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RESEARCH ARTICLE

Inflammation biomarkers and Alzheimer's disease: A pilot study using NULISAseq

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Abstract

INTRODUCTION: Increasing evidence links amyloid beta (Aβ) aggregation with inflammation. This pilot study investigated the use of an immunoassay panel to map biomarker changes in patients with Alzheimer's disease (AD). Furthermore, we evaluated the stability of protein quantification after multiple freeze–thaw cycles (FTCs).

METHODS: The nucleic acid-linked immuno-sandwich assay (NULISA) inflammation panel measured 203 proteins in serum samples of individuals with ($n = 31$) and without ($n = 31$) AD pathology. Linear models, adjusted for age and sex, contrasted protein expression across groups.

RESULTS: After multiple-testing adjustments, glial fibrillary acidic protein ($p < 0.001$) and S100A12 ($p < 0.001$) were significantly changed in the presence of AD pathology. Furthermore, they correlated with cerebrospinal fluid biomarkers (phosphorylated

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tau-181 [p-tau181], tau, and Aβ42). Additional markers were nominally changed between groups. Five FTCs caused minimal changes in measurements with the NULISA inflammation panel.

DISCUSSION: Monitoring of inflammation in AD, using the 200-plex NULISA panel, demonstrates changes in peripherally circulating inflammation-related proteins. Contrary to previous reports, FTCs had minimal impact on the quantification of inflammatory markers.

KEYWORDS
freeze–thaw cycles, GFAP, inflammation, multiplexed-immunoassays, novel blood biomarkers, NULISA, S100A12, sample stability

Highlights

- The novel nucleic acid-linked immuno-sandwich assay (NULISA) inflammation panel, which includes 200 protein biomarkers, was used.
- The panel was used for the first time in serum from patients with Alzheimer's disease (AD).
- The protein S100A12 was identified as a potential biomarker for AD.
- Inflammation markers were stable in up to five freeze–thaw cycles.

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, with patient numbers expected to rise due to increasing life expectancy and an aging population.[1] Neuropathologically, AD is characterized by the accumulation of extracellular amyloid beta (Aβ) plaques and intracellular tau tangles that lead to neuronal death.[2] Moreover, these protein accumulations are immunogenic, triggering an immune response primarily mediated by microglia and astrocytes.[3] Our recent work also supported a model where an interaction between Aβ and inflammation influenced greater tau spreading and subsequent cognitive decline than in individuals without baseline elevated inflammation.[4] In this context, research on AD-related inflammation has highlighted the need for reliable biomarkers. Positron emission tomography (PET) tracers for the translocator protein (TSPO) have been developed to evaluate central nervous system inflammation, showing increased uptake in patients with mild cognitive impairment (MCI) who are Aβ PET-positive compared with Aβ PET-negative individuals.[5] However, the specificity of TSPO as a microglial marker remains debated.[6]

In cerebrospinal fluid (CSF), glial fibrillary acidic protein (GFAP), chitinase-3-like protein 1 (YKL-40), and soluble triggering receptor expressed on myeloid cells 2 (sTREM2) have been identified as potential inflammation markers.[7–9] The transition to blood-based biomarkers reflecting amyloid, tau, and neurodegeneration pathologies in AD has developed at a fast pace during recent years. However, identifying reliable blood biomarkers for brain inflammation has proven more challenging due to the proteins potentially being degraded before reaching systemic circulation, the diluting effect by peripheral release, and limitations in immunoassay sensitivity.[10]

The novel nucleic acid-linked immuno-sandwich assay (NULISA) platform offers high multiplexing and, in principle, attomolar sensitivity, making it more suitably placed for profiling inflammation responses in blood. This study investigates the 200-plex NULISA inflammation panel to identify novel blood biomarkers for neuroinflammation in AD.

2 | METHODS

2.1 | Study population

Sixty-two serum samples were collected from de-identified routine clinical chemistry samples by the Institute of Neuroscience & Physiology, Department of Psychiatry & Neurochemistry, University of Gothenburg (Mölndal, Sweden), according to protocols approved by the regional ethics committee at the University of Gothenburg (EPN 140811). Samples were selected based on the availability of CSF measures of core AD biomarkers. The amyloid status of the patient was determined by the ratio of CSF Aβ42 over phosphorylated tau-181 (p-tau181) measured on the Lumipulse 600II (Fujirebio, Belgium) with the ratio's cutoff set at <10.25. Demographics are represented in Table 1.

For the freeze–thaw cycle (FTC) experiment, we selected five plasma de-identified routine clinical chemistry samples by the Institute of Neuroscience & Physiology, Department of Psychiatry & Neurochemistry, University of Gothenburg (Mölndal, Sweden). The plasma samples consisted of an independent set, from which we had previous results with the inflammation panel. For the selection of the five samples, the measurements of S100A12 and GFAP were taken into consideration to achieve a spread of values.

