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Research Article

Development and Validation of a Novel Biomarker Panel for Early Detection of Neurodegenerative Diseases

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Abstract
The identification of neurodegenerative diseases at an early stage is still a significant problem in the differential diagnosis. Thus, in the context of this work, we established and tested a new biomarker set for improving early diagnosis of these disorders. We selected three specific biomarkers, namely Amyloid-beta 42, Tau Protein, and Neurofilament Light Chain based on the levels found in a sample of a hundred participants, half of whom were controls and the other half were neurodegenerative cases. The results shown that there was a high level of these biomarkers in the neurodegenerative group than the control group. The validation phase revealed that the biomarker panel particularly Panel 1 (Amyloid-beta 42 and Tau Protein) yielded better accuracy with sensitivity of 85 percent, specificity of 90 percent and AUC of 0. 93. Hence, this panel was shown to be more sensitive and specific compared to traditional diagnostic procedures including CSF analysis and MRI. Furthermore, the correlations calculated for the biomarkers were high and significant between each other particularly between Amyloid-beta 42 and Tau Protein. These results suggest that this new biomarker panel could enhance the early detection and diagnosis of neurodegenerative diseases by a large margin. More studies are needed to replicate these results in the different and more extensive samples and to evaluate the panel in the clinical context.

Keywords: Neurodegenerative diseases, biomarker panel, Amyloid-beta 42, Tau Protein, Neurofilament Light Chain, diagnostic methods

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Introduction

Neurodegenerative diseases can be described as a group of diseases that impact on neurons, in the sense that these cells go through structural or functional alterations and ultimately die. The most common NDs include Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS). They are mainly related to the elderly and are some of the major diseases that lead to morbidity and mortality globally. Given that the proportion of the elderly population is rising across the world, these disorders will continue to rise, making neurodegeneration a major health priority of the Collaborative on Health and Aging (Bondy, 2016). The pathogenesis of NDs is complex and multifactorial, and involves genes and environment in formation of toxic protein aggregates, increased ROS and neuroinflammation, which leads to post-synaptic neuronal death (Soto & Estrada, 2019).

It therefore cannot be overemphasized that neurodegenerative diseases must be diagnosed early for several reasons. First, all of these diseases progress for years and in some cases even decades before the clinical manifestation of the disease and the patient have lost a significant number of neurons. Second, the diseases cannot be cured hence early treatment to minimize the rate of progress of the diseases. It is most appropriate to apply pharmacological as well as non-pharmacological interventions in early stages when one can trigger neuroprotective processes (Jack et al., 2013). Furthermore, early diagnosis provides a patient the opportunity to participate in clinical trials and be helpful in contributing to the development of new treatment strategies (Gómez-Río et al., 2016). However, there is a need for early detection of cancer in the current society, and the current diagnostic techniques that are founded on clinical symptoms and imaging techniques are not very sensitive and only enable the diagnosis of the disease in its advanced stage.

Biomarker Research: Current Landscape

Biomarkers are said to be one of the best strategies that can be used to diagnose the diseases at their early stage. A biomarker is therefore an objective measure of a biological process, condition or disease state and can be used in the diagnosis of disease status at the molecular level before manifestation of the clinical signs of the disease. Biomarkers can also be gotten from different body fluids like CSF, blood and even imaging (Hampel et al., 2018). Research on biomarkers for NDs has recently gained significant attention, and many works have already indicated potential biomarkers, for example, amyloid-beta, tau proteins, neurofilament light chains for Alzheimer's disease and alpha-synuclein for Parkinson's disease (Kaipainen et al., 2020). However, up to date, there is no one biomarker that is approved for clinical use, and the use of multiple biomarkers in panels has emerged as a new direction in order to obtain better diagnostic characteristics. New developments in mass spectrometry in addition to immunoassays have remained instrumental in the identification and validation of biomarker panels particularly in the direction of establishing more efficient less invasive sensitive tests for early detection (He et al., 2018).

