BALNEO RESEARCH Balneo and PRM Research Journal English Edition
e ISSN 2734 - 8458 p ISSN 2734 - 844X ROMANIAN ASSOCIATION OF BALNEOLOGY

Research article

Unsupervised Machine Learning of Automated Brain Volumetry Uncovers Three Volumetric Endophenotypes in Cognitive Impairment

Albert Vamanu [2], Liana Rada Borza[6], Livia Livinț-Popa[7], Alexandra Mastaleru [1], Roxana Covali [1*],Robert-Valentin Bilcu [1], Sofia Alexandra Socolov-Mihaita [3], Lucia Corina Dima-Cozma [1], Romica Sebastian Cozma [1], Roxana Serban [1], Raluca Olariu [1], Alexandru Patrascu [4], Cătălin-Alexandru Chihaia [5] and Alin Ciubotaru [1]

Citation: Vamanu A., Borza L.R., Livinț-Popa L., Mastaleru A., Covali R., Bilcu R.V., Socolov-Mihaita S.A., Dima-Cozma L.C., Cozma R.S., Serban R., Olariu R., Patrascu A., Chihaia C.A., Ciubotaru A. - Unsupervised Machine Learning of Automated Brain Volumetry Uncovers Three Volumetric Endophenotypes in Cognitive Impairment
Balneo and PRM Research Journal **2026**, *17(2): 1062*

Academic Editor(s):
Constantin Munteanu

Reviewer Officer:
Viorela Bembea

Production Officer:
Camil Filimon

Received: 16.05.2026
Published: 10.07.2026

Reviewers:
Alexandra Ecaterina Ciota
Himena Zippenfening

Publisher's Note:
Balneo and PRM Research Journal stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1 Grigore T. Popa University of Medicine and Pharmacy, 700115 Iasi, Romania;
2 Basic and Clinical Neuroscience Department, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London SE5 8AF, UK;
3 Doctoral School, Carol Davila University of Medicine and Pharmacy, 020021 Bucharest, Romania;
4 Apollonia University, 700511 Iasi, Romania;
5 9th Psychiatric Clinic for Chronic Patients, "Socola" Institute of Psychiatry, Iasi, Romania;
6 Honorary Clinical Lecturer in Psychiatry, Faculty of Medicine - Imperial College London, UK;
7 Department of Neuroscience, "Iuliu Hațieganu" University of Medicine and Pharmacy, 400083 Cluj-Napoca, Romania

* Correspondence: ana.covali@umfiasi.ro

Abstract: Cognitive impairment and dementia represent a heterogeneous group of disorders with variable patterns of brain atrophy. Automated brain volumetry combined with machine learning offers the potential to identify biologically meaningful subgroups beyond traditional clinical classifications. This study aimed to characterize cerebral volumetric profiles and identify distinct volumetric endophenotypes in individuals with cognitive impairment using unsupervised machine learning. This retrospective cross-sectional study included 78 participants with cognitive impairment who underwent T1-weighted three-dimensional magnetic resonance imaging (MRI). Automated brain volumetry was performed using mdbrain software (version 2.2), which provides normalized percentile values for 42 brain regions adjusted for age, sex, and total intracranial volume. Unsupervised k-means clustering was applied to volumetric percentiles to identify distinct endophenotypes. Cluster validation was performed using elbow method, silhouette analysis, Calinski-Harabasz index, and Davies-Bouldin index. Discriminative features were identified using ANOVA with effect size calculations (η^2). A multinomial logistic regression model was developed to predict cluster membership. The cohort comprised 48 (61.5%) females and 30 (38.5%) males. Substantial inter-individual variability was observed across all brain regions, with hippocampal percentiles ranging from 0.1 to 99.8 (median: 25.6). Clustering analysis revealed three distinct volumetric endophenotypes: Cluster 1 (Preserved, n=27, 34.6%) characterized by percentiles above the 40th percentile; Cluster 2 (Moderate Atrophy, n=31, 39.7%) with percentiles in the 10th–30th range; and Cluster 3 (Severe Atrophy, n=20, 25.6%) with hippocampal percentiles below the 5th percentile and ventricular percentiles above the 85th percentile. Hippocampal volumes demonstrated the strongest discriminative ability (left hippocampus: F=68.4, η^2=0.64; right hippocampus: F=65.2, η^2=0.63), followed by temporal (η^2=0.60–0.61) and ventricular volumes (η^2=0.57–0.58). Interhemispheric asymmetry was minimal (median absolute difference: 3.4–7.2 percentile points). A parsimonious model using four volumetric features (left hippocampus, left temporal, left ventricle, and right frontal percentiles) achieved excellent classification accuracy (87.2%, cross-validated AUC=0.91). Automated brain volumetry combined with unsupervised machine learning identified three distinct volumetric endophenotypes in cognitive impairment, primarily discriminated by hippocampal, temporal, and ventricular volumes. These findings suggest that cognitive impairment manifests through heterogeneous patterns of structural brain involvement, with potential implications for prognosis and

personalized care. The high accuracy of a parsimonious four-feature model supports the clinical utility of focused volumetric assessment for risk stratification. Future longitudinal studies with multimodal biomarker integration are warranted to validate these findings and establish their prognostic value.

Keywords: brain volumetry; cognitive impairment; dementia; machine learning; clustering analysis; hippocampal atrophy; endophenotypes; neuroimaging; artificial intelligence; precision medicine

1. Introduction

Major neurocognitive disorders, collectively referred to as dementia, represent one of the most pressing public health challenges of the 21st century, with an estimated 55 million individuals affected worldwide a figure projected to triple by 2050 due to demographic aging [1, 2]. While cognitive decline in domains such as memory, executive function, and language constitutes the core diagnostic features, the clinical and socioeconomic burden of dementia is disproportionately driven by its associated neuropsychiatric manifestations [3].

Beyond cognitive deterioration, up to 90% of individuals with dementia experience Behavioral and Psychological Symptoms of Dementia (BPSD), including agitation, apathy, depression, psychosis, and sleep disturbances [4]. These symptoms are strongly correlated with reduced quality of life, accelerated institutionalization, caregiver burnout, and substantially increased healthcare expenditures [5, 6]. The neurobiological underpinnings of both cognitive decline and BPSD are complex, involving region-specific patterns of neurodegeneration that vary across dementia subtypes and disease stages [7, 8].

In this context, the identification of objective, quantifiable biomarkers that correlate with cognitive severity and predict disease progression has become a research priority. Automated brain volumetry, leveraging deep learning algorithms applied to T1-weighted magnetic resonance imaging (MRI), has emerged as a powerful tool for quantifying regional brain atrophy with high precision and reproducibility [9, 10]. Unlike qualitative visual assessment, automated volumetry provides normalized percentile values adjusted for age, sex, and total intracranial volume (ICV), enabling precise characterization of atrophy patterns across distinct brain regions [11].

Previous studies have established that hippocampal atrophy is a robust biomarker for Alzheimer's disease, while distinct patterns of frontal, temporal, or parietal atrophy correspond to other dementia subtypes such as frontotemporal dementia or dementia with Lewy bodies [12-15]. However, the multidimensional relationships between regional brain volumes across the entire cerebrum and the graded severity of cognitive impairment ranging from mild to moderate to severe remain incompletely characterized. Furthermore, whether distinct volumetric endophenotypes exist within cognitively impaired populations, and whether such clusters can predict the risk of cognitive worsening, represents a critical gap in the literature [12-15].

The advent of artificial intelligence (AI) and machine learning (ML) in neuroimaging has opened new avenues for data-driven phenotyping. Unsupervised learning techniques, such as clustering algorithms, can identify natural groupings within patient populations based on multidimensional volumetric data, potentially revealing biologically meaningful subtypes that transcend traditional clinical classifications [15, 16]. Such an approach aligns with the Research Domain Criteria (RDoC) framework, which seeks to link observable biological features to clinical phenomena [17].

1.1. Aim and Objectives

Aim: This study aims to characterize cerebral volumetric profiles in patients with cognitive impairment using automated brain volumetry and to identify distinct volumetric endophenotypes through unsupervised machine learning, with the ultimate goal of informing prognostic risk stratification pending longitudinal validation.

Specific Objectives:

1. To quantify regional brain volumes (total brain, gray matter, white matter, cortical gray matter, frontal, parietal, temporal, occipital lobes, hippocampus, thalamus, brainstem, and lateral ventricles) using automated volumetry in a cohort stratified by cognitive severity (mild: 40%, moderate: 40%, severe: 20%).
2. To identify distinct volumetric endophenotypes (clusters) through unsupervised machine learning (k-means clustering) based on multidimensional percentile data, controlling for age, sex, and total intracranial volume.
3. To evaluate the association between baseline volumetric percentiles and severity of cognitive impairment using multivariate regression models.
4. To develop a predictive risk model for cognitive worsening based on volumetric cluster membership and regional atrophy patterns.

1.2. Novelty Elements

This study introduces several novel contributions to the existing literature:

First, while previous research has predominantly focused on single-structure volumetry (e.g., hippocampus) for diagnostic classification, we employ a comprehensive, whole-brain approach encompassing 42 distinct brain regions, including cortical and subcortical structures, brainstem compartments, and ventricular systems. This multidimensional perspective allows for the identification of complex atrophy patterns rather than isolated structural changes.

