08 e–07. Lines: green – known interaction from text mining; pink – known interaction experimentally determined; black – co-expression among the proteins. IGFBP2, Insulin-like growth factor-binding protein 2; TF, Serotransferrin; APOB, Apolipoprotein B-100; CRP, C-reactive protein; INS, insulin (C-peptide fragment); CXCL8, interleukin 8; IL3, interleukin 3; CCL1, C-C motif chemokine 1 (TLSP 1-309); CCL18, Pulmonary and Activation-Regulated Chemokine (PARC).

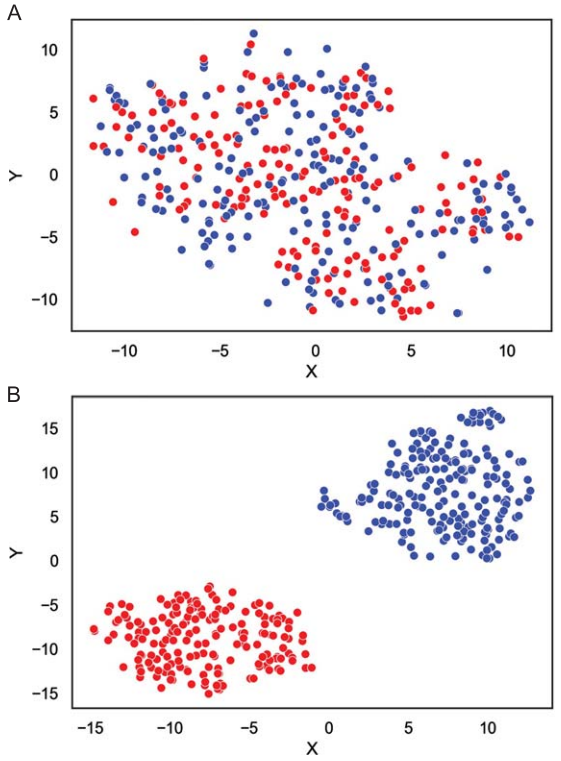

Fig. 5. t-Distributed stochastic neighbor embedding visualizations of the model. A) t-Distributed stochastic neighbor embedding visualization of the individuals, clustered based on their features values (i.e., protein concentrations in the blood). Red dots represent individuals who have converted to AD dementia, whereas blue dots represent individuals who remained as MCI. B) t-Distributed stochastic neighbor embedding visualization of the individuals, clustered based on Shapley values. This visualization represents the ability of our model to separate these two groups of individuals.

blue dots represent stable MCI individuals. To build these visualizations, we applied the t-Distributed Stochastic Neighbor Embedding algorithm [30] for dimensionality reduction. Figure 5A represents the raw proteins' concentrations for each individual. We observe no clear distinction between both groups, reflecting what might be observed in attempting to draw linear correlations between the 12 proteins. On the other hand, Fig. 5B represents the corresponding marginal contributions of each protein to the model, named Shapley values [27], reflecting all the non-linear interactions between these 12 proteins involved in the decision process of our model. In this scenario, there is a visual clear separation between both groups.

Figure 6 represents two examples of the SHAP decision plot of correct predictions made by the model for a stable MCI individual (A) and an MCI-to-AD dementia individual (B). SHAP decision plots show how the model arrives at its predictions (i.e., how it makes decisions). The x-axis represents the model's predicted probability for each individual, and the vertical line marks the model's base value. The y-axis lists the model's proteins, and the individual's prediction is represented by a colored line. Protein measures are printed next to the prediction line for reference. Starting at the bottom of the plot, the prediction line shows how the SHAP values (i.e., the protein effects) accumulate from the cutoff to arrive at the model's final score at the top of the plot. The inclination of the line for each protein shows both the direction (positive or negative) and the magnitude of each protein's effect on the prediction. At the top of the plot, the observations converge at the final predicted value.

As we can see in the top x-axis of Fig. 6, the left individual has received a probability of about 0.25, while the right received about 0.76. We can see in these figures how each protein adds to the decision of the model: in the left individual, for example, the CRP level of 1.542 μg/mL decreased the model's risk probability, while the IGFBP-2 of 6.943 ng/mL and the serotransferrin of 35.183 mg/dL increased it. PARC of 7.891 ng/ml helped to decrease the model's