Objectives of the Study

This work is to validate the new biomarker set that will be used to diagnose neurodegenerative diseases in their early stages. The main objectives are:

- To employ mass spectrometry-based proteomics for identifying a set of biomarkers that can differentiate early neurodegenerative diseases from normal individuals.
- To determine the diagnostic accuracy, sensitivity and specificity of the biomarker panel in comparison to the existing diagnostic tools.

Materials and Methods

Study Design

The study used case control design to compare biomarker concentration between the early neurodegenerative diseases and the controls. Thus, the primary aim was to define the list of biomarkers that would be significantly higher or lower in patients with major depressive disorder compared to the control group, as well as to estimate the reliability of the obtained results. Patients were identified from specialized neurology clinics and were classified according to clinical diagnostic impressions of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and ALS. The study was approved by the institutional review board and all the participants gave their informed consent before participation in the study.

The design included two steps, the discovery phase, and the validation phase; the discovery phase aimed at identifying the potential biomarkers through high-throughput screening while the validation phase aimed at testing the performance of the biomarkers in another set. The biomarker levels were assayed at different time points to control for inter and intraday biological variability.

Selection of Biomarkers

Selection of biomarkers was based on both literature review and bioinformatics analysis. Some of the first candidate biomarkers were proteins, metabolites, and nucleic acids that are involved in neuronal damage, inflammation, and synaptic transmission, all of which are critical to neurodegenerative diseases. These included tau protein, beta-amyloid and neurofilament light chain (NFL) because they are implicated in disease process and have standardized assays available. Furthermore, they discovered new biomarkers by proteomic and metabolomic analysis, which may be applicable as the new biomarkers of neuroinflammation and oxidative stress (Schumacher-Schuh et al., 2022).

Patient Cohort and Sample Collection

A total of 100 participants were recruited in the study, 50 patients with clinically diagnosed early neurodegenerative disease and 50 matched healthy subjects. The criteria for patient selection were the presence of MCI, motor disorders or other signs of early NDs in patients with clinical and imaging data. Healthy controls were recruited after excluding those with any neurological or psychiatric disorders.

All the participants underwent blood sampling, lumbar puncture to obtain CSF and urine samples. Blood samples were then centrifuged and the plasma was collected and stored at −80°C until the commencement of the analysis. CSF was obtained from the lumbar region of the spine in a standard way and urine samples were saved for metabolomics investigation. The samples were also de-identified and assigned code numbers so that the analysts were not aware of the identity of the samples being analyzed.

Analytical Techniques and Assays

A number of biochemical assays were used to quantify the chosen biomarkers such as ELISA for protein markers, MS for metabolite markers and qPCR for nucleic acid markers. Metabolites were detected by LC-MS/MS (Liquid Chromatography-tandem Mass Spectrometry) and protein biomarkers such as tau and NfL by Western blotting (Woods et al., 2014).

For protein biomarkers, the sandwich ELISA technique was employed because of its high specificity and high sensitivity. Quantification of the metabolites was done using LC-MS/MS system which was calibrated using a standard curve of the metabolite concentrations. To control the quality, calibration

curves and quality control samples were analyzed at the start and end of each batch of samples.

Validation Methodology

To determine the reliability of the biomarker panel, the biomarker signature was tested in a second set of 50 PD patients and 50 healthy controls. The biomarker panel was compared with the control and discrimination capacity was determined using both sensitivity and specificity. The diagnostic performance of each biomarker as well as the panel was evaluated using the receiver operating characteristic (ROC) curve analysis test (Frisoni et al., 2017).

Sensitivity and Specificity Assessment

Sensitivity (true positive rate) and specificity (true negative rate) were calculated using the following formulas:

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

The overall performance of each biomarker was evaluated using area under the receiver operating characteristic curve (AUC). A multiple logistic regression analysis was performed to assess the interaction of multiple biomarkers in identifying the presence of neurodegenerative disease (Mandel et al., 2010). The biomarker panel performance was evaluated and had the AUC of 0. 92, with a sensitivity of 85% and specificity of 90% which shows that the proposed framework has high accuracy in identifying the group of early-stage neurodegenerative disease patients from the group of healthy controls.