TABLE 1 Demographics and key characteristics of the participants.

	AD ($N = 31$)	Control ($N = 31$)
Age, mean (SD)	76 (± 7.2)	69 (± 12)
Sex, female, *n* (%)	12 (38.7%)	22 (71.0%)
p-tau (ng/L), mean (SD)	100 (± 31)	43 (± 8.5)
t-tau (ng/L), mean (SD)	760 (± 300)	260 (± 59)
Aβ42 (ng/L), mean (SD)	410 (± 95)	820 (± 130)

Abbreviation: Aβ, amyloid beta; AD, Alzheimer's disease; SD, standard deviation.

RESEARCH IN CONTEXT

1. **Systematic review**: Searching the available databases (e.g. PubMed and ScienceDirect), we did not encounter previous research, using the nucleic acid-linked immunosandwich assay (NULISA) inflammation panel, to identify biofluid biomarkers of inflammation in Alzheimer's disease (AD). In addition, we performed a search regarding the pre-analytical stability of inflammation markers, which was often reported as poor.
2. **Interpretation**: Our findings show the promise of using multiplex technologies for inflammation biomarker measurements. We confirm that glial acidic fibrillary protein level is elevated in AD and we identify, for the first time, S100A12 as reduced. We also report how inflammatory markers are stable after repeated freeze–thaw cycles (FTCs).
3. **Future Directions**: Our pilot study reveals how multiplexed assays could be implemented in the investigation of novel inflammation biomarkers. To further expand our findings, larger and more deeply phenotyped cohorts should be used, which would be essential to assess our biomarker candidates for example, S100A12. Longer FTCs should be carried out, as perhaps our protocol was not sufficiently severe to degrade the proteins.

2.2 | NULISA analysis

The NULISAseq inflammation panel was performed on the patient samples at Alamar Biosciences (Fremont, CA, USA) as described previously.[11] Briefly, serum samples stored at −80°C were thawed on ice and centrifuged at 10,000 × *g* for 10 min as recommended by the manufacturer. For the 200-plex inflammation panel NULISAseq, 10 µL of each sample was measured in a singlet format. Targets of the panel are in Table S1. A Hamilton-based automation instrument was used to perform the NULISA workflow, starting with immunocomplex formation with DNA-barcoded capture and detection antibodies. The subsequent steps involved capturing and washing immunocomplexes on paramagnetic oligo-dT beads, releasing them into a low-salt buffer, and capturing and washing on streptavidin beads. Proximal ends of DNA strands on each immunocomplex were ligated with T4 DNA ligase, generating a DNA reporter. For inflammation panel NULISAseq, sample-specific barcodes were also incorporated into the DNA reporter during this step. For NULISAseq, DNA reporters containing both target-specific and sample-specific barcodes were pooled and subjected to polymerase chain reaction (PCR) amplification, followed by purification and sequencing on an Illumina NextSeq 2000 (Illumina, Inc.).

The assay includes by default two sets of positive controls named sample control (SC) and inter-plate control (IPC), one set of negative controls (NC; samples that do not contain the target proteins or analytes of interest), and the well internal control (IC) in each of the 96 wells. The ICs are used to control for well-to-well variation intra-plate, whereas the NCs are used to calculate the plate-specific limit of detection (LOD) for each protein target, by taking the mean added of three times the standard deviation (SD) of the unlogged normalized counts of these samples (a detailed description on the use of these control samples, NULISA protein quantification (NPQ) and LOD calculations can be found here[11]).

For FTC measurements, analysis was carried out on the new 250-plex version of the inflammation panel (Table S1) and measurements were performed on the ARGO automated analyzer at the Department of Psychiatry & Neurochemistry, University of Gothenburg (Mölndal, Sweden). The five plasma samples selected for the protein stability test were divided into aliquots that then underwent different rounds of FTCs, whereby they were placed on dry ice for 20 min and thawed for 20 min. The process was carried out a maximum of five times. All samples were analyzed in one run carried out on the same day once the last FTC was completed.

2.3 | Statistical analysis

All statistical analysis and figures were undertaken using R software, version 3.6.3. Initially the data were quality-controlled based on sample coefficient of variation (CV) and on the detection of sample outliers using principal component analysis (PCA—control vs AD pathology) and target outliers. At this stage, six samples were excluded: four samples showing the internal QC ± 40% from the median read number in the well, one sample was determined as an outlier by PCA analysis, and one sample was exluded as the signal from the protein midkine (MDK) was determined as an outlier (NPQ value >28), possibly affecting other measurements from the same sample. The latter was determined by checking the distributions of the $\log_2$ protein expression values normalized to the spiked in intra-well mCherry control + interplate control. Therefore, the differential expression analysis was performed on 56 samples (27 AD and 29 controls) with all 203 targets of the inflammation panel used for the analysis. Furthermore, normality was assessed with QQ-plots and histograms and homogeneity of variances confirmed with the Levene test prior to parametric analyses. To investigate the differential expression of proteins between

