NfL-Based Clustering Analysis in Biologically Defined Alzheimer's Disease According to the 2024 NIA-AA Criteria

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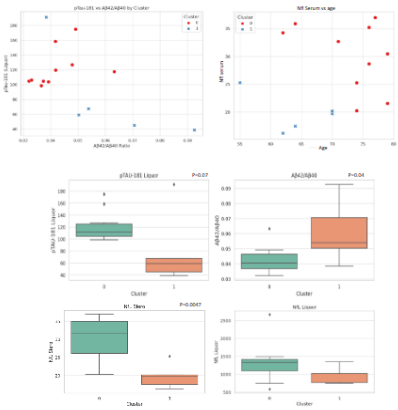
OBJECTIVES

Alzheimer's disease (AD) is now biologically defined by the 2024 NIA-AA criteria based on the presence of core biomarkers of amyloid plaques and tau tangles in cerebrospinal fluid (CSF) or imaging. This framework allows identification of the biological state of AD independent of clinical symptoms. We aimed to explore the biological heterogeneity within a cohort of AD patients selected according to CSF core biomarkers and to relate these data-driven groups to clinical severity and additional biomarker profiles.

AD Patients	n=15
M:F	2:1
Mean age (SD)	70,3 (±7,2)
Mean MMSE	21,4
Stage distribution (%)	Stage 3: 40% Stage 4: 46,6% Stage 5: 6,7% Stage 6; 6,7%

MATERIALS & METHODS

We included 15 patients biologically defined as having AD based on CSF core biomarkers (Aβ42/40 ratio and pTau-181) according to the 2024 NIA-AA criteria, independent of clinical phenotype, from the Policlinico of Bari Memory Clinic. CSF and serum Neurofilament Light Chain (NfL), total Tau, pTau-181, and Aβ42/40 ratio were measured using Lumipulse® assays (Fujirebio). An unsupervised k-means cluster analysis was performed using standardized values of CSF NfL, serum NfL, and age, yielding two clusters. Clusters were compared for clinical and biomarker differences using Mann-Whitney U tests. AT(N) classification was updated considering Lumipulse-specific thresholds: A+ (Aβ42/40 ratio < 0.069), T+ (pTau > 65 pg/mL), N+ (NfL > 1000 pg/mL).



RESULTS

Cluster 0 (n = 10) included older patients with significantly higher serum NfL (p = 0.0047) and lower Aβ42/40 ratio (p = 0.0400), consistent with a typical biological AD profile. Cluster 1 (n = 5) included younger patients with lower NfL levels and greater biomarker heterogeneity. CDR and MMSE did not differ significantly between clusters. Cluster 0 included 8 patients classified as A+T+N+ based on updated criteria, while cluster 1 showed a more heterogeneous distribution.

DISCUSSION

Despite similar cognitive profiles, cluster analysis revealed two biologically distinct AD subgroups. Cluster 0 likely represents typical late-onset AD with full amyloid-tau positivity, while Cluster 1 may represent early-onset or atypical variants with incomplete biomarker expression. These findings highlight the utility of combining NfL and age for biological stratification, complementing the 2024 NIA-AA core biomarker approach.

CONCLUSION

Unsupervised clustering of biologically defined AD patients based on NfL and age identifies distinct biological subgroups not captured by clinical severity alone. These findings support the integration of NfL into multimodal biomarker-based precision stratification strategies in Alzheimer's disease.

References:
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