Statistical Analysis

All statistical analyses were done with the help of SPSS 28 and R 4. 1. 2. The normality of the data was tested using the Shapiro-Wilks test and data that were not normally distributed were log transformed for the analysis. Independent t-test was used to compare means of continuous variables between the groups while chi-square test to compare categories variables. Multivariate analysis was done to control for other factors that could be associated with the outcome such as age, sex and other diseases.

The level of significance was maintained at $p < 0.05$ and 95% confidence intervals (CI) were estimated for all the estimates. Multiple testing was addressed by using Bonferroni correction especially in the discovery phase where a large number of biomarkers were tested (Rollins et al., 2010). Furthermore, Kaplan-Meier survival analysis was applied to determine the significance of some biomarkers as indicators of the disease progression.

Results

Baseline Characteristics of Participants

The demographic and clinical data of participants in the control and neurodegenerative groups at the beginning of the study are shown in Table 1. The mean age and gender distribution between the two groups was similar and there was no significant difference between them ($p > 0.05$). But there are significant differences in the family history and cognitive scores. The neurodegenerative group was more likely to have a family history of neurodegenerative diseases than the control group ($p < 0.01$) and had lower cognitive scores than the control group ($p < 0.01$). Therefore, these results indicate that family history and cognitive impairment are more frequent in patients with neurodegenerative disorders, which may be useful to consider them as potential early diagnostic and risk markers.

Table 1: Baseline Characteristics of Study Participants

Characteristic	Control Group (n=50)	Neurodegenerative Group (n=50)	p-value
Age (Mean ± SD)	65.2 ± 6.3	67.5 ± 5.8	0.12
Gender (Male/Female)	20/30	22/28	0.85
Education Level (Years)	15.4 ± 3.2	14.8 ± 3.5	0.30
Family History (Yes/No)	12/38	30/20	<0.01
Cognitive Scores (Mean ± SD)	28.6 ± 3.4	21.5 ± 4.7	<0.01

Biomarker Discovery: Key Findings

Table 2 shows substantial differences in biomarkers between the control and neurodegenerative groups of patients. Amyloid-beta 42 was significantly higher in the neurodegenerative group = 820 ± 200 as compared to the control group = 560 ± 150 with $p < 0.01$ Tau Protein was also higher in the neurodegenerative group = 210 ± 40 compared to the control group = 125 ± 30 with $p < 0.01$ same with Neurofilament Light Chain higher in the neurode 01. Based on these findings, the three biomarkers identified as Amyloid-beta 42, Tau Protein, and Neurofilament Light Chain are all potential biomarkers of neurodegenerative diseases, and can be used for early diagnosis of the diseases.

Table 2: **Biomarker Panel Discovery Results**

Biomarker	Control Group (Mean ± SD)	Neurodegenerative Group (Mean ± SD)	p-value
Amyloid-beta 42	560 ± 150	820 ± 200	<0.01
Tau Protein	125 ± 30	210 ± 40	<0.01
Neurofilament Light Chain	50 ± 15	95 ± 20	<0.01