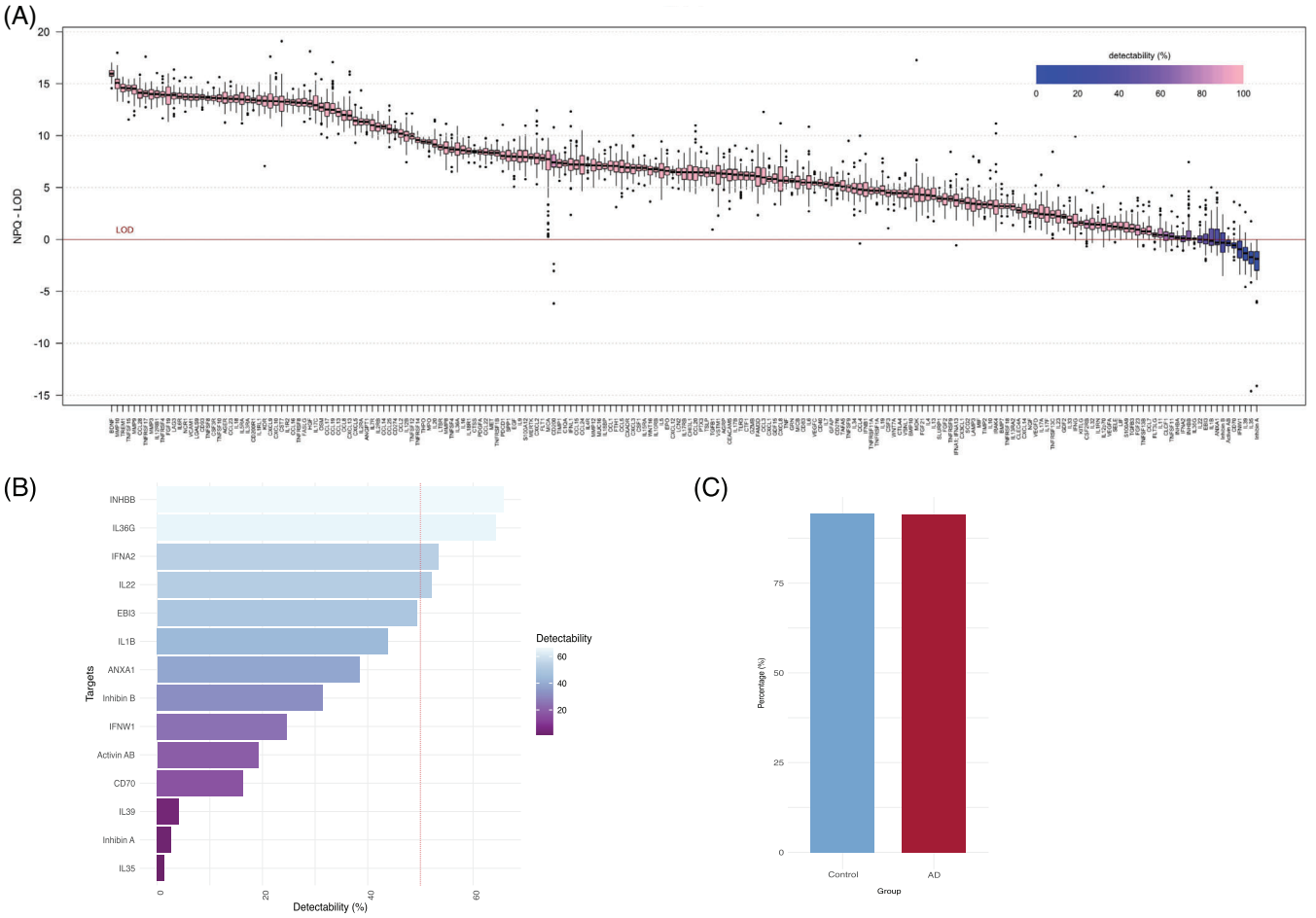

FIGURE 1 Performance of the measurements on the NUcleic Linked Immuno-Sandwich Assay platform. (A) Box plots showing the detectability of the targets of the panel. The *y*-axis displays NPQ–LOD, where values greater than 0 indicate detectability. The central line on each box represents the median, whereas the lower and upper edges correspond to the 25th and 75th percentiles. (B) A list of targets within the panel that showed a lower detectability; the dashed line is set at 50%. (C) The overall detectability of proteins in the panel was unaffected by the patient group. AD, Alzheimer's disease; LOD, limit of detection; NPQ, NULISA Protein Quantification; NULISA, nucleic linked immuno-sandwich assay.