Second, we utilize unsupervised machine learning (k-means clustering) to derive data-driven volumetric endophenotypes, moving beyond a priori clinical classifications. This approach enables the discovery of biologically meaningful patient subgroups that may not align with traditional cognitive severity categories, potentially revealing distinct neurodegenerative trajectories [15].

Third, this study integrates volumetric percentiles normalized for age, sex, and total intracranial volume, leveraging a large reference cohort (n = 6,371) to ensure accurate, individualized assessments. This normalization is critical for distinguishing pathological atrophy from normal age-related changes.

Fourth, we develop a predictive risk model linking baseline volumetric cluster membership to the risk of cognitive worsening, addressing a critical gap in prognostic neuroimaging. Such predictive analytics represent a paradigm shift from reactive diagnosis to proactive risk stratification [18].

1.3. Practical Clinical Applications

The findings of this study hold several potential clinical applications:

Enhanced Diagnostic Accuracy: By providing objective, quantitative percentiles for regional brain volumes, clinicians can augment visual radiological assessment with precise numerical data, reducing inter-rater variability and improving diagnostic confidence in early-stage cognitive impairment.

Personalized Risk Stratification: Identification of distinct volumetric endophenotypes enables clinicians to stratify patients based on their specific atrophy profiles, facilitating more accurate prognostic counseling regarding the likelihood and timeline of cognitive decline progression.

Targeted Monitoring: Patients identified as high-risk based on their volumetric cluster can be prioritized for more frequent clinical follow-up, cognitive assessments, and early intervention strategies, potentially delaying institutionalization.

Informed Therapeutic Decision-Making: Volumetric profiles may guide treatment selection by identifying patients who might benefit most from specific pharmacological or non-pharmacological interventions. For example, patients with predominant hippocampal atrophy may be optimal candidates for cholinesterase inhibitors, while those with frontal-predominant patterns might benefit from behavioral interventions targeting apathy and executive dysfunction.

Objective Outcome Measurement: In clinical trials, volumetric percentiles can serve as quantitative, objective endpoints for assessing treatment efficacy in slowing neurodegeneration, complementing traditional cognitive scales which are subject to practice effects and rater variability.

Resource Allocation: Healthcare systems can utilize volumetric risk stratification to allocate limited resources such as specialist consultations, home care services, or institutional placement to patients with the highest predicted risk of rapid decline.

2. Materials and Methods

2.1. Study Design and Population

This was a retrospective, cross-sectional cohort study designed to characterize cerebral volumetric profiles in patients with cognitive impairment using automated brain volumetry and unsupervised machine learning. The study protocol was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all participants or their legal guardians prior to enrollment.

A total of 78 participants were included in the final analysis. The cohort consisted of individuals with cognitive impairment who underwent brain MRI as part of their diagnostic evaluation. Demographic characteristics, including age and sex, were recorded for all participants.

Inclusion criteria were: (1) age ≥ 50 years; (2) clinical diagnosis of mild cognitive impairment or major neurocognitive disorder (dementia) according to DSM-5 criteria; (3) availability of a high-quality T1-weighted 3D MRI sequence suitable for automated volumetry; (4) complete volumetric data for all 42 brain regions.

Exclusion criteria were: (1) presence of large territorial stroke, intracranial mass, or other structural lesions interfering with volumetric analysis; (2) history of traumatic brain injury with loss of consciousness > 30 minutes; (3) active substance use disorder; (4) contraindications to MRI; (5) severe motion artifacts on MRI scans precluding accurate segmentation; (6) missing volumetric data for > 5% of brain regions.

2.2. MRI Acquisition Protocol

All participants underwent brain MRI on a 1.5 or 3.0 Tesla scanner using a standardized protocol. Scanner field strength (1.5T vs. 3.0T) was recorded for each participant and included as a covariate in sensitivity analyses to assess its potential influence on volumetric measurements; no significant effect of field strength on cluster assignment was identified. The core sequence for volumetric analysis was a three-dimensional T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence with the following parameters: repetition time (TR) = 2300 ms, echo time (TE) = 2.98 ms, inversion time (TI) = 900 ms, flip angle = 9°, field of view (FOV) = 256 × 256 mm, matrix size = 256 × 256, slice thickness = 1.0 mm with no gap, yielding isotropic voxel dimensions of 1.0 × 1.0 × 1.0 mm^3. All scans were reviewed by a board-certified neuroradiologist to ensure adequate image quality and absence of significant artifacts.

2.3. Automated Brain Volumetry: Software and Technical Specifications

Image processing was performed using the "mdbrain" software (version 2.2) from Mediaire GmbH, Berlin, Germany. This software is approved as a medical device in accordance with the European Medical Devices Directive (CE marking) and is compliant with the European Commission requirements for medical software. The system performs automated brain volumetry for different brain segments and lobes using native T1-weighted 3D MRI sequences as input.

The segmentation algorithm employs a custom deep learning model based on the U-Net architecture a convolutional neural network (CNN) specifically designed for biomedical image segmentation. The U-Net architecture consists of a contracting path to capture context and a symmetric expanding path that enables precise localization, allowing the model to segment anatomical structures with high spatial accuracy. The model was trained on a large, diverse dataset of manually annotated MRI scans to ensure robust performance across different scanner types, acquisition parameters, and patient populations.

The mdbrain software generates volumetric measurements for 42 distinct brain regions. For each region, the software outputs both absolute volume (in milliliters) and

normalized percentile values. Percentiles are derived by comparing the individual's volumes to a reference cohort of healthy individuals (n = 6,371; age range 10–97 years) stratified by age, sex, and total intracranial volume (ICV). This normalization approach accounts for physiological variations in brain size due to demographic factors, enabling the identification of pathological atrophy independent of normal age-related changes.

For each participant, both absolute volumes (mL) and percentiles were exported from the mdbrain platform for subsequent statistical analysis.

2.4. Volumetric Measures

The primary volumetric measures extracted for this study included:

Global measures:

- Total brain volume (TBV)
- Total gray matter volume (GM)
- Total white matter volume (WM)
- Cortical gray matter volume (cGM)

Lobar measures (bilateral):

- Frontal lobe (left and right)
- Parietal lobe (left and right)
- Occipital lobe (left and right)
- Temporal lobe (left and right)

Subcortical structures (bilateral):

- Hippocampus (left and right)
- Thalamus (left and right)

Brainstem structures:

- Midbrain
- Pons

Ventricular measures:

- Lateral ventricle (left and right)

For each region, both absolute volumes (mL) and percentiles were analyzed. Percentile values were used as the primary input for clustering analyses, as they provide age- and sex-adjusted normalization.

2.5. Statistical Analysis

Statistical analyses were performed using R software (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria) and Python (version 3.11, Python Software Foundation, Wilmington, DE, USA). All statistical tests were two-tailed, and a significance threshold of $\alpha = 0.05$ was adopted. Correction for multiple comparisons was applied where appropriate using the Benjamini-Hochberg false discovery rate (FDR) method.

2.5.1. Descriptive Statistics

Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for non-normally distributed data. Normality was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Categorical variables were expressed as frequencies and percentages. Descriptive statistics were calculated for all global volumetric measures (absolute volumes) and regional volumetric percentiles.

2.5.2. Correlation Analysis

To examine the relationships between volumetric percentiles across different brain regions, Spearman's rank correlation analysis was performed. Correlation coefficients were interpreted as: negligible ($|\rho| < 0.1$), weak ($0.1 \leq |\rho| < 0.3$), moderate ($0.3 \leq |\rho| < 0.5$), strong ($0.5 \leq |\rho| < 0.7$), and very strong ($|\rho| \geq 0.7$). Correlation matrices were generated for all 42 brain regions, with particular focus on:

- Interhemispheric correlations for homologous structures
- Hippocampal correlations with lobar regions
- Ventricular correlations with parenchymal structures
- Brainstem correlations with subcortical structures

Confidence intervals for correlation coefficients were calculated using Fisher's z-transformation with 1,000 bootstrap replicates.

2.5.3. Unsupervised Machine Learning: Clustering Analysis

To identify distinct volumetric endophenotypes within the cohort, we performed unsupervised clustering using the k-means algorithm. The input feature matrix consisted of volumetric percentiles for all 42 brain regions.

Preprocessing steps included:

1. **Handling of missing values:** Participants with > 5% missing volumetric data were excluded. No participants met this exclusion criterion. For the remaining dataset, there were no missing values, as the mdbrain software provides complete volumetric data for all regions.
2. **Standardization:** All percentile features were standardized to have zero mean and unit variance (z-score normalization) to ensure equal weighting of all regions regardless of their absolute value ranges. This transformation was performed using the StandardScaler function in scikit-learn.
3. **Outlier detection:** Multivariate outliers were identified using Mahalanobis distance with a threshold of $p < 0.001$. No participants were excluded based on this criterion.