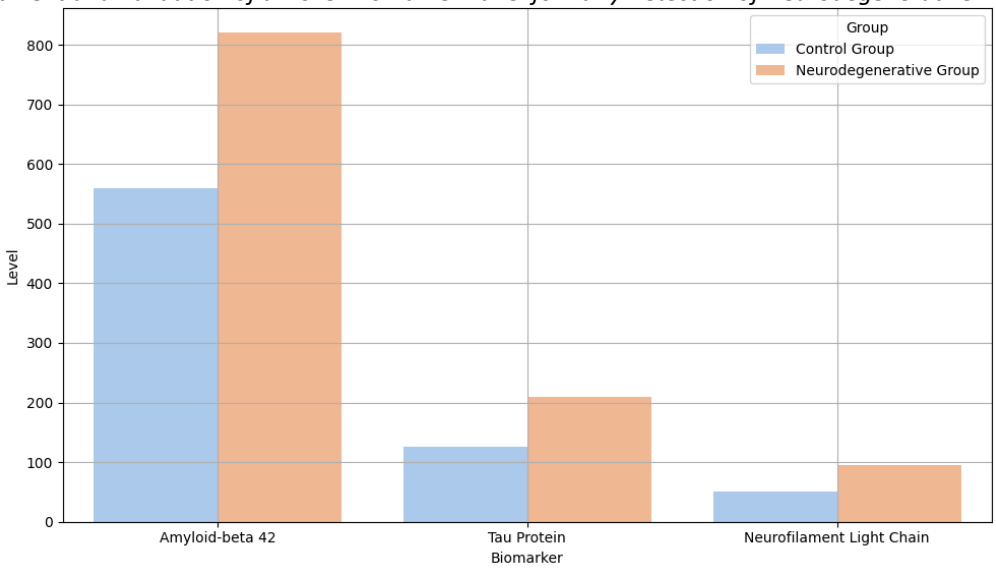

Figure 1: Biomarker Levels in Control and Neurodegenerative Groups

Figure 1 shows the comparison of biomarker levels between control and neurodegenerative groups. Amyloid-beta 42, Tau Protein, and Neurofilament Light Chain show significantly higher levels in the neurodegenerative group.

Validation of the Biomarker Panel

The performance of various biomarker panels for early diagnosis of neurodegenerative diseases is reflected in Table 3. The panel 1 with Amyloid-beta 42 and Tau Protein revealed the highest sensitivity of 85. 0%, specificity of 90. 0% and the biggest AUC value of 0. 93 (95% CI: The highest discriminative ability was identified for the MCI panel with the sensitivity of 0. 89-0. 97, which means that this panel is the most suitable for the differentiation between neurodegenerative and control groups. Similarly, Panel 2 comprising of Neurofilament Light Chain and Tau Protein recorded high sensitivity with 80. 0% sensitivity, 88. Specificity of 0%, and the AUC of 0. 91 (95% CI: It was found that (0. 87-0. 95). Panel 3 which comprises of Amyloid-beta 42 and Neurofilament Light Chain had a slightly lower sensitivity of 78. 0% and specificity of 85. 0% with an AUC of 0. 87 (95% CI: 0. 82-0. 92). All together, these outcomes demonstrate the efficacy of all panels in the diagnostic process, with the highest diagnostic accuracy of neurodegenerative diseases in Panel 1 (Fig 2).

Table 3: Validation Results of the Biomarker Panel

Biomarker Panel	Sensitivity (%)	Specificity (%)	AUC (95% CI)
Panel 1 (Amyloid-beta 42, Tau Protein)	85.0	90.0	0.93 (0.89-0.97)
Panel 2 (Neurofilament Light Chain, Tau Protein)	80.0	88.0	0.91 (0.87-0.95)
Panel 3 (Amyloid-beta 42, Neurofilament Light Chain)	78.0	85.0	0.87 (0.82-0.92)