AD and control groups, a linear modeling analysis (lmNULISAseq package) was performed. The model included groups, age, and sex as explanatory variables. The results were visualized in volcano plots, with false discovery rate (FDR)–corrected (Benjamini–Hochberg) *p* values (Figure 1A). Unadjusted *p* values are also reported in Table S2. For the resulting significant biomarkers from the differential expression analysis, Pearson correlation was conducted to examine the relationship between serum GFAP and S100A12 and CSF biomarkers (Aβ42 and p-tau181). To evaluate the ability of GFAP and S100A12 to distinguish groups, we performed a receiver operating characteristic (ROC) analysis using a logistic regression model and 95% confidence intervals (CIs) were calculated using the DeLong method.

To evaluate the effect of FTCs on protein quantification, Wilcoxon rank-sum tests compared protein levels at each cycle to the respective baseline quantification. In addition, average NPQ values, across all protein targets, were calculated and visualized over different FTCs. The non-parametric Friedman test was used to test whether there was a significant change in protein quantifications across FTCs.

3 | RESULTS

3.1 | NULISA performance

In the AD and control serum samples, 95.1% of the proteins in the panel were detectable in at least one sample (Figure 1A). Overall, most proteins in the panel were measurable, specifically, 189 of 203 targets (93.1%) were detectable in >70% of samples (Figure 1B). The percentage of NPQ values higher than LOD was 94.0% in the control and AD groups (Figure 1C).

3.2 | Identification of differential levels of inflammation proteins

In total, 15 significantly differentially expressed proteins were observed in the AD group (Figure 2B). This includes, six upregulated proteins— glial fibrillary acidic protein (GFAP) ($p\text{-value}_{unadj} < 0.001$),

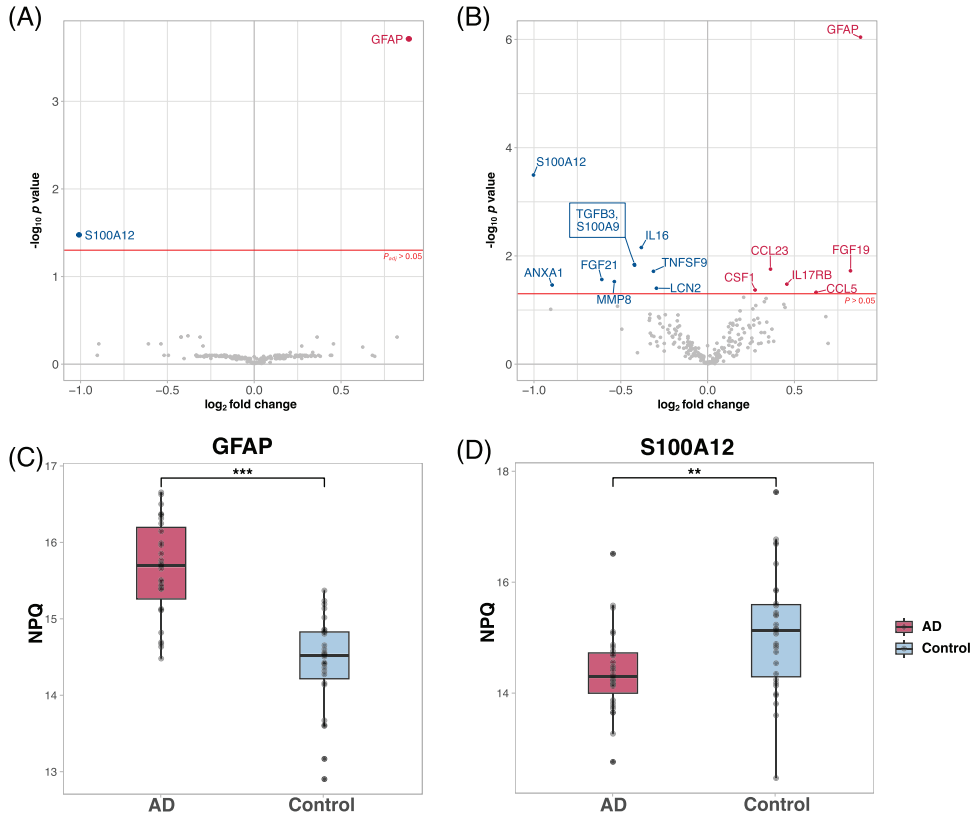

FIGURE 2 (A) Volcano plot showing the log2 fold change on the *x*-axis and the log10 of the FDR-adjusted *p*-values on the *y*-axis. Only significant proteins (p-value < 0.05, downregulated in blue and upregulated in red) are displayed. The volcano plot utilized FDR-corrected values to account for multiple testing. (B) Volcano plot showing the log2 fold change on the *x*-axis and the log10 of the unadjusted *p*-values on the *y*-axis. The volcano plot utilized unadjusted *p*-values to illustrate the raw statistical significance of the results. (C, D) box plots comparing the levels of GFAP and S100A12 in the two patient groups. AD, Alzheimer's disease; FDR, false discovery rate; GFAP, glial fibrillary acidic protein; NPQ, NULISA protein quantification; NULISA, nucleic acid linked immuno-sandwich assay.