Determination of optimal cluster number:

The optimal number of clusters (k) was determined using a combination of four validation methods:

- **Elbow method:** The within-cluster sum of squares (WCSS) was plotted as a function of k (k = 1–10). The elbow point, defined as the value of k where the rate of decrease in WCSS substantially slows, was identified as the optimal number of clusters.
- **Silhouette analysis:** The average silhouette width was computed for each k (k = 2–10). The silhouette width measures how similar a point is to its own cluster compared to other clusters, with values ranging from -1 to 1. Higher values indicate better-defined clusters. The optimal k was selected as the value maximizing the average silhouette width.
- **Calinski-Harabasz index:** Also known as the variance ratio criterion, this index calculates the ratio of between-cluster dispersion to within-cluster dispersion. Higher values indicate better cluster separation. The index was computed for k = 2–10, and the optimal k was selected as the value maximizing the index.
- **Davies-Bouldin index:** This index measures the average similarity between clusters, where lower values indicate better separation. The index was computed for k = 2–10, and the optimal k was selected as the value minimizing the index.

Clustering algorithm implementation:

K-means clustering was performed using the KMeans class in scikit-learn with the following parameters:

- Number of clusters: k = 3 (as determined by validation metrics)
- Initialization: k-means++ to improve convergence
- Number of initializations: n_init = 10
- Maximum iterations: max_iter = 300
- Random state: random_state = 42 for reproducibility

Clustering stability assessment:

Cluster stability was assessed using bootstrap resampling with 1,000 iterations. For each bootstrap sample (sampling with replacement, n = 78), the k-means algorithm was reapplied, and cluster assignments were compared to the original solution using the adjusted Rand index (ARI). A mean ARI > 0.8 was considered indicative of stable cluster solutions.

Cluster characterization:

Following cluster identification, each cluster was characterized by its centroid (mean vector of standardized percentiles) and by the median and interquartile range

of original percentiles for each brain region. Clusters were labeled based on their volumetric profiles (Preserved, Moderate Atrophy, Severe Atrophy).

2.5.4. Discriminative Feature Analysis

To identify the brain regions that most strongly discriminated between clusters, we performed one-way analysis of variance (ANOVA) comparing mean percentiles across the three clusters for each brain region. For regions where the assumption of normality was violated, the Kruskal-Wallis test was used as a non-parametric alternative.

Effect sizes were calculated using eta-squared (η^2) for ANOVA, interpreted as: small ($\eta^2 \geq 0.01$), medium ($\eta^2 \geq 0.06$), and large ($\eta^2 \geq 0.14$) effects. For each region, the F-statistic or H-statistic, p-value, and effect size were reported.

Post-hoc pairwise comparisons between clusters were conducted using Tukey's honest significant difference (HSD) test for ANOVA and Dunn's test with Bonferroni correction for Kruskal-Wallis. Regions were ranked by their discriminative ability based on F-statistic and η^2.

2.5.5. Interhemispheric Asymmetry Analysis

To assess whether atrophy patterns were symmetric or asymmetric across hemispheres, we calculated the absolute difference between left and right percentiles for each paired structure (hippocampus, frontal lobe, parietal lobe, temporal lobe, occipital lobe, thalamus, and lateral ventricle). For each structure, we reported:

- Median absolute difference
- Interquartile range (IQR)
- Range (minimum–maximum)

Participants with absolute differences exceeding the 90th percentile were examined individually to identify potential cases of atypical asymmetry.

2.5.6. Predictive Modeling for Cluster Membership

To identify the minimal set of volumetric features that could accurately predict cluster membership, we performed multinomial logistic regression with elastic net regularization using the glmnet package in R. The outcome variable was cluster membership (three categories: Cluster 1, Cluster 2, Cluster 3).

Feature selection:

The initial feature set included all 42 volumetric percentiles. Elastic net regularization, which combines L1 (lasso) and L2 (ridge) penalties, was used for feature selection. The regularization parameter λ was selected using 10-fold cross-validation with the one-standard-error rule to minimize overfitting. The mixing parameter α was set to 0.5 to balance between lasso and ridge penalties.

Model specification:

The final model included the following predictors selected through regularization:

- Left hippocampus percentile
- Left temporal lobe percentile
- Left lateral ventricle percentile
- Right frontal lobe percentile

Model performance evaluation:

Model performance was evaluated using:

- **Overall accuracy:** Proportion of correctly classified participants
- **Sensitivity per cluster:** Proportion of participants correctly classified within each cluster
- **Cross-validated area under the receiver operating characteristic curve (AUC-ROC):** Calculated using one-vs-rest approach with 10-fold cross-validation repeated 100 times. AUC values were interpreted as: 0.5–0.7 (poor), 0.7–0.8 (acceptable), 0.8–0.9 (excellent), > 0.9 (outstanding).

Odds ratios:

For each predictor, odds ratios (OR) with 95% confidence intervals were calculated comparing Cluster 2 versus Cluster 1 and Cluster 3 versus Cluster 1. Wald tests were used to assess statistical significance.

2.6. Ethical Considerations

The study protocol was approved by the institutional ethics committee at the University of Medicine and Pharmacy "Grigore T. Popa", and all participants provided written informed consent before enrollment.

All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 2013. Written informed consent was obtained from all participants or their legally authorized representatives. All data were anonymized prior to analysis to ensure participant confidentiality.

3. Results

3.1. Demographic Characteristics of the Study Cohort

A total of 78 participants were included in the final analysis. The cohort consisted of individuals with cognitive impairment, with volumetric data available for all 42 brain regions per participant. Demographic characteristics are presented in Table 1. Sex distribution showed a predominance of female participants (n = 48, 61.5%) compared to male participants (n = 30, 38.5%). Mean age was 73.4 ± 8.7 years (range: 52–91 years). MRI acquisitions were performed on 1.5T (n = 34, 43.6%) and 3.0T (n = 44, 56.4%) scanners; sensitivity analyses confirmed no significant effect of field strength on cluster assignment. Full demographic characteristics are presented in Table 1.

Table 1. Demographic and Clinical Characteristics of the Study Cohort

Characteristic	Total Cohort (N = 78)
Female	48 (61.5%)
Male	30 (38.5%)
Age, years (mean ± SD)	73.4 ± 8.7 (range 52–91)

3.2. Global Volumetric Measures

Global volumetric measures, including total brain volume (TBV), total gray matter (GM), total white matter (WM), and cortical gray matter (cGM), are presented in Table 2. Substantial inter-individual variability was observed across all global measures, reflecting the heterogeneous nature of neurodegenerative processes in this cohort.

Table 2. Global Volumetric Measures

Volumetric Measure (mL)	Mean ± SD	Median (IQR)	Range (Min–Max)
Total Brain Volume (TBV)	1132.4 ± 137.8	1139.5 (1048.3–1242.8)	741.3–1388.0
Total Gray Matter (GM)	483.6 ± 68.4	482.3 (445.0–523.5)	283.2–625.9
Total White Matter (WM)	648.8 ± 74.2	649.0 (597.2–690.9)	458.1–847.9
Cortical Gray Matter (cGM)	446.2 ± 58.1	445.8 (407.5–482.4)	322.2–559.3

SD, standard deviation; IQR, interquartile range.

3.3. Regional Volumetric Percentiles

To account for age, sex, and total intracranial volume, normalized percentile values for each brain region were analyzed. Table 3 presents the descriptive statistics for all volumetric percentiles across the entire cohort.

3.3.1. Hippocampal Volume Percentiles

Hippocampal percentiles showed wide variability across the cohort, reflecting the spectrum of neurodegenerative involvement. Left hippocampal percentiles ranged from 0.1 to 99.8, with a median of 25.6 (IQR: 3.6–58.7). Right hippocampal percentiles ranged from 0 to 99.9, with a median of 26.2 (IQR: 3.7–61.8). The substantial proportion of participants with very low hippocampal percentiles (< 10th percentile) indicates significant hippocampal atrophy in a subset of the cohort.

3.3.2. Lobar Volume Percentiles

Frontal lobe percentiles demonstrated significant variability, with left frontal percentiles ranging from 0.1 to 100 (median: 19.8, IQR: 3.5–66.2) and right frontal percentiles ranging from 0 to 100 (median: 23.6, IQR: 4.6–71.6). The wide range suggests heterogeneous frontal involvement across participants.

Temporal lobe percentiles showed similar patterns, with left temporal percentiles ranging from 0.1 to 99.9 (median: 28.9, IQR: 6.8–67.3) and right temporal percentiles ranging from 0 to 100 (median: 34.2, IQR: 8.9–72.1).

Parietal lobe percentiles ranged from 0 to 97.3 (left) and 0 to 99.8 (right), with medians of 12.6 and 13.9, respectively. Occipital lobe percentiles showed the highest median values among lobar regions (left: 38.6, right: 41.8), consistent with relative preservation of visual cortex.

3.3.3. Subcortical and Brainstem Structures

Thalamic percentiles ranged from 0 to 100 for both hemispheres, with medians of 17.9 (left) and 16.8 (right). Brainstem structures showed relatively higher percentiles compared to cortical regions, with midbrain median percentile of 28.6 (IQR: 19.3–46.9) and pons median percentile of 34.2 (IQR: 20.4–60.1).

3.3.4. Ventricular Volumes

Lateral ventricular percentiles were markedly elevated in a subset of participants, with left ventricle percentiles ranging from 0 to 100 (median: 59.8, IQR: 16.4–90.2) and right ventricle percentiles ranging from 0 to 100 (median: 64.5, IQR: 18.7–92.5). The high median values reflect compensatory ventricular enlargement secondary to parenchymal atrophy.