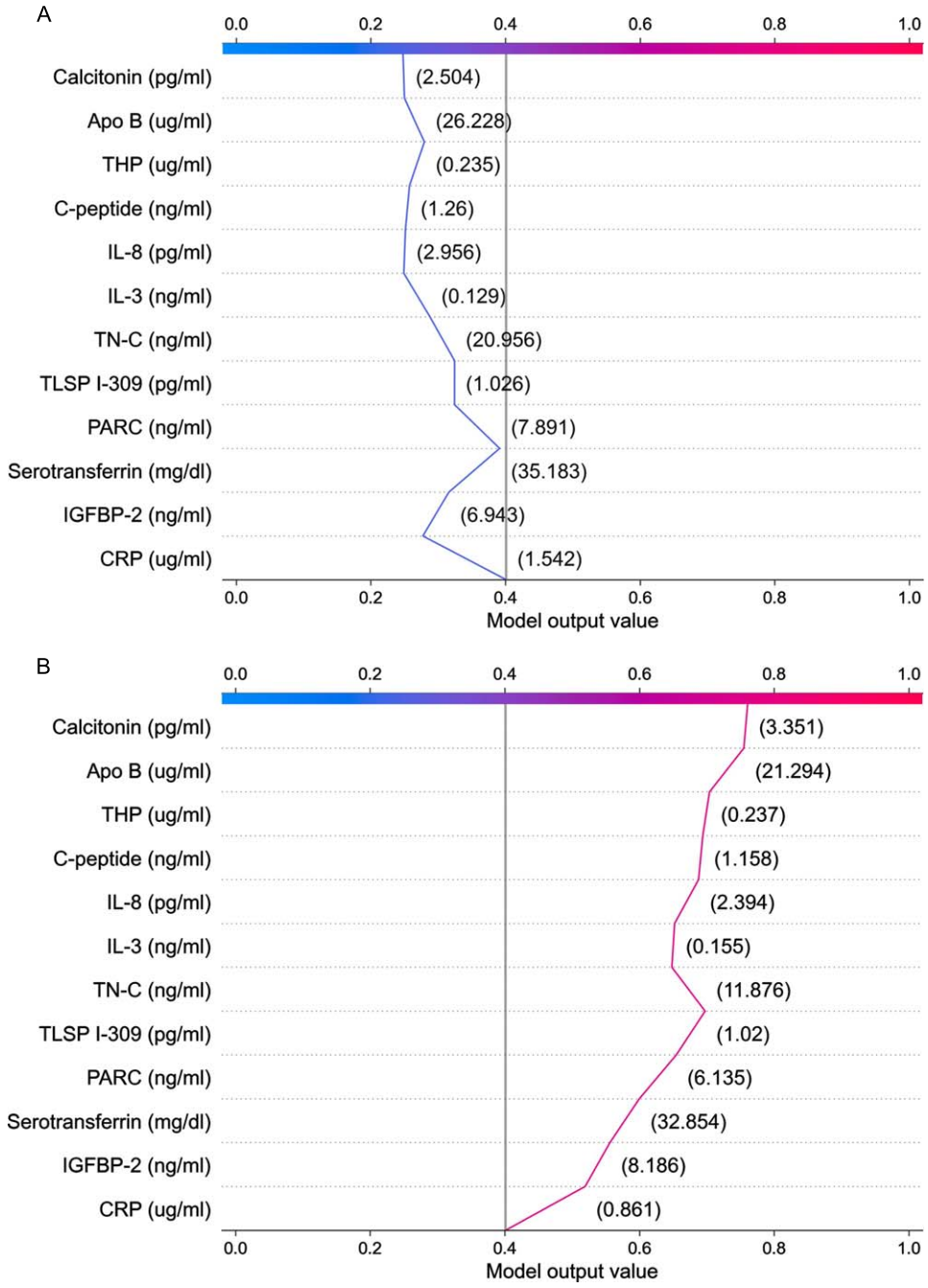

Fig. 6. SHAP decision plots showing the proteins' measures and their individual addition to the model risk prediction of two individuals: (A) stable MCI individual and (B) a MCI-to-dementia converter.

risk probability. TLSP I-309, IL-8, C-peptide, and calcitonin had almost no effect on the decision. TN-C, IL-3, and ApoB decreased the model's risk probability slightly, while THP increased it slightly. The addition of the other proteins to the model's final decision is slight.

DISCUSSION

As seen in the machine learning model explanations, the concentrations of the 12 described proteins in each MCI individual may increase or decrease the risk of conversion to AD dementia. Several

protein-protein interactions were identified, which suggests that many proteins contribute to a shared function. Most of these interactions relate to pathways associated with the inflammatory process (chemokine and cytokine receptors), which suggest that inflammation is an important mechanism for AD conversion.

For most of these 12 proteins, there is no well-established pathophysiological link and therefore our discussion will be essentially speculative. We firstly discuss CRP, THP, IL-8, and PARC, proteins whose lower concentrations may increase the risk of conversion to AD dementia, according to the SHAP Summary Plot. Then we discuss serotransferrin and IL-3, proteins that have the opposite trend. In machine learning models, complex non-linear relationships can happen between features. Because of this, sometimes it is difficult to understand a linear pattern in an isolated feature in the SHAP Summary Plot. That is the case of IGFBP-2, TLSP 1-309, TN-C, ApoB, calcitonin, and C-peptide, the last discussed proteins.