chemokine ligand 5 (CCL5) (p-value$_{unadj}$ = 0.046), chemokine ligand 23 (CCL23) (p-value$_{unadj}$ = 0.017), fibroblast growth factor 19 (FGF19) (p-value$_{unadj}$ = 0.019), interleukin-17 receptor B (IL17RB) (p-value$_{unadj}$ = 0.033), and colony stimulating factor 1 (CSF1) (p-value$_{unadj}$ = 0.043), and 9 downregulated proteins—S100A12 (p-value$_{unadj}$ < 0.001), transforming growth factor beta-3 (TGFB3) (p-value$_{unadj}$ = 0.015), S100A9 (p-value$_{unadj}$ = 0.014), interleukin 16 (L16) (p-value$_{unadj}$ = 0.007), annexin A1 (ANXA1) (p-value$_{unadj}$ = 0.035), fibroblast growth 21 (FGF21) (p-value$_{unadj}$ = 0.027), matrix metalloproteinase-8 (MMP8) (p-value$_{unadj}$ = 0.03), tumor necrosis factor superfamily 9 (TNFSF9) (p-value$_{unadj}$ = 0.019), and lipocalin-2 (LCN2) (p-value$_{unadj}$ = 0.040). After applying FDR correction, only S100A12 was found to be significantly reduced in patients with AD, by a log-fold change of 0.5 (p-value$_{adj}$ = 0.031) (Figure 2A). Conversely, GFAP was significantly increased with a log-fold change of 1.84 (p-value$_{adj}$ < 0.001) (Figure 2C). Adjusted and unadjusted *p*-values for the top 15 targets are presented in Table S2. The box plots comparing the levels of nominally significant proteins in the two patient groups are presented in Figure S1.

3.3 | Correlation of differentially measured biomarkers with core AD CSF biomarkers

GFAP had a strong positive correlation with CSF p-tau181 (Spearman rho = 0.69; p < 0.001) and negative correlation with CSF Aβ42 (Spearman rho = 0.71; p < 0.001). S100A12 also showed significant, albeit weaker, correlations (p-tau181: Spearman rho = −0.36, p = 0.0053; Aβ42: Spearman rho = 0.38; p < 0.0067) (Figure 3).

3.4 | Discrimination of patient groups through the NULISA inflammation panel

We then probed whether the inflammation component of AD had discriminative properties to distinguish between AD and age-matched controls. We performed a ROC to assess this, which resulted in GFAP having strong discriminatory properties (area under the curve [AUC] = 0.93, CI = 0.872–0.955; Figure 4A). S100A12 showed a more moderate result as a biomarker (AUC = 0.72,