Table 3. Descriptive Statistics for Regional Volumetric Percentiles (N = 78)

Region	Mean ± SD	Median (IQR)	Range (Min–Max)
Hippocampus			
Left Hippocampus	32.1 ± 29.4	25.6 (3.6–58.7)	0.1–99.8
Right Hippocampus	33.5 ± 30.2	26.2 (3.7–61.8)	0–99.9
Frontal Lobe			
Left Frontal	32.4 ± 30.8	19.8 (3.5–66.2)	0.1–100
Right Frontal	35.8 ± 31.4	23.6 (4.6–71.6)	0–100
Parietal Lobe			
Left Parietal	23.5 ± 25.8	12.6 (2.8–38.7)	0–97.3
Right Parietal	24.9 ± 26.7	13.9 (3.1–41.5)	0–99.8
Occipital Lobe			
Left Occipital	44.2 ± 30.4	38.6 (14.2–74.3)	0–99.9
Right Occipital	45.1 ± 30.8	41.8 (15.4–75.6)	0–100
Temporal Lobe			
Left Temporal	36.4 ± 30.1	28.9 (6.8–67.3)	0.1–99.9

Right Temporal	38.5 ± 30.6	34.2 (8.9–72.1)	0–100
Subcortical			
Left Thalamus	27.9 ± 26.4	17.9 (5.2–46.4)	0–100
Right Thalamus	27.6 ± 26.6	16.8 (4.8–46.7)	0–100
Brainstem			
Midbrain	34.8 ± 25.3	28.6 (19.3–46.9)	0–97.7
Pons	39.7 ± 27.8	34.2 (20.4–60.1)	0–99.9
Ventricular			
Left Lateral Ventricle	56.4 ± 31.2	59.8 (16.4–90.2)	0–100
Right Lateral Ventricle	59.3 ± 30.8	64.5 (18.7–92.5)	0–100

3.4. Correlation Analysis Between Brain Regions

To examine the relationships between volumetric percentiles across different brain regions, we performed Spearman's rank correlation analysis. The correlation matrix revealed strong interhemispheric correlations for homologous structures, as well as distinct patterns of correlation between cortical and subcortical regions.

3.4.1. Hippocampal Correlations

Left and right hippocampal percentiles showed a very strong positive correlation ($\varrho = 0.89$, $p < 0.001$), indicating symmetric involvement in most participants. Hippocampal percentiles also demonstrated strong positive correlations with temporal lobe percentiles (left hippocampus-left temporal: $\varrho = 0.74$, $p < 0.001$; right hippocampus-right temporal: $\varrho = 0.71$, $p < 0.001$) and moderate correlations with frontal lobe percentiles (left hippocampus-left frontal: $\varrho = 0.52$, $p < 0.001$).

3.4.2. Lobar Correlations

Strong correlations were observed between homologous lobar regions across hemispheres: frontal lobes ($\varrho = 0.91$, $p < 0.001$), parietal lobes ($\varrho = 0.88$, $p < 0.001$), temporal lobes ($\varrho = 0.87$, $p < 0.001$), and occipital lobes ($\varrho = 0.84$, $p < 0.001$). These strong interhemispheric correlations suggest that atrophy patterns tend to be relatively symmetric in this cohort.

3.4.3. Ventricular Correlations

Lateral ventricular percentiles showed strong negative correlations with hippocampal percentiles (left ventricle-left hippocampus: $\varrho = -0.58$, $p < 0.001$; right ventricle-right hippocampus: $\varrho = -0.55$, $p < 0.001$), reflecting the expected inverse relationship between parenchymal atrophy and ventricular enlargement. Ventricular percentiles also demonstrated moderate negative correlations with temporal lobe percentiles ($\varrho = -0.47$ to -0.51) and frontal lobe percentiles ($\varrho = -0.38$ to -0.43).

Table 4. Selected Correlation Coefficients Between Key Brain Regions

Region Pair	Spearman's ρ	95% CI	p-value
Interhemispheric Correlations			
Left Hippocampus – Right Hippocampus	0.89	[0.83–0.93]	< 0.001
Left Frontal – Right Frontal	0.91	[0.86–0.94]	< 0.001
Left Parietal – Right Parietal	0.88	[0.82–0.92]	< 0.001
Left Temporal – Right Temporal	0.87	[0.80–0.91]	< 0.001
Left Occipital – Right Occipital	0.84	[0.76–0.89]	< 0.001
Hippocampus – Lobar Correlations			

Left Hippocampus – Left Temporal	0.74	[0.61–0.83]	< 0.001
Left Hippocampus – Left Frontal	0.52	[0.34–0.67]	< 0.001
Left Hippocampus – Left Parietal	0.44	[0.24–0.60]	< 0.001
Left Hippocampus – Left Occipital	0.28	[0.06–0.48]	0.014
Hippocampus – Subcortical Correlations			
Left Hippocampus – Left Thalamus	0.48	[0.29–0.64]	< 0.001
Right Hippocampus – Right Thalamus	0.45	[0.25–0.61]	< 0.001
Ventricular – Parenchymal Correlations			
Left Ventricle – Left Hippocampus	-0.58	[-0.72 – -0.41]	< 0.001
Left Ventricle – Left Temporal	-0.51	[-0.66 – -0.32]	< 0.001
Right Ventricle – Right Hippocampus	-0.55	[-0.70 – -0.37]	< 0.001
Brainstem – Subcortical Correlations			
Midbrain – Left Thalamus	0.41	[0.21–0.58]	< 0.001
Pons – Left Thalamus	0.38	[0.17–0.56]	< 0.001

3.5. Unsupervised Machine Learning: Identification of Volumetric Endophenotypes

To identify distinct volumetric endophenotypes within the cohort, we performed k-means clustering analysis using volumetric percentiles from all 42 brain regions as input features. Preprocessing included standardization (z-score normalization) of all percentile features to ensure equal weighting.

3.5.1. Optimal Cluster Number Determination

The optimal number of clusters was determined using a combination of four validation methods, as presented in Figure 1:

- **Elbow method:** The within-cluster sum of squares (WCSS) curve showed an inflection point at k = 3, with diminishing returns beyond this point.
- **Silhouette analysis:** The average silhouette width was maximized at k = 3 (silhouette score = 0.52), indicating good cluster separation.
- **Calinski-Harabasz index:** The highest ratio of between-cluster to within-cluster dispersion was observed at k = 3 (F = 124.7).
- **Davies-Bouldin index:** The minimum average similarity between clusters was achieved at k = 3 (DB index = 0.68).

Based on these criteria, a three-cluster solution was selected. Cluster stability assessment using bootstrap resampling (1,000 iterations) confirmed robust solutions, with a mean adjusted Rand index (ARI) of 0.84 (range: 0.79–0.91), exceeding the prespecified stability threshold of ARI > 0.80.

3.5.2. Cluster Distribution and Characteristics

The three clusters were characterized as follows:

- **Cluster 1 (n = 27, 34.6%):** "Preserved Volumetric Profile" – characterized by volumetric percentiles generally above the 40th percentile across most regions, with preserved hippocampal and temporal volumes.
- **Cluster 2 (n = 31, 39.7%):** "Moderate Atrophy Profile" – characterized by moderate reductions in volumetric percentiles, particularly in hippocampal, temporal, and frontal regions (percentiles in the 10th–30th range).
- **Cluster 3 (n = 20, 25.6%):** "Severe Atrophy Profile" – characterized by marked reductions across all regions, with hippocampal and temporal percentiles below the 5th percentile and elevated ventricular percentiles (> 85th percentile).

Table 5 presents the volumetric characteristics of each cluster for key brain regions.

Table 5. Cluster-Specific Volumetric Profiles for Key Regions

Region	Cluster 1 (Preserved) n = 27	Cluster 2 (Moderate Atrophy) n = 31	Cluster 3 (Severe Atrophy) n = 20	p-value*
Hippocampus				
Left Hippocampus	62.4 (48.2–78.6)	18.7 (9.4–31.2)	2.1 (0.4–4.8)	< 0.001
Right Hippocampus	64.1 (49.8–79.3)	19.2 (10.1–32.5)	2.3 (0.5–5.1)	< 0.001
Frontal Lobe				
Left Frontal	68.7 (54.2–82.4)	22.4 (11.8–38.7)	3.2 (0.8–8.9)	< 0.001
Right Frontal	70.2 (56.7–84.1)	24.1 (12.9–40.2)	3.8 (1.1–9.6)	< 0.001
Temporal Lobe				
Left Temporal	71.3 (58.4–84.9)	28.6 (16.4–44.7)	4.1 (1.4–9.8)	< 0.001
Right Temporal	72.8 (59.7–86.2)	30.1 (17.8–46.2)	4.6 (1.7–10.3)	< 0.001
Parietal Lobe				
Left Parietal	52.6 (38.9–69.4)	12.8 (5.2–24.6)	2.3 (0.6–6.2)	< 0.001
Right Parietal	54.1 (40.2–71.2)	13.6 (5.8–26.1)	2.7 (0.8–7.1)	< 0.001
Thalamus				
Left Thalamus	51.8 (38.4–68.9)	16.4 (7.6–29.8)	3.9 (1.2–9.4)	< 0.001
Right Thalamus	50.9 (37.6–67.4)	15.8 (7.1–28.6)	3.6 (1.0–8.7)	< 0.001
Lateral Ventricle				
Left Ventricle	24.6 (12.8–38.9)	68.4 (52.7–84.2)	91.2 (84.6–96.8)	< 0.001
Right Ventricle	26.8 (14.2–41.2)	71.2 (55.4–86.7)	93.4 (87.1–97.9)	< 0.001
Brainstem				
Midbrain	54.2 (41.6–69.8)	32.4 (21.8–46.7)	18.6 (9.4–29.2)	< 0.001
Pons	59.8 (47.2–74.6)	38.9 (27.4–54.2)	22.4 (12.8–34.6)	< 0.001

Kruskal-Wallis test. Data presented as median (interquartile range). All p-values < 0.001.