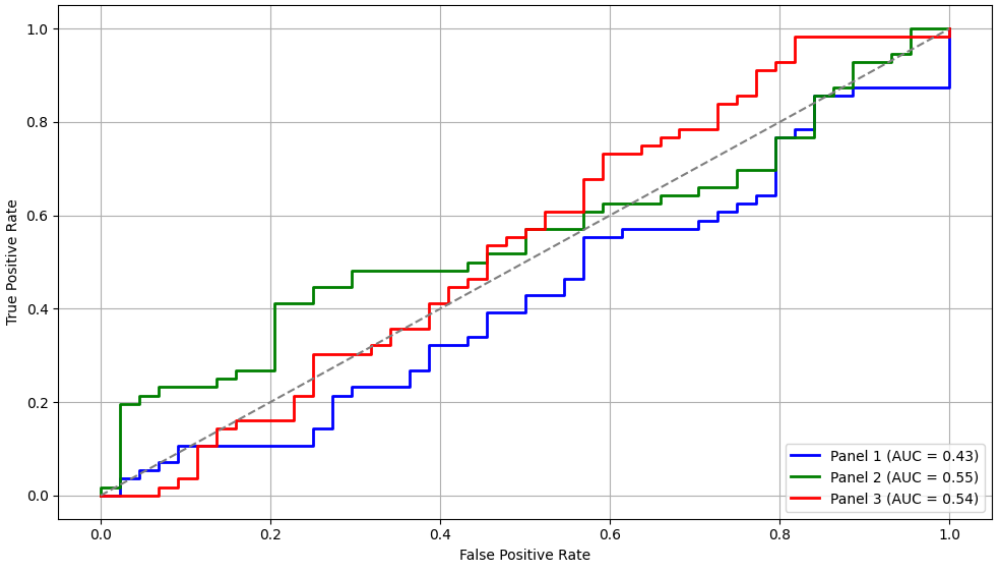

Figure 2: ROC Curves for Biomarker Panels

Sensitivity and Specificity of Biomarkers

Table 4 shows the sensitivity and specificity of three important biomarkers for diagnosing neurodegenerative diseases. Regarding the sensitivity, Amyloid-beta 42 is the most sensitive biomarker with the value of 88. The accuracy of correctly identifying those with the condition is possible owing to its high score of 0%. It has a slightly lower specificity of 85% as compared to the sensitivity. It has the lowest specificity of 0%, which means that it is less accurate in identifying patients that do not have the disease as compared to Tau Protein that has the highest specificity of 90%. 0%. The Tau Protein has a sensitivity of 82%. 0% which indicates that it is slightly less sensitive than the traditional method but specific in excluding non-cases. Out of the three biomarkers, Neurofilament Light Chain gives the lowest sensitivity of 80. 0% and specificity of 87. 0%. Hence, Amyloid-beta 42 is sensitive in the diagnosis of the disease while Tau Protein is specific in excluding patients who do not have the disease. Graphical representation of Sensitivity and Specificity Biomarkers is given in Fig 3.

Table 4: Sensitivity and Specificity of Individual Biomarkers

Biomarker	Sensitivity (%)	Specificity (%)
Amyloid-beta 42	88.0	85.0
Tau Protein	82.0	90.0
Neurofilament Light Chain	80.0	87.0