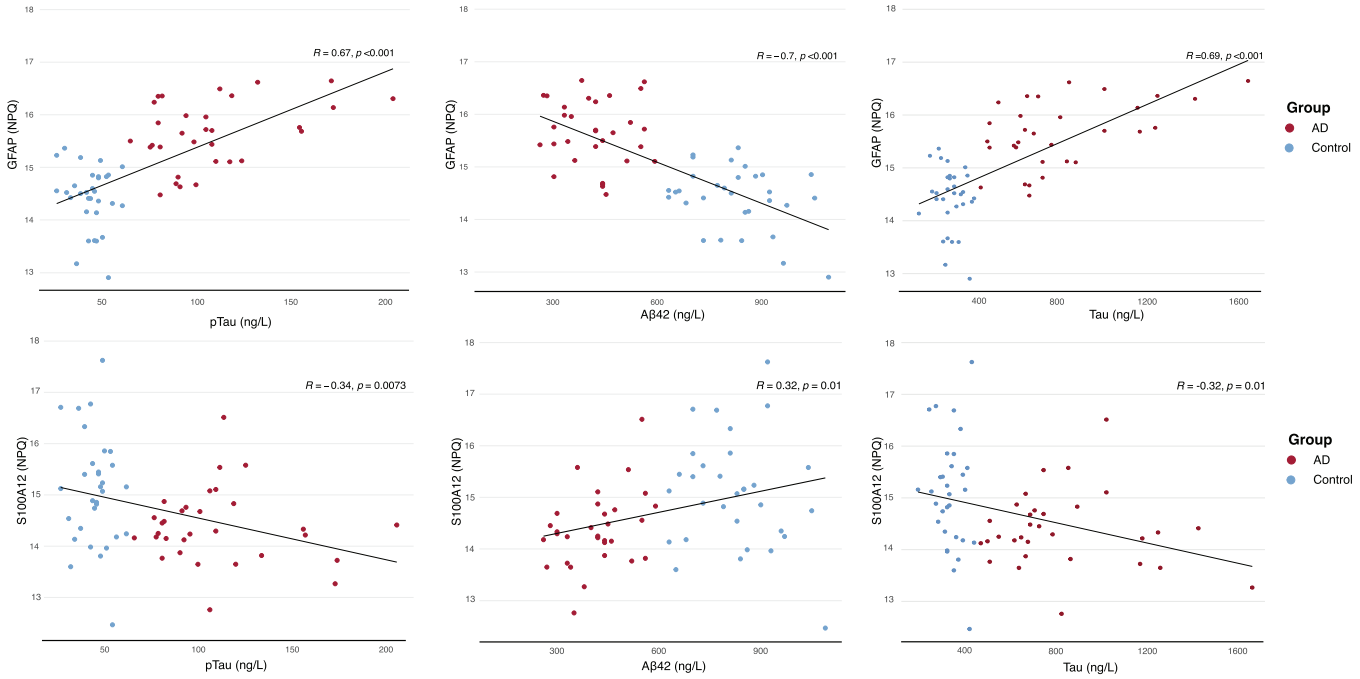

FIGURE 3 Correlations between the differentially expressed proteins GFAP and S100A12 and the core CSF biomarkers within the same patient are significant. AD, Alzheimer's disease; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; LOD, limit of detection; NPQ, NULISA protein quantification; NULISA, nucleic acid linked immuno-sandwich assay.

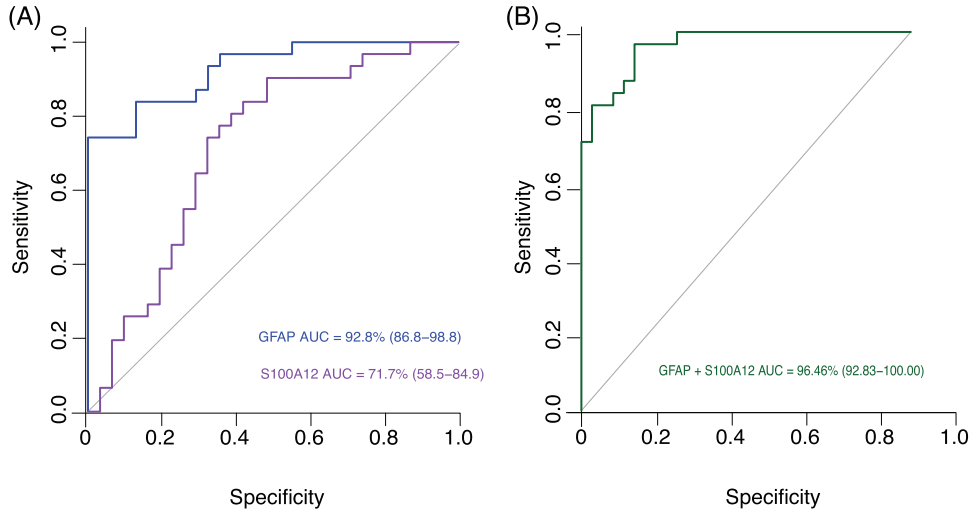

FIGURE 4 (A) ROC curve analyss, showing the ability of the two significantly different proteins to predict the diagnostic group of the sample. The blue line represents the discriminative power of GFAP alone in distinguishing between the AD and control groups, the purple line represents the discriminative power of S100A12 alone in distinguishing between AD and control groups. (B) ROC curve representing the combined discriminative power of GFAP and S100A12. The combined model considers both markers simultaneously to assess their joint ability to differentiate between the AD and control groups. AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; ROC, receiver-operating characteristic.

CI = 0.59–0.86; Figure 4A). A combination of the two proteins with the highest change (GFAP, S100A12, Figure 4B) had the highest predictive strength (AUC = 0.97, 0.93–1.00), which was better than GFAP alone but not a statistically significant improvement (DeLong $p = 0.09$).

3.5 | Effect of FTCs on the measurements of inflammation panel proteins

Within the panel, we assessed the stability of the two targets that emerged as significantly changed from our previous screening, namely