3.6. Discriminative Features of Volumetric Clusters

To identify the brain regions that most strongly discriminated between clusters, we performed ANOVA with post-hoc comparisons across the three clusters. The top discriminative features, ranked by F-statistic and effect size (η^2), are presented in Table 6.

Hippocampal volumes emerged as the most discriminative features, with left hippocampus ($F = 68.4$, $\eta^2 = 0.64$) and right hippocampus ($F = 65.2$, $\eta^2 = 0.63$) showing the largest effect sizes. Temporal lobe volumes followed closely (left temporal: $F = 59.7$, $\eta^2 = 0.61$; right temporal: $F = 57.3$, $\eta^2 = 0.60$). Ventricular volumes also demonstrated strong discriminative ability (left ventricle: $F = 52.8$, $\eta^2 = 0.58$; right ventricle: $F = 50.1$, $\eta^2 = 0.57$).

Frontal lobe volumes showed moderate discriminative ability (left frontal: $F = 41.2$, $\eta^2 = 0.52$; right frontal: $F = 39.6$, $\eta^2 = 0.51$), while occipital lobe volumes showed the weakest discriminative ability (left occipital: $F = 12.4$, $\eta^2 = 0.24$; right occipital: $F = 11.8$, $\eta^2 = 0.23$), consistent with the relative preservation of visual cortex in many neurodegenerative processes.

Table 6. Top Discriminative Features Between Clusters (ANOVA)

Region	F-statistic	p-value	η^2 (Effect Size)	Post-hoc Differences*
Left Hippocampus	68.4	< 0.001	0.64	C1 > C2 > C3
Right Hippocampus	65.2	< 0.001	0.63	C1 > C2 > C3
Left Temporal	59.7	< 0.001	0.61	C1 > C2 > C3
Right Temporal	57.3	< 0.001	0.60	C1 > C2 > C3
Left Ventricle	52.8	< 0.001	0.58	C3 > C2 > C1
Right Ventricle	50.1	< 0.001	0.57	C3 > C2 > C1
Left Frontal	41.2	< 0.001	0.52	C1 > C2 > C3
Right Frontal	39.6	< 0.001	0.51	C1 > C2 > C3
Left Parietal	35.8	< 0.001	0.48	C1 > C2 > C3
Right Parietal	34.2	< 0.001	0.47	C1 > C2 > C3
Left Thalamus	31.6	< 0.001	0.45	C1 > C2 > C3
Right Thalamus	30.2	< 0.001	0.44	C1 > C2 > C3
Midbrain	22.4	< 0.001	0.37	C1 > C2 > C3
Pons	19.8	< 0.001	0.34	C1 > C2 > C3
Left Occipital	12.4	< 0.001	0.24	C1 > C2 > C3
Right Occipital	11.8	< 0.001	0.23	C1 > C2 > C3

C1, Cluster 1 (Preserved); C2, Cluster 2 (Moderate Atrophy); C3, Cluster 3 (Severe Atrophy). All post-hoc comparisons significant at $p < 0.05$ after Bonferroni correction.

3.7. Interhemispheric Asymmetry Analysis

To assess whether atrophy patterns were symmetric or asymmetric across hemispheres, we calculated the absolute difference between left and right percentiles for each paired structure. Results are presented in Table 7.

Hippocampal asymmetry was relatively low, with a median absolute difference of 4.2 percentile points (IQR: 1.8–8.9), indicating generally symmetric involvement. Similarly, lobar structures showed low asymmetry, with median absolute differences ranging from 3.6 to 6.8 percentile points. Thalamic asymmetry was also low (median: 3.4, IQR: 1.2–7.2).

These findings suggest that in this cohort, volumetric atrophy patterns are predominantly symmetric, with no evidence of systematic lateralization. Occasional participants showed larger asymmetries (> 15 percentile points), but these were not consistently associated with any particular structure.

Table 7. Interhemispheric Asymmetry in Volumetric Percentiles

Structure	Median Absolute Difference (L–R)	IQR	Range (Min–Max)
Hippocampus	4.2	1.8–8.9	0.1–28.6
Frontal Lobe	5.6	2.4–11.2	0.1–34.2
Parietal Lobe	4.8	1.9–10.1	0.1–29.8
Temporal Lobe	5.1	2.2–10.8	0.1–32.4
Occipital Lobe	6.8	2.8–13.4	0.1–38.6
Thalamus	3.4	1.2–7.2	0.1–22.4
Lateral Ventricle	7.2	3.1–14.8	0.1–41.2

3.8. Cluster classification model for Cluster Membership

To identify the minimal set of volumetric features that could accurately replicate full 42-region cluster assignment using a parsimonious feature set, we performed a multinomial logistic regression with elastic net regularization. It is important to note

that this model predicts cluster membership as derived from the unsupervised analysis of the same dataset; it does not constitute a prospective prognostic model for cognitive outcomes. External validation in independent cohorts is necessary before clinical deployment. The final model, presented in Table 8, achieved excellent internal classification accuracy.

Using only four volumetric features (left hippocampus percentile, left temporal percentile, left ventricle percentile, and right frontal percentile), the model achieved:

- Overall accuracy: 87.2% (68/78 participants correctly classified)
- Sensitivity for Cluster 1: 88.9% (24/27)
- Sensitivity for Cluster 2: 87.1% (27/31)
- Sensitivity for Cluster 3: 85.0% (17/20)
- Cross-validated AUC: 0.91 (95% CI: 0.85–0.96)

Table 8. Multinomial Logistic Regression Model for Cluster Membership Prediction

Predictor	Cluster 2 vs. Cluster 1		Cluster 3 vs. Cluster 1	
	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Left Hippocampus Percentile	0.89 (0.84–0.94)	< 0.001	0.76 (0.68–0.84)	< 0.001
Left Temporal Percentile	0.92 (0.87–0.97)	0.002	0.81 (0.74–0.89)	< 0.001
Left Ventricle Percentile	1.08 (1.03–1.14)	0.003	1.18 (1.09–1.28)	< 0.001
Right Frontal Percentile	0.94 (0.89–0.99)	0.018	0.85 (0.78–0.93)	< 0.001

Model Performance: Overall accuracy = 87.2%, Cross-validated AUC = 0.91 (95% CI: 0.85–0.96). CI, confidence interval.

4. Discussion

Authors This study provides a comprehensive characterization of cerebral volumetric profiles in a cohort of 78 individuals with cognitive impairment, using automated brain volumetry and unsupervised machine learning to identify distinct volumetric endophenotypes. Our findings demonstrate that: (1) there is substantial heterogeneity in regional atrophy patterns across individuals with similar clinical profiles; (2) unsupervised clustering reveals three distinct volumetric endophenotypes preserved, moderate atrophy, and severe atrophy that are primarily discriminated by hippocampal, temporal, and ventricular volumes; (3) interhemispheric asymmetry is minimal across most structures, suggesting symmetric neurodegenerative processes; and (4) a parsimonious set of four volumetric features (left hippocampus, left temporal, left ventricle, and right frontal percentiles) can accurately predict cluster membership with 87.2% accuracy.

4.1. The Heterogeneity of Brain Atrophy in Cognitive Impairment

One of the central findings of this study is the substantial inter-individual variability in regional brain volumes, even within a cohort selected for cognitive impairment. The range of hippocampal percentiles spanned from 0.1 to 99.8, indicating that some participants with cognitive impairment had preserved hippocampal volumes while others exhibited severe atrophy [19]. This heterogeneity challenges the notion of a single, uniform neurodegenerative trajectory and supports the concept of multiple biological pathways leading to cognitive decline [20].

The wide variability observed across all brain regions is consistent with the emerging literature on biological subtypes of dementia. Previous studies have demonstrated that Alzheimer's disease, the most common cause of dementia, can present with distinct patterns of atrophy hippocampal-sparing, typical, and limbic-predominant variants each associated with different clinical presentations and rates of progression

[21, 22]. Our clustering analysis identified three distinct volumetric endophenotypes that may correspond to these biological subtypes, though clinical characterization will be necessary to confirm such associations. Specifically, Cluster 1 (Preserved) may represent individuals with very early-stage neurodegeneration, non-neurodegenerative cognitive impairment (such as psychiatric or metabolic aetiology), or slow-progressing disease. Cluster 2 (Moderate Atrophy), with hippocampal-temporal predominance, is most consistent with the typical Alzheimer's disease pattern. Cluster 3 (Severe Atrophy) likely represents advanced multi-domain neurodegeneration, potentially corresponding to the limbic-predominant or typical AD subtypes with additional white matter burden, or mixed-aetiology dementia. Formal etiological characterization using amyloid and tau biomarkers will be essential to validate these assignments.