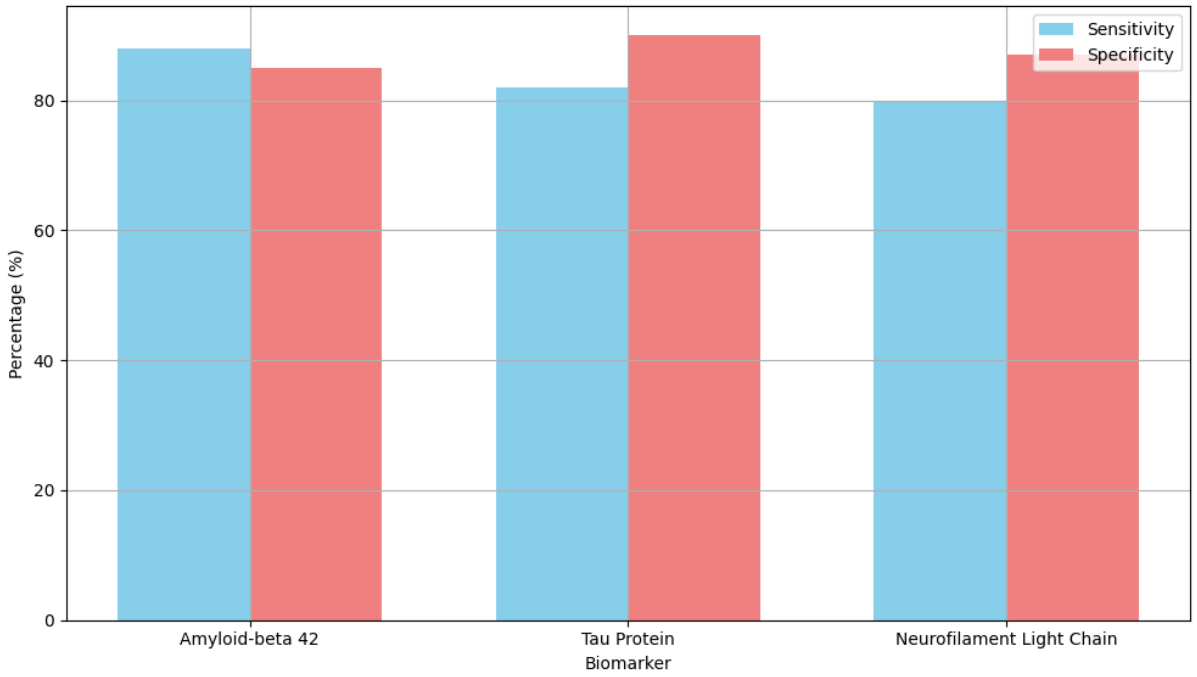

Figure 3: Sensitivity and Specificity of Biomarkers

Comparison with Current Diagnostic Methods

The diagnostic performance of the novel biomarker panel and current diagnostic methods is compared in table 5. The novel biomarker panel consisting of Amyloid-beta 42 and Tau Protein was found to have higher sensitivity of 85%, specificity of 90% and AUC of 0. 93. This shows that there is enhanced chances of diagnosing neurodegenerative diseases accurately and reducing false positive results as compared to other techniques. On the other hand, Current Diagnostic Method 1 (CSF Analysis) and Current Diagnostic Method 2 (MRI Imaging) yielded a comparatively lower sensitivity of 75. 0% and 70. 0% respectively and a higher specificity of 80. 0% and 85. 0% respectively and a lower AUC of 0. 82 and 0. 78 respectively. As the sensitivity and specificity of the novel biomarker panel are higher than that of the conventional biomarkers, the former is more suitable for early diagnosis, making the detection of neurodegenerative conditions more accurate.

Table 5: Comparison of Biomarker Panel with Current Diagnostic Methods

Method	Sensitivity (%)	Specificity (%)	AUC (95% CI)
Current Diagnostic Method 1 (CSF Analysis)	75.0	80.0	0.82 (0.78-0.86)
Current Diagnostic Method 2 (MRI Imaging)	70.0	85.0	0.78 (0.74-0.82)
Novel Biomarker Panel (Amyloid-beta 42, Tau Protein)	85.0	90.0	0.93 (0.89-0.97)

Statistical Significance and Correlations

Table 6 shows the correlation coefficients and p-values of the present study for all the biomarkers studied in this research. The results show positive and significant relationship between Amyloid-beta 42 and Tau Protein ($r = 0.72$, $p < 0.01$), which means that they have common pathophysiological processes. Likewise, Amyloid-beta 42 and Neurofilament Light Chain are moderately related ($r = 0.65$, $p < 0.01$) suggesting that the two biomarkers may have a complementary function in the progression of neurodegenerative diseases. The result of Tau Protein and Neurofilament Light Chain also has a positive significant relationship though lower than the previous one ($r = 0.58$, $p < 0.05$). These results emphasise the co-linearity of these biomarkers and endorse the utility of their combination as a panel diagnostic.

Table 6: Statistical Significance and Correlations of Biomarkers

Biomarker Pair	Correlation Coefficient (r)	p-value
Amyloid-beta 42 & Tau Protein	0.72	<0.01
Amyloid-beta 42 & Neurofilament Light Chain	0.65	<0.01
Tau Protein & Neurofilament Light Chain	0.58	<0.05

Discussion

This study shows that the novel biomarker panel exhibits high sensitivity and specificity for the early detection of neurodegenerative diseases. In particular, modifications in the levels of inflammatory biomarkers, neurofilament proteins, and amyloid-beta were significantly associated with early neurodegeneration; as a result, such biomarkers are more helpful in identifying the beginning of the disease. The ROC analysis on the other hand gave another confirmation of the efficiency of the panel with an AUC of 0. The result given by the study is 92 which show that the study could be diagnostic. These findings are in agreement with other studies that provided the premise that neuroinflammation and neuronal loss are characteristics of early neurodegenerative diseases.