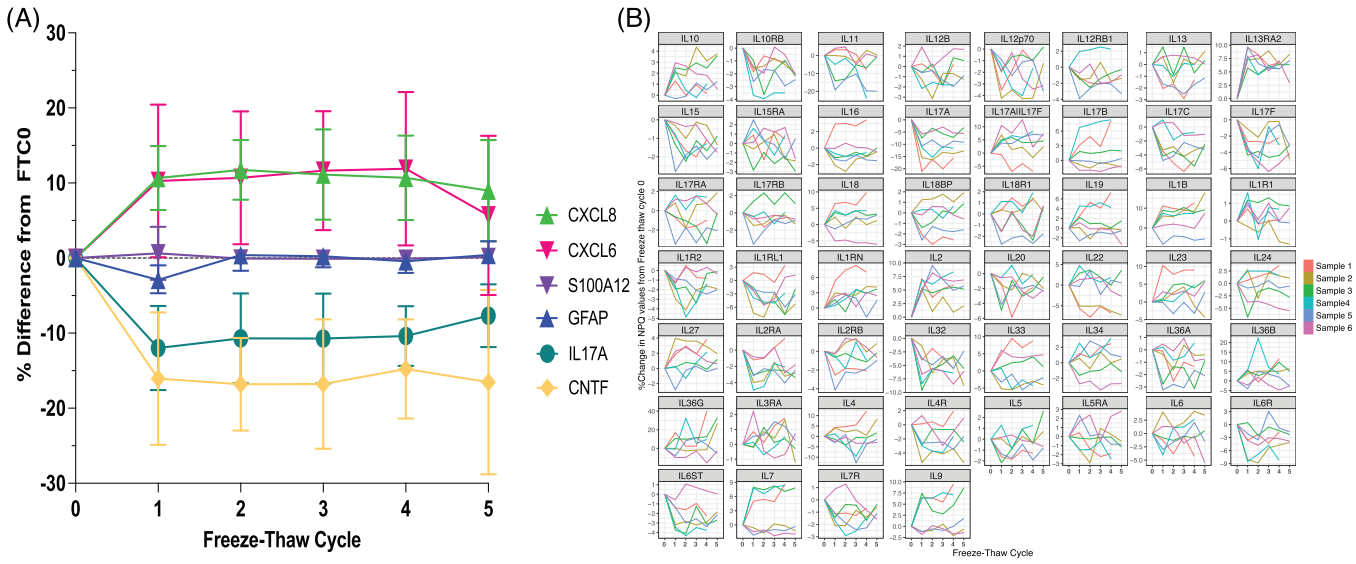

FIGURE 5 (A) The percentage difference with median ± 95% CI is displayed for the two targets in the panel that showed the highest increase (CXCL8; CXCL6) and highest decrease (IL17A; CNTF) in measurement. Our two biomarker candidates (GFAP; S100A12) showed very little fluctuations between FTCs. (B) The effect of FTCs on the interleukins is shown as the percentage difference from cycle 0. None of the proteins showed significant differences. FTCs, freeze–thaw cycles; GFAP, glial fibrillary acidic protein.

S100A12 and GFAP (Figure 5A).In the five plasma samples we tested, both proteins showed no change in up to five FTCs. The proteins that showed the highest percentage increase (CXCL8 and CXCL6) and decrease (IL17A and CNTF) had a mean change from the first measurement of around ± 10%. This change, and that of other interleukins in the panel, was not found to be significant (Friedman test, $p = 0.96$) (Figure 5B).

4 | DISCUSSION

In this pilot study, we report the significant changes of inflammation protein biomarkers in serum utilizing the NULISA platform. Specifically, we identified two proteins, GFAP and S100A12, that were significantly dysregulated after multiple testing adjustments. We also observed a number of nominally changed inflammation proteins that warrant further investigation in larger studies.

Elevated blood GFAP has been widely implicated in AD,[12–14] where levels have already aided the characterization of astrogliosis in patients with AD. In studies comparing several AD cohorts, GFAP showed a moderate ability to separate individuals based on their amyloid status (ROC-AUC = 0.69–0.86).[7,14,15] Our results, which are biased toward extreme case selection, demonstrated an AUC of 0.93. In our study, serum GFAP correlated significantly with the core AD CSF markers, increasing with a higher amyloid load. Initial evidence points toward plasma GFAP being a dynamic biomarker; in the TRIALBLAZER phase III trial for the anti-amyloid therapy donanemab, it showed a 12% decrease after 12 weeks of treatment compared to the 6% increase in the placebo group.[16] This was interpreted as a reduction in astrocytic activation among responders to treatment. In this trial and other cohorts, plasma GFAP also correlated positively with changes in amyloid PET measurements, highlighting a potential role of GFAP as a biomarker to monitor AD progression.[12,15]

The S100 family has previously been identified as embedded within amyloid plaques in AD mouse models and, specifically, S100A12 was co-stained in plaques, neurons, and glia in genetic and sporadic AD human brain tissue.[17,18] Therefore, a reduction in measured S100A12 could follow a mechanism similar to that of Aβ42, whereby it is believed to decrease in biofluids as it is trapped in plaques.[19] Previous research in traumatic brain injury (TBI) and stroke uncovered a connection between S100A12 and the extent of brain insults, with a higher level of S100A12 in blood correlating with poorer patient outcomes.[20–22] Our results in patients with AD contrast with such acute insults, suggesting that brain amyloid pathology does not exhibit traits of an acute inflammation. This could explain why there was only a low, yet significant, correlation between S100A12 and biomarkers of amyloid pathology, which have been shown to mirror disease stage and severity.[23]