4.2. Hippocampus as the Primary Discriminator of Volumetric Profiles

Across all analyses, hippocampal volumes emerged as the most discriminative feature between clusters and showed the strongest correlations with other brain regions. This finding aligns with the well-established role of the hippocampus in memory function and its vulnerability to neurodegenerative pathology, particularly in Alzheimer's disease [23, 24].

The very strong correlation between left and right hippocampal volumes ($\varrho = 0.89$) indicates that hippocampal atrophy tends to be symmetric in this cohort. This symmetry is consistent with the pattern observed in typical Alzheimer's disease, where bilateral hippocampal involvement is the norm [25]. However, the presence of participants with asymmetric hippocampal volumes (absolute differences up to 28.6 percentile points) suggests that a subset of individuals may have atypical patterns that could represent distinct etiologies, such as frontotemporal dementia or vascular pathology [26].

The strong correlation between hippocampal and temporal lobe volumes ($\varrho = 0.74$) is expected given the anatomical continuity of these structures. The entorhinal cortex, which serves as the primary input-output gateway to the hippocampus, is itself part of the medial temporal lobe and is similarly vulnerable to early neurodegenerative changes [27]. This anatomical and pathological linkage explains why hippocampal and temporal volumes are so tightly coupled in our data.

4.3. Frontal and Parietal Involvement Across Severity Levels

Frontal lobe volumes showed a progressive decline across clusters, with Cluster 3 (severe atrophy) demonstrating median frontal percentiles below the 5th percentile. This finding is consistent with the well-documented involvement of frontal regions in advanced dementia, where executive dysfunction, apathy, and behavioral disturbances become increasingly prominent [28].

Parietal lobe volumes, while also reduced in severe atrophy, showed a somewhat less pronounced decline compared to frontal and temporal regions. This pattern is consistent with the topography of neurodegeneration in typical Alzheimer's disease, where temporoparietal involvement is characteristic but may be less severe than hippocampal and temporal pathology in early-to-moderate stages [29].

Occipital lobe volumes showed the weakest discriminative ability between clusters and the smallest effect sizes in ANOVA comparisons ($\eta^2 = 0.23–0.24$). This relative preservation of visual cortex is consistent with the clinical observation that primary visual functions are typically spared until very late stages of most dementia syndromes [30]. The occipital lobe is primarily affected in posterior cortical atrophy (a variant of Alzheimer's disease) and in dementia with Lewy bodies, but these represent a minority of cases [31].

4.4. Ventricular Enlargement as a Marker of Parenchymal Loss

Lateral ventricular volumes demonstrated a strong inverse correlation with hippocampal and temporal volumes, and ventricular percentiles were markedly elevated in Cluster 3 (median > 90th percentile). Ventricular enlargement is a well-established indirect marker of cerebral atrophy, reflecting the compensatory expansion of the ventricular system following loss of adjacent parenchyma [32].

The strong discriminative ability of ventricular volumes (η^2 = 0.57–0.58) suggests that ventricular measurements may serve as useful surrogate markers of disease severity when direct parenchymal measurements are not available. This is particularly relevant in clinical settings where automated volumetry may not be routinely available, and qualitative assessment of ventricular size on standard clinical MRI can provide valuable information [33].

4.5. Subcortical and Brainstem Involvement

Thalamic volumes showed moderate discriminative ability between clusters (η^2 = 0.44–0.45), with significant reductions in Cluster 3. The thalamus is a critical relay station for cortical-subcortical connectivity, and thalamic involvement has been documented in multiple dementia syndromes, including Alzheimer's disease, vascular dementia, and frontotemporal dementia [34, 35]. The observed thalamic atrophy in severe cases likely reflects both direct neurodegenerative pathology and secondary degeneration following cortical deafferentation.

Brainstem structures (midbrain and pons) showed relatively preserved volumes even in Cluster 3, though significant differences were still observed across clusters. This pattern is consistent with the notion that brainstem pathology is typically less severe than cortical pathology in most dementia syndromes, except in conditions such as progressive supranuclear palsy or multiple system atrophy, where brainstem involvement is a defining feature [36].

4.6. Clinical Implications of Volumetric Endophenotypes

The identification of three distinct volumetric endophenotypes has several potential clinical implications. First, the presence of a "preserved" cluster (Cluster 1) within a cohort of individuals with cognitive impairment suggests that some patients with clinical cognitive deficits may have minimal structural brain changes on volumetric analysis. This subgroup may represent individuals with mild cognitive impairment due to causes other than neurodegenerative disease, such as psychiatric conditions, metabolic disturbances, or early-stage disease where structural changes have not yet become apparent [37].

Second, the "moderate atrophy" cluster (Cluster 2) likely represents individuals in the intermediate stages of neurodegenerative disease, where structural changes are evident but not yet severe. This subgroup may be most amenable to disease-modifying interventions, as there remains substantial brain tissue that could potentially be preserved [38].

Third, the "severe atrophy" cluster (Cluster 3) represents individuals with advanced structural brain damage, where therapeutic efforts may need to focus on symptomatic management and supportive care rather than disease modification [39].

The high accuracy of our predictive model (87.2%) using only four volumetric features suggests that parsimonious volumetric assessment focusing on hippocampus, temporal lobe, frontal lobe, and ventricles may be sufficient for clinical risk stratification. This has practical implications for resource-limited settings where comprehensive 42-region volumetry may not be feasible.

4.7. Comparison with Previous Studies

Our findings are consistent with previous studies that have used unsupervised machine learning to identify subtypes in neurodegenerative diseases. Sano et al. applied k-means clustering to multidimensional sensor and behavioural data to identify distinct phenotypes in Alzheimer's disease and related dementias, demonstrating that unsupervised approaches can reveal clinically meaningful subgroups that transcend traditional diagnostic categories [15]. Similarly, our approach applied to structural volumetric MRI identified three clusters that align with the expected spectrum of disease severity, while extending prior work by grounding endophenotypes in quantitative neuroimaging rather than behavioural signal alone.

The strong discriminative role of hippocampal volumes in our study aligns with extensive literature establishing the hippocampus as a key biomarker in Alzheimer's disease [23, 24]. The effect sizes observed for hippocampal volumes (η^2 = 0.63–0.64) are

among the largest reported in volumetric clustering studies, underscoring the central role of hippocampal integrity in cognitive impairment.

The symmetric nature of atrophy observed in our cohort is consistent with previous reports that typical Alzheimer's disease is characterized by relatively symmetric involvement [25]. However, the presence of some asymmetry in our data highlights the importance of considering individual variability and the possibility of atypical presentations.

4.8. Methodological Considerations

The use of automated volumetry with mdbrain software (version 2.2) provided standardized, reproducible measurements across all participants. The software's reference cohort (n = 6,371, age range 10–97 years) allowed for age- and sex-adjusted percentile calculations, which is essential for distinguishing pathological atrophy from normal age-related changes [40-41].

The U-Net deep learning architecture employed by mdbrain represents a state-of-the-art approach to medical image segmentation [42]. The ability of convolutional neural networks to learn hierarchical features from imaging data enables accurate segmentation even in the presence of anatomical variability and pathology. It should be noted, however, that while the mdbrain reference cohort spans ages 10–97 years, cognitive impairment predominantly affects individuals aged 65 years and above. The normative percentile estimates for elderly participants are based on a subset of the full reference cohort, and future validation of age-band-specific normative accuracy in cognitively impaired populations would strengthen the foundation of the percentile-based approach used here.

Our clustering methodology, combining multiple validation metrics (elbow method, silhouette analysis, Calinski-Harabasz index, Davies-Bouldin index), provided robust support for the three-cluster solution. The consistency across these different metrics increases confidence that the identified clusters represent genuine structure in the data rather than artifacts of the clustering algorithm.

4.9. Limitations of the Study

Several limitations of this study should be acknowledged.

First, we identified three distinct volumetric clusters, we cannot definitively state that these clusters correspond to mild, moderate, and severe cognitive impairment without clinical validation. Future studies with concurrent cognitive assessments are needed to establish the clinical relevance of these volumetric endophenotypes [43].

Second, this study did not include a control group of healthy individuals without cognitive impairment. The inclusion of such a control group would have allowed for direct comparison of volumetric percentiles between cognitively impaired and healthy populations, enabling the determination of what constitutes "abnormal" atrophy at the individual level [44].

Third, the cross-sectional design of this study limits our ability to make inferences about disease progression. While we identified three clusters that likely represent different stages of disease severity, we cannot determine whether individuals progress from Cluster 1 to Cluster 2 to Cluster 3 over time. Longitudinal studies with repeated volumetric assessments are necessary to characterize trajectories of volumetric change [45].

Fourth, the sample size (n = 78) is relatively modest for clustering analyses, which typically benefit from larger sample sizes to ensure stable cluster solutions. Although our cluster validation metrics indicated good separation and stability, replication in larger, independent cohorts is necessary to confirm the generalizability of our findings.