Systemic inflammation has been shown to play a role in cognitive decline and AD, particularly in the dementia stage [31, 32]. Although CRP is synthesized in response to inflammatory stimuli, previous studies have suggested that CRP levels might decrease before or during the development of AD [33–35]. In fact, Yarchoan et al. (2013) showed decreased CRP levels in MCI individuals compared to controls, thus reinforcing the hypothesis of changes in CRP levels during disease progression, with an additional decline observed in dementia stages [35].

Tamm-Horsfall protein (THP) is a multifunctional protein critical for modulating renal ion channel activity, salt/water balance, renal and systemic inflammatory response, intertubular communication, mineral crystallization, and bacterial adhesion. THP deficiency is related to urinary and kidney impairment. In clinical settings, THP can be used as a theranostic biomarker and a target for modulation to improve patient outcomes [36]. Clinical studies demonstrate that patients with chronic kidney disease (CKD) are more prone to cognitive impairment and AD, and the degree of cognitive impairment is closely related to CKD progression and renal failure [36, 37]. As far as we know, there are no specific studies that relate THP to the pathophysiology of AD.

IL-8 is a proinflammatory cytokine produced by several types of cells. It also occurs in the brain, being released from microglia in response to inflammatory stimuli. Although Baune et al. (2008) study showed that elevated cytokine IL-8 levels were linked with poorer performance in memory, cognitive speed, and motor function domains [38], what is contradictory to our findings, a recent meta-analysis study reported lower levels of IL-8 in blood from MCI patients in comparison to controls [39], what could reinforce the hypothesis of changes in IL-8 levels during disease progression.

PARC (CCL18) participates in the homing of lymphocytes and dendritic cells to microenvironmental of the secondary lymphoid organs. Within an inflammatory stimulus, PARC mediates the attraction of naïve T cells toward a primary immune response. As far as we know, there is no evidence of the direct role of PARC (CCL18) in cognitive impairment. However, considering the involvement of PARC on inflammation, lower levels in MCI patients that developed AD could be associated with their deficiency in counterbalancing the excess of immunological reactions [40].

Serotransferrin is an abundant blood glycoprotein produced in the liver that binds and transports iron. Increased serotransferrin levels are seen in association with low iron levels and iron deficiency anemia. Previous studies with the ADNI cohort have found that high plasma and CSF serotransferrin levels were associated with faster cognitive decline in participants with MCI and AD, as well as facilitated Aβ deposition and acceleration of the pathophysiological process [41, 42].

IL-3 is a cytokine that regulates the production of several blood-cell types. IL-3 plays an important role in the nervous system and appears to be important in several chronic inflammatory diseases. There are few studies investigating IL-3 in AD and most of them indicate that it has a neuroprotective role [43], in contrast to what our SHAP graph has shown. However, some studies have suggested that IL-3 increase may have a relationship with AD. Sankar et al. (2020) combined a mouse model of AD amyloid pathology (APP/PS1) with diabetes to study the effect of cortical changes in cytokine proteins. Their results suggested that AD pathology associated with diabetes yielded upregulation of IL-3. Moreover, circulating levels of $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, glucose, and insulin all correlated with this cytokine expression in the brain, suggesting a strong relationship between peripheral changes and brain pathology [44].

IGFBP-2 is the predominant IGF-binding protein in the brain. Elevated levels of IGFBP-2 are considered to inhibit the neuroprotective effects provided by IGF-I and IGF-II. High peripheral levels of IGFBP-2 have been associated with an increased risk of AD

in the Framingham Offspring cohort study [45] and were also found to be associated with reduced hippocampal volume and weakened cognitive function [46].

TLSP 1-309 (CCL1) acts as a potent chemoattractant for monocytes and lymphocytes through binding to its receptor CCR8 and has been proposed to contribute to macrophage and lymphocyte recruitment and activation in several inflammatory diseases [47]. Jorda et al. (2019) study showed a decrease in TLSP 1-309 expression and an increased expression of its receptor CCR8 in APP/PS1 mice compared to wild-type mice. This data indicates that TLSP 1-39 works as a mediator between neurons and microglia in the CNS [48].

Tenascin-C (TNC) is a hexameric, multimodular extracellular matrix protein with several molecular forms that are created through alternative splicing and protein modifications. Hasanzadeh et al. (2021) found significantly higher mean serum levels of TNC in the AD group compared to the healthy group ($p < 0.001$), showing that evaluating serum levels of TNC could be utilized for diagnosis and monitoring of AD patients [49]. However, Minta et al. (2019) found no difference between serum levels of TNC in the AD group compared to the healthy group but found significantly higher levels of TNC in women than in men in the AD group ($p = 0.02$) [50]. These findings suggest a relationship between TNC and AD, although the mechanism is not completely clear.