Combining GFAP and S100A12 numerically improved the discrimination of the AD group versus controls compared to GFAP and S100A12 alone. Despite this not being a significant improvement ($p = 0.09$), it suggests a trend toward improved predictive strength. Combining biomarkers is a widely used strategy in complex diseases, such as AD, as it potentially adds information from complementary biological processes, in this case GFAP, as reflecting astrocytic activity, and S100A12, associated with more general immune activation. Given the restricted statistical power of this pilot study, further validation in larger cohorts will be necessary to confirm these findings and to better understand how these proteins together could enhance diagnostics in the clinical practice.

Several other proteins could be observed when lowering the threshold of the differential analysis, which enabled us to explore more inflammatory proteins of interest in AD pathophysiology. Indeed,

these proteins have been linked previously to pathological processes present in AD, for example, microglial modulation and neuroprotection, which confirms their potential importance in future research scenarios. It is important to also mention that findings should be interpreted with caution given the intrinsic risks of considering the results of multiple testing without multiple correction adjustment. Bearing this in mind, our results included an increase in, for example, CCL5 and CSF1, and a decrease in MMP8, which were implicated in the regulation of microglia activity.[24–28] Conversely, the growth factor FGF21, which is known to perform neuroprotective functions, was reduced.[29,30] Indeed, higher levels of this marker were found in a centenarian offspring cohort but not in patients with AD who were part of the study, suggesting it to be a candidate biomarker of healthy aging.[31]

Further benefits of multiplexing the measurement of proteins in a single experiment are the reduction of analytical and pre-analytical variation. The platform is standardized for all proteins, instead of relying on multiple instruments, and there is no need to freeze samples between the various analyses. For example, in a meta-analysis by Liu et al., three FTCs were identified as the threshold to not affect protein quantification significantly, whereas further FTCs may selectively degrade proteins and skew results.[32] In our FTC experiment, we measured ≈200 inflammation markers simultaneously and did not see a sharp change in the general quantification of proteins, even in ones that had been reported previously in the literature as sensitive, such as cytokines (Figure 4).[33,34] When comparing to the first measurement, the most affected proteins showed a median difference of ± 10%, which is less than what is often reported.[33] Moreover, our prospective biomarkers selected from the panel, GFAP and S100A12, were almost unaffected by repeated snap-freezing cycles. Our finding, related to GFAP being stable to FTCs, matches previous data with a comparable snap-freezing protocol. The study reported GFAP displaying a non-significant decreasing trend in up to seven FTCs in serum.[35] When tested in plasma with a longer 24 h freeze cycle at −80°C, there was a reported recovery of 113% at the fourth FTC.[36] This suggests that there could be a matrix-dependent effect on GFAP; therefore further studies should be carried out with more samples, including serum ones, and longer FTCs, as our snap-freezing might not have caused some of the cold denaturation stress that long-term storage might induce in the proteins.[37]

5 | CONCLUSION

With our study implementing the NULISA inflammation panel, we add to the growing body of evidence that blood GFAP concentration reflects reactive astrogliosis in AD. Second, we report a reduction of serum S100A12,[38] warranting further research on this protein as a candidate biomarker, and a number of inflammation-related candidates. Limitations of our study design included the low number of samples and the lack of characterizations beyond the amyloid status of the patients, for example, cognitive status. All participants in our study were being evaluated for cognitive complaints; therefore our approach does not exclude that the non-AD control group may have included patients with non-AD neurodegenerative conditions. Thus, our findings can only be implicated in AD and general neuroinflammation markers would not be apparent in this study. This could partly explain why only two targets were firmly identified as being differentially expressed. Indeed, further studies are required with more deeply phenotyped cohorts and healthy age-matched controls. However, lowering the statistical stringency of the analysis revealed additional differentially expressed proteins. For these targets, the existing literature frequently shows contradictions. This is not only due to the complexity of neuroinflammatory processes in the context of AD, but also to the susceptibility of pre-analytical and analytical variations. Therefore, a panel on the NULISA platform could represent a significant tool in standardization and harmonization of measurements, ensuring greater consistency and confidence in the results when monitoring a patient's disease progression.

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CONFLICT OF INTEREST STATEMENT

H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside the submitted work). K.B. has served as a consultant and on advisory boards for Abbvie, AC Immune, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, Sanofi and Siemens Healthineers; has served on data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. The other authors report no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The study was approved by the ethics committee at the University of Gothenburg (EPN 140811), as samples were prepared from de-identified left-over aliquots from clinical routine analyses.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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