Fifth, the lack of detailed clinical characterization, including dementia subtype diagnosis (e.g., Alzheimer's disease, frontotemporal dementia, vascular dementia, dementia with Lewy bodies), limits our ability to link specific volumetric patterns to underlying etiologies. Different dementia subtypes have distinct atrophy signatures, and future studies should incorporate detailed diagnostic information to refine cluster interpretation [46].

Sixth, the study cohort was predominantly female (61.5%), which may limit generalizability to male populations. Sex differences in brain structure and dementia risk are well-documented, and future studies should ensure balanced representation to enable sex-specific analyses [47].

Seventh, while the mdbrain software provides robust automated segmentation, the performance of deep learning segmentation algorithms can vary across different scanner types, field strengths, and acquisition protocols. All MRI scans in this study were acquired on a 1.5 or 3.0 Tesla scanner with a standardized protocol, but external validation on data from different scanners would strengthen the generalizability of our findings.

Eighth, our clustering analysis used percentile data, which are normalized for age and sex. While this normalization is advantageous for comparing individuals across demographic groups, it may obscure age-related changes that are themselves biologically meaningful. Future studies could explore clustering using absolute volumes to capture different aspects of brain structure. Ninth, the absence of biomarker confirmation (amyloid PET, tau PET, CSF amyloid-β/tau) means that cluster assignments cannot be directly linked to specific underlying neuropathologies. The A/T/N framework recommends biomarker-confirmed diagnostic categorization, and future work should integrate such data to anchor volumetric endophenotypes to their biological substrates [49]. Tenth, the multinomial logistic regression model predicts cluster membership as derived internally from the same dataset; it is not a prospective prognostic model for cognitive outcomes, and its clinical utility is contingent on external validation demonstrating that cluster assignment carries genuine predictive meaning for individual patient trajectories.

4.10. Future Directions for Research

The findings of this study suggest several promising directions for future research.

First, prospective longitudinal studies with repeated volumetric assessments are needed to characterize the trajectories of volumetric change across the identified clusters. Such studies would determine whether the clusters represent stable subgroups or stages of a single progressive process, and would enable the identification of predictors of rapid decline [48].

Second, integration of multimodal biomarkers including cerebrospinal fluid biomarkers (amyloid-β, tau), neuroimaging biomarkers (amyloid PET, tau PET), and genetic markers (APOE ε4 status) with volumetric data could refine cluster definitions and provide mechanistic insights into the biological processes driving different atrophy patterns [49].

Third, the incorporation of clinical data, including cognitive assessments, behavioral symptoms (BPSD), and functional status, would establish the clinical relevance of volumetric clusters. Understanding how volumetric profiles relate to real-world outcomes such as quality of life, caregiver burden, and institutionalization—would inform clinical decision-making [50].

Fourth, development and validation of prediction models for individual-level outcomes, such as risk of rapid progression or response to specific treatments, represents a critical next step. Machine learning approaches that integrate volumetric data with clinical, genetic, and biomarker information could enable personalized risk stratification [51].

Fifth, application of advanced machine learning techniques, such as deep learning on raw imaging data or longitudinal trajectory modeling, could reveal more nuanced patterns of brain change beyond the regional volumetric features examined here. Techniques such as convolutional neural networks applied to whole-brain images may capture subtle patterns of atrophy not reflected in regional summaries [52].

Sixth, validation of our findings in independent, diverse cohorts is essential. Replication in cohorts with different demographic compositions, dementia subtypes, and

healthcare settings would establish the generalizability of the three-cluster solution and its clinical utility.

Seventh, investigation of the biological mechanisms underlying different volumetric endophenotypes should be pursued. Post-mortem neuropathological correlation studies could determine whether the clusters correspond to distinct underlying pathologies, such as Alzheimer's disease pathology, vascular pathology, or mixed pathologies [53].

Eighth, exploration of the utility of volumetric clustering for clinical trial enrichment represents an important translational application. Stratifying patients by volumetric profile could enable more efficient clinical trials by identifying subgroups most likely to respond to specific interventions or those at highest risk of progression [54].

Conclusions

This study demonstrates that automated brain volumetry, combined with unsupervised machine learning, can identify distinct volumetric endophenotypes in individuals with cognitive impairment. Analysis of 78 participants revealed three clusters preserved, moderate atrophy, and severe atrophy that are primarily discriminated by hippocampal, temporal, and ventricular volumes. The strong discriminative ability of hippocampal volumes ($\eta^2 = 0.64$) underscores the central role of this structure in cognitive impairment, while the substantial inter-individual variability across all brain regions highlights the heterogeneity of neurodegenerative processes.

The finding of three distinct volumetric profiles suggests that cognitive impairment may manifest through different patterns of structural brain involvement, with potential implications for prognosis and treatment. The high accuracy (87.2%) of a parsimonious predictive model using only four volumetric features suggests that focused volumetric assessment may be sufficient for clinical risk stratification.

Prospective longitudinal investigations, integration with multimodal biomarkers, and validation in independent cohorts are essential next steps.

In conclusion, automated volumetry with machine learning analysis represents a powerful approach to characterizing brain structure in cognitive impairment, enabling the identification of biologically meaningful subgroups that may inform prognosis and guide personalized care. As the field moves toward precision medicine in dementia, such data-driven phenotyping approaches will become increasingly important for stratifying patients, enriching clinical trials, and ultimately improving outcomes for individuals living with cognitive impairment.

Author Contributions: Conceptualization, A.V. and A.C.; methodology, A.V. and A.C.; software, L.R.B.; validation, R.C., A.C. and R.V.B; formal analysis, L.C.D.C.; investigation, R.S.C.; resources, R.S.; data curation, R.O.; writing—original draft preparation, A.C. and A.C.; writing—review and editing, A.P., A.C. and C.A.C.; visualization, A.V; supervision, A.C.; project administration, L.L.P. All authors have read and agreed to the published version of the manuscript..

Funding: This research received no external funding.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Clinical Rehabilitation Hospital Iasi 22/16 November 2022 and Research Ethics Committee of U.M.F. "Gr. T. Popa" no. 265/1 February 2023.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. Dementia. 2023. Available online: https://www.who.int/news-room/fact-sheets/detail/dementia (accessed on 20 February 2026).
2. GBD 2019 Dementia Forecasting Collaborators. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the Global Burden of Disease Study 2019. Lancet Public Health2022, 7, e105–e125. https://doi.org/10.1016/S2468-2667(21)00249-8
3. Cerejeira, J.; Lagarto, L.; Mukaetova-Ladinska, E.B. Behavioral and psychological symptoms of dementia. Frontiers in Neurology 2012, 3, 73. https://doi.org/10.3389/fneur.2012.00073
4. Kales, H.C.; Gitlin, L.N.; Lyketsos, C.G. Assessment and management of behavioral and psychological symptoms of dementia. BMJ 2015, 350, h369. https://doi.org/10.1136/bmj.h369
5. Feast, A.; Moniz-Cook, E.; Stoner, C.; Charlesworth, G.; Orrell, M. A systematic review of the relationship between be-havioral and psychological symptoms (BPSD) and caregiver well-being. International Psychogeriatrics2016, 28, 1761–1774. https://doi.org/10.1017/S1041610216000922
6. Wimo, A.; Seeher, K.; Cataldi, R.; et al. The worldwide costs of dementia in 2019. Alzheimer's & Dementia 2023, 19, 2865–2873. https://doi.org/10.1002/alz.12901
7. Lanctôt, K.L.; Amatniek, J.; Ancoli-Israel, S.; et al. Neuropsychiatric signs and symptoms of Alzheimer's disease: New treatment paradigms. Alzheimer's & Dementia: Translational Research & Clinical Interventions 2017, 3, 440–449. https://doi.org/10.1016/j.tri.2017.07.001
8. Theleritis, C.; Siarkos, K.; Katirtzoglou, E.; Politis, A. A systematic review of non-pharmacological treatments for apathy in dementia. International Journal of Geriatric Psychiatry 2018, 33, e177–e192. https://doi.org/10.1002/gps.4786
9. Wen, J.; Thibeau-Sutre, E.; Diaz-Melo, M.; et al. Convolutional neural networks for classification of Alzheimer's disease: Overview and reproducible evaluation. Medical Image Analysis 2020, 63, 101694. https://doi.org/10.1016/j.media.2020.101694
10. Topol, E.J. High-performance medicine: The convergence of human and artificial intelligence. Nature Medicine2019, 25, 44–56. https://doi.org/10.1038/s41591-018-0300-7
11. Qiu, S.; Joshi, P.S.; Miller, M.I.; et al. Development and validation of an interpretable deep learning framework for Alz-heimer's disease classification. Brain 2020, 143, 1920–1933. https://doi.org/10.1093/brain/awaa137
12. Deardorff, W.J.; Grossberg, G.T. Behavioral and psychological symptoms in Alzheimer's dementia and vascular demen-tia. In Handbook of Clinical Neurology; 2019; Volume 165, pp. 5–32. https://doi.org/10.1016/B978-0-444-64012-3.00002-3
13. Fischer, C.E.; Ismail, Z.; Youakim, J.M.; et al. Validation of the Neuropsychiatric Inventory Questionnaire (NPI-Q) in the nursing home setting. Journal of the American Medical Directors Association 2021, 22, 1300–1304.e2. https://doi.org/10.1016/j.jamda.2020.09.026
14. Ienca, M.; Fabrice, J.; Elger, B.; et al. Intelligent assistive technology for Alzheimer's disease and other dementias: A sys-tematic review. Journal of Alzheimer's Disease 2017, 56, 1301–1340. https://doi.org/10.3233/JAD-161037
15. Sano, M.; Zhu, C.W.; Yang, T.; et al. Unsupervised machine learning identifies behavioral phenotypes of Alzheimer's disease and related dementias. Alzheimer's & Dementia: Translational Research & Clinical Interventions 2022, 8, e12329. https://doi.org/10.1002/trc2.12329
16. Davoudi, A.; Azimi, I.; Rahmani, A.M.; Liljeberg, P. A predictive model for aggressive behavior in dementia using environmental and physiological sensors. IEEE Transactions on Biomedical Circuits and Systems 2022, 16, 272–283. https://doi.org/10.1109/TBCAS.2022.3164782
17. Insel, T.R.; Cuthbert, B.N. Brain disorders? Precisely. Science 2015, 348, 499–500. https://doi.org/10.1126/science.aab2358
18. Bejenariu, A.; Ciobotaru, A.; Gireada, B.; et al. The Artificial Intelligence Revolution in Behavioral and Psychological Symptoms of Dementia: A Comprehensive Narrative Review on Digital Phenotyping and Predictive Analytics. Balneo and PRM Research Journal 2026, 17(1), 980.
19. Jack, C.R.; Bennett, D.A.; Blennow, K.; et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14:535–562. doi:10.1016/j.jalz.2018.02.018.
20. Ferreira, D.; Nordberg, A.; Westman, E. Biological subtypes of Alzheimer disease: A systematic review and meta-analysis. Neurology. 2020;94:436–448. doi:10.1212/WNL.0000000000009058.
21. Murray, M.E.; Graff-Radford, N.R.; Ross, O.A.; et al. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol. 2011;10:785–796. doi:10.1016/S1474-4422(11)70156-9.
22. Whitwell, J.L.; Dickson, D.W.; Murray, M.E.; et al. Neuroimaging correlates of pathologically defined subtypes of Alzheimer's disease: a case-control study. Lancet Neurol. 2012;11:868–877. doi:10.1016/S1474-4422(12)70200-4.
23. Jack, C.R.; Petersen, R.C.; Xu, Y.C.; et al. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease. Neurology. 1997;49:786–794. doi:10.1212/wnl.49.3.786.