Apolipoprotein B-100 (ApoB) is a glycoprotein that circulates in the plasma as the major protein component of low-density lipoprotein (LDL). Apolipoprotein B-100 (APOB) was observed to be decreased in AD compared to controls in two proteomic studies [51]. In contradiction, significantly higher levels of ApoB were found in AD patients compared to controls [52]. Overexpressed human ApoB protein was also related to cerebrovascular lesions, apoptosis, and neurodegeneration [53], as well as impairment of the episodic-like memory, associated with disorganization of the neuronal microtubule network, increase in astrogliosis and lipid peroxidation in the brain regions associated with AD in transgenic mice [54]. It is important to remark that ApoB data should be interpreted with caution since there is a difference in the frequency of *APOE* alleles between the two groups (Table 1) and the presence of the $\varepsilon 4$ allele is associated with higher LDL ApoB levels [55].

The peptide calcitonin (CT) is a hypocalcemic hormone. Lehallier et al. (2016) found no significant difference in levels of calcitonin between individuals with stable MCI and those with progression to AD. However, a combination of apolipoprotein A-II and cortisol levels in plasma and fibroblast growth factor 4, heart-type fatty acid-binding protein, calcitonin, and tumor necrosis factor-related apoptosis-inducing ligand receptor 3 (TRAIL-R3) in CSF allowed for the prediction of disease status 3 years ahead with 80% accuracy [56]. Calcitonin gene-related peptide (CGRP) is a thirty-seven-amino acid regulatory neuropeptide resulting from a different merging of the CGRP gene, which also includes calcitonin. The Singh et al (2017) study showed that exogenous administration of CGRP inhibits infiltration of macrophages and expression of various inflammatory mediators, such as intercellular adhesion molecule (ICAM)-1, which attenuates the consequence of inflammation in AD, thus concluding that selective agonists of CGRP receptors may become the potential candidates for the treatment of AD [57].

C-peptide is a cleavage product of proinsulin that acts on different types of cells, such as endothelial cells. Lower levels of C-peptide in MCI patients who developed AD dementia could be associated with two mechanisms: 1) a functional interaction between C-peptide and insulin [58] since glucose uptake and utilization in the brain and neuronal cells are stimulated by insulin, insulin deficiency, or insulin resistance could dysregulate energy metabolism and thereby contribute to the pathogenesis of AD [59]. In agreement, de la Monte et al. (2019) showed that impairments in brain glucose uptake and utilization are detectable in pre-symptomatic stages of AD [59]. 2) C-peptide induces nitric oxide production with its subsequent release by platelets and endothelium, as well as the interaction with erythrocytes, leading to the generation of adenosine triphosphate and inhibition of cytokine release [58]. Consequently, lower C-peptide levels could be related to the inflammatory process, one of the main risk factors of AD.

Non-invasive plasma biomarkers may turn out to be a widely used method in the near future, allowing early and confident diagnosis of AD. Moreover, identifying the MCI subjects who have a higher probability of converting to AD dementia may aid in the development of new treatments and prevention strategies targeting the early stages of the disease. However, these proteins are not clearly linked to what we currently know about the pathophysiology of AD-associated neurodegeneration. So, it is crucial

to remember that: 1) not every phenomenon in the periphery reflects what is actually going on in the brain, so the findings can be epiphenomenal. Thus, further studies are needed to clarify possible pathophysiological links with the reported proteins; 2) many of the markers are not currently used in the clinic, but were measured in a research context; thus, the clinical applicability is not demonstrated; 3) it is essential that the results are replicated in independent samples, preferably with biological (PET-amyloid, PET-tau) or pathological confirmation of the diagnosis.

The present study has some limitations, namely, the limited sample size, coming from a single study; the use of some proteins for which the relationship with AD pathophysiology is still hypothetical; the limited timeframe for prediction - given that longitudinal studies with follow-up of cases of MCI (not just secondary to AD) describe an annual conversion rate of 12 to 15% [60], the conversion to dementia would be between 48 and 60% in four years; and the absence of external validation of the model.

In conclusion, our model is able to identify prodromal AD among MCI individuals up to four years before symptoms onset. This model was developed using 12 plasma proteins (ApoB, Calcitonin, C-peptide, CRP, IGFBP-2, Interleukin-3, Interleukin-8, PARC, Serotransferrin, THP, TLSP 1-309, and TN-C) measured with a highly sensitive technique (Luminex platform) obtained from a very well clinically characterized population, with well-defined outcomes, from one of the most important longitudinal studies of AD (ADNI). Moreover, the results were rigorously verified using repeated cross-validation and reached high-performance metrics in the experiments. We consider that the present findings may contribute to improving the early diagnosis of AD, using a more accessible and less invasive method, and to a better understanding of the pathophysiology of the disease.

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