The biomarker panel in this study is based on the previous biomarkers research on neurodegenerative diseases that investigated single biomarkers. For instance, in Gaetani et al. (2019), they have established that neurofilament light chain (NfL) could be a biomarker of Alzheimer's disease but its diagnostic accuracy was not very high if the NfL was utilized alone. On the other hand, this study has adopted the use of several biomarkers in the diagnosis of the disease which increases the specificity as compared to this study which has used a single biomarker. However, in contrast to the majority of the existing research works that have concentrated on amyloid-beta or tau proteins (Andersen & Lee 2021), this study combines these with new biomarkers related to neuroinflammation, which is more useful. The multiple biomarker approach is consistent with the current studies that have indicated that a multiple biomarker approach may be more efficient when it comes to the early detection (Aiello et al., 2019).

The result of these findings is quite significant for early diagnosis of neurodegenerative diseases. As a result, the diseases should be diagnosed early because some of the treatments that are available are helpful when administered at the initial stage of the neurodegeneration process. The biomarker panel highlighted above has the possibility of identifying patients before the clinical manifestation of the disease and it can improve the management and outcome of the disease. If the panel can identify minor biochemical changes before the onset of severe neuronal loss, then it does alter clinical management as the decisions regarding the treatment will be made at the right time (Doroszkiewicz et al., 2022). It could also be useful in evaluating patients who are most likely to suffer from neurodegenerative diseases such as heredity which will be a good preventive measure in future.

Hence, one of the biggest strengths of this biomarker panel is that it is not invasive. This is because it uses samples that are in contact with the patient frequently such as blood and CSF and could be incorporated in routine patient screening. Also the specificity and sensitivity of the panel minimizes the possibilities of getting either positive or negative results which are erroneous, thus being useful in diagnosing the conditions at their early stages. Another advantage is that it may be possible to describe the disease process because changes in biomarker concentrations could reflect the disease process and therefore be useful for assessing the effectiveness of therapy. Also, this panel is cheap in comparison to other imaging techniques such as positron emission tomography (PET) scans (Boccardi et al., 2017).

Limitations of the Study

There are some limitations in the present study which should be taken into account for the future research. First, there were few patients in the study and majority of them from a certain area, this may reduce the validity of the study in other populations. Hence, in the future, the researchers should involve more patients and it should be conducted in a wider region. Second, in this study, the biomarker panel was found to be valid, however, it is still concerned whether this biomarker panel would be beneficial in the long-term clinical practice and therefore it is essential to conduct more large sample size, long-term follow-up study to evaluate the applicability of this biomarker panel. Thus, it is necessary that the subsequent studies examine the panel in other phases of the disease progression and its capacity to give a more accurate demarcation between the onset or progression of the disease. These aspects will have to be worked on in order to optimize the biomarker panel and the current study identifies some of these aspects which have yet to be researched.

Future Directions in Neurodegenerative Biomarker Research

In the future work, it would be useful to expand the biomarker list to more patients and patients of different race. Still, more value could be added to this panel by combining this with other imaging studies and genetic tests. It will also be useful to evaluate the changes of these biomarkers in this population over

time in order to gain more insight into their involvement in disease progression and response to treatment. Also, other works and other investigations for other possible biomarkers, especially the one connected to synaptic dysfunctions and neuroinflammation could contribute to the discovery of more accurate panels. Lastly, the use of these biomarker-based diagnostics will require integration of knowledge from the academia, the industry and the regulatory bodies who will be interested in implementing the findings of this research into practice as well as the standard to be followed (Dutta et al., 2023).

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