24. Frisoni, G.B.; Fox, N.C.; Jack, C.R.; Scheltens, P.; Thompson, P.M. The clinical use of structural MRI in Alzheimer disease. Nat. Rev. Neurol. 2010;6:67–77. doi:10.1038/nrneurol.2009.215.
25. Barnes, J.; Scahill, R.I.; Schott, J.M.; et al. Does Alzheimer's disease affect hippocampal asymmetry? Neurology. 2005;64:1724–1729. doi:10.1212/01.WNL.0000161918.74655.4F.
26. Shi, F.; Liu, B.; Zhou, Y.; et al. Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. Hippocampus. 2009;19:1055–1064. doi:10.1002/hipo.20573.
27. Braak, H.; Braak, E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82:239–259. doi:10.1007/BF00308809.
28. Salmon, E.; Lekeu, F.; Garraux, G.; et al. Metabolic correlates of apathy in Alzheimer's disease. Neurology. 2006;67:856–860. doi:10.1212/01.wnl.0000233225.46958.b5.
29. Nestor, P.J.; Fryer, T.D.; Smielewski, P.; Hodges, J.R. Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. Ann. Neurol. 2003;54:343–351. doi:10.1002/ana.10659.
30. Crutch, S.J.; Lehmann, M.; Schott, J.M.; et al. Posterior cortical atrophy. Lancet Neurol. 2012;11:170–178. doi:10.1016/S1474-4422(11)70289-7.
31. Ossenkoppele, R.; Jansen, W.J.; Rabinovici, G.D.; et al. Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. JAMA. 2015;313:1939–1949. doi:10.1001/jama.2015.4669.
32. Nestor, S.M.; Rupsingh, R.; Borrie, M.; et al. Ventricular enlargement as a possible measure of Alzheimer's disease pro-gression validated using the Alzheimer's disease neuroimaging initiative database. Brain. 2008;131:2443–2454. doi:10.1093/brain/awn146.
33. Carmichael, O.T.; Kuller, L.H.; Lopez, O.L.; et al. Ventricular volume and dementia progression in the Cardiovascular Health Study. Neurobiol. Aging. 2007;28:389–397. doi:10.1016/j.neurobiolaging.2006.01.006.
34. de Jong, L.W.; van der Hiele, K.; Veer, I.M.; et al. Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. Brain. 2008;131:3277–3285. doi:10.1093/brain/awn278.
35. Zarei, M.; Patenaude, B.; Damoiseaux, J.; et al. Combining shape and connectivity analysis: an MRI study of thalamic degeneration in Alzheimer's disease. Neuroimage. 2010;49:1–8. doi:10.1016/j.neuroimage.2009.09.001.
36. Jellinger, K.A. Neuropathology of atypical parkinsonism. J. Neural Transm. 2002;109:1033–1055. doi:10.1007/s007020200087.
37. Petersen, R.C. Mild cognitive impairment as a diagnostic entity. J. Intern. Med. 2004;256:183–194. doi:10.1111/j.1365-2796.2004.01388.x.
38. Sperling, R.A.; Aisen, P.S.; Beckett, L.A.; et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:280–292. doi:10.1016/j.jalz.2011.03.003.
39. Livingston, G.; Huntley, J.; Sommerlad, A.; et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396:413–446. doi:10.1016/S0140-6736(20)30367-6.
40. Sano, M.; Zhu, C.W.; Yang, T.; et al. Unsupervised machine learning identifies behavioral phenotypes of Alzheimer's disease and related dementias. Alzheimers Dement. (N Y). 2022;8:e12329. doi:10.1002/trc2.12329.
41. Mediaire GmbH. mdbrain Software User Manual, Version 2.2. Berlin, Germany; 2024.
42. Ronneberger, O.; Fischer, P.; Brox, T. U-Net: Convolutional Networks for Biomedical Image Segmentation. In: *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015*. Springer International Publishing; 2015:234–241. doi:10.1007/978-3-319-24574-4_28.
43. Beach, T.G.; Monsell, S.E.; Phillips, L.E.; Kukull, W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. J. Neuropathol. Exp. Neurol. 2012;71:266–273. doi:10.1097/NEN.0b013e31824b211b.
44. Schwarz, C.G.; Gunter, J.L.; Wiste, H.J.; et al. A large-scale comparison of cortical thickness and volume methods for measuring Alzheimer's disease severity. Neuroimage Clin. 2016;11:802–812. doi:10.1016/j.nicl.2016.05.017.
45. Jack, C.R.; Shiung, M.M.; Weigand, S.D.; et al. Brain atrophy rates predict subsequent clinical conversion in normal el-derly and amnestic MCI. Neurology. 2005;65:1227–1231. doi:10.1212/01.wnl.0000180958.22678.91.
46. Harper, L.; Barkhof, F.; Fox, N.C.; Schott, J.M. Using visual rating to diagnose dementia: a critical evaluation of MRI at-rophy scales. J. Neurol. Neurosurg. Psychiatry. 2015;86:1225–1233. doi:10.1136/jnnp-2014-310090.
47. Ferretti, M.T.; Iulita, M.F.; Cavedo, E.; et al. Sex differences in Alzheimer disease — the gateway to precision medicine. Nat. Rev. Neurol. 2018;14:457–469. doi:10.1038/s41582-018-0032-9.
48. Jack, C.R.; Knopman, D.S.; Jagust, W.J.; et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013;12:207–216. doi:10.1016/S1474-4422(12)70291-0.
49. Jack, C.R.; Bennett, D.A.; Blennow, K.; et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87:539–547. doi:10.1212/WNL.0000000000002923.
50. Kales, H.C.; Gitlin, L.N.; Lyketsos, C.G. Assessment and management of behavioral and psychological symptoms of dementia. BMJ. 2015;350:h369. doi:10.1136/bmj.h369.

51. Vieira, S.; Pinaya, W.H.L.; Mechelli, A. Using deep learning to investigate the neuroimaging correlates of psychiatric and neurological disorders. Neurosci. Biobehav. Rev. 2017;74:58–75. doi:10.1016/j.neubiorev.2017.01.002.
52. Litjens, G.; Kooi, T.; Bejnordi, B.E.; et al. A survey on deep learning in medical image analysis. Med. Image Anal. 2017;42:60–88. doi:10.1016/j.media.2017.07.005.
53. Schneider, J.A.; Arvanitakis, Z.; Leurgans, S.E.; Bennett, D.A. The neuropathology of probable Alzheimer disease and mild cognitive impairment. Ann. Neurol. 2009;66:200–208. doi:10.1002/ana.21706.
54. Cummings, J.; Lee, G.; Zhong, K.; et al. Alzheimer's disease drug development pipeline: 2021. Alzheimers Dement. (N Y). 2021;7:e12179. doi:10.1002/trc2.12179.