# The Biochemical Biomarkers Determination in Alzheimer Dementia

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*Alzheimer's disease (AD) is a complex neuropsychiatric condition characterized by progressive cognitive symptoms.The social and psychological costs associated with the care of AD patients are very high; therefore, significant funds for research in this field are being invested worldwide. The accuracy of medical methods for establishing the diagnosis of AD with specific neuropathology is very important. Biomarkers can detect AD in their pre-clinical stage, monitor the disease progression, and detectmore objectively the treatment response. We performed a retrospective study of 100 patients admitted to the Gheorghe Preda Psychiatric Hospital of Sibiu with the diagnosis of Mild Cognitive Impairment and Alzheimer's Dementia. The diagnosis was established according to the Diagnostic and Statistical Manual Disorders-4th edition criteria and the severity of the condition was determined by the Mini Mental State Examination. Our retrospective study revealed that in a certain period of time, most hospitalized patients with AD had the most days of hospitalization; they were patients in severe stage, due to the needs of multidisciplinary care and the multiple possibilities offered by medical staff. At present, AD research, in addition to finding therapeutic remedies in the clinical stages of AD, also aims at primary and secondary prevention strategies, including the detection of biomarkers in the pre-clinical stage of AD.*

*Keywords:Alzheimer's disease, diagnosis, amyloidplaques, protein*

Alzheimer's disease (AD) is a complex neuropsychiatric condition characterized by progressive cognitive symptoms and a very well-studied neuropathology consisting mainly in amyloid plaques and neurofibrillary tangles [1].

The accuracy of clinical methods for establishing the diagnosis of AD with specific neuropathology is rather low, with a sensitivity of 71 to 80% and a specificity between 44 and 70% (according to the histopathological criteria used) [2].

For a more objective diagnosis, in the case of AD, we have several biomarkers: magnetic resonance imaging (MRI), Fluoro-deoxy-glucose positron emission tomography (FDG-PET) and proteins in cerebrospinal fluid (CSF) [3]. MRI and PET do not directly measure the neuropathological indicators of the disease, so the most valid measurements remain those of Amyloid $\beta_{42}$ protein ($A\beta_{42}$),phosphorylated tau protein (p-tau) and total tau protein (t-tau) in CSF [4].

Biomarkers can detect AD in their pre-clinical stage, monitor the disease progression, detect treatment response more objectively [5].

According to WHO statistics, in the period 2000-2010, the number of deaths due to AD increased by 68%, while other diseases, including cardiovascular diseases and cancer decreased [6]. The social and psychological costs associated with the care of AD patients are very high; it is expected that in the United States, it will exceed $ 1 trillion by 2050 [7],therefore, significant funds for research in this field are being invested worldwide.

*Pathophysiology of Alzheimer Dementia*

Amyloid plaques are composed of amyloid fibrils with a surrounding of dystrophic neurites and activated astrocytes and microglia. The main protein of amyloid plaques is the Aβ peptide. Aβ is generated from Aβ precursor protein (APP) when a β secretase cleaves to form c99, a membrane-bound C-terminal fragment [8]. Furthermore, c99 is cleaved by γ- secretase and generates Aβ 40 and Aβ 42 [9]. The aggregation of Aβ peptides is enhanced by excess free amount of Zn, Fe, Cu, Al.

An imbalance between the production and clearance or degradation or clearance of Aβ in the brain [10] is the initiating event in AD. Aβ peptides are promising candidate biological markers because they may exist in solution and can be detected in CSF and plasma [11]. Further reactive oxygen species (ROS) and inflammation promotes loss of synaptic integrity, neuronal death and progressive neurodegeneration [12].

Neurofibrillary tangles are formed by the hyper phosphorylation and aggregation of tau protein of numerous serine and threonine residues [13]. The tau protein is a constituent of neuronal microtubules which are cell structures responsible for the motility of proteins and organelles within the neuron [14]. Abnormal tau hyperphosphorylation deposits in AD are observed within neurons, NFTs, or dystrophic neurites present in neuritic plaques [15]. Because of aberrant phosphorylation, tau protein cannot bind and stabilize the microtubules, which is likely to induce axonal [16].

There was no correlation between the amount of amyloid plaque and the level of dementia. Certain patients

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who were diagnosed with AD did not show amyloid plaques, whereas in cognitively healthy individuals, amyloid plaques were not identified at autopsy. At the same time, the existence of the amyloid plaque in patients presenting preclinical AD can be excluded. Amyloid plaque formation is believed to mark the event of generating more toxic and soluble forms of Aβ. T-tau is a good predictive marker to make a difference between stable *Mild cognitive impairment* (MCI) and MCI that converts to AD with a sensitivity of 90% and 100% specificity. The cognitive decline in MCI patients correlates with P-tau concentrations in CSF [17]. Also, the increased level of T-tau in CSF also reflects the severity of dementia [18].

*Evolution of assessing biomarkers in Alzheimer disease*

Because CSF is in a contact with cerebral tissues, then the pathological brain changes can be reflected as a source of biomarkers [19]. The term *biomarker* was described as any neurochemical indicator that is used to diagnose Alzheimer's disease in the clinical and pre-clinical disease stages [20]. $A\beta_{42}$and t-tau have been considered as diagnostic biomarkers as follows: T-tau levels are elevated and $A\beta_{42}$ levels decreased in CSF of AD patients [21]. Decreased $A\beta_{42}$ levels in AD are believed to be caused by deposition of Aβ42 in senile plaque [22].

These biomarkers were measured using the ELISA technique, including a T-tau protein assay that measures all of tau protein isoforms [23], respectively of the phosphorylated tau protein at the level of *threonine*-181 [24] and the 42-amino acid forms of Aβ [25].

The first trials that examined CSF Aβ level focused on the total Aβ in CSF and did not distinguish between the Aβ isoforms. Some studies have shown a slight decrease in total Aβ [26] or have not detected any change in the total Aâ level in AD [27]. There are two main variants of Aâ: the shorter version presenting Val-40 (Aβ40) at the C-terminus end and the longer variant having Ala-42 (Aβ42) at the C-terminus end. Of these, Aβ42 was found to aggregate much faster than Aβ40, being the predominant form found in the amyloid plaques [28].

The first study assessing CSF A beta 42 levels showed significantly lower levels in AD patients relative to controls. Because A beta 42 is preferentially deposited in brain tissue of patients with AD, it is suggested that this reduction is due to the diminished clearance; in addition, tau levels were increased in AD patients. Neither A beta 42 nor tau levels were apparently influenced by the ApoE genotype [29].

Subsequently most studies have confirmed these findings [30]. Increased CSF Aβ42 levels in mild AD and its decline has been registered with the disease progression [31] and with brain atrophy [32].

The first study of the T-tau protein in the CSF as a biomarker for AD revealed an increased marked level of this one in AD [33]. At least 30 phosphorylation sites of tau protein have been identified. Several studies showing a marked increase in CSF P-tau targeted phosphorylation of several epitopes, such as threonine 181 + 231 [34], threonine 181 [35], threonine 231 + serine 235, serine 199 [36], threonine 231 [37].

In a study evaluating the combination of CSF P-tau 181 and Aβ42, the sensitivity was 86% and the specificity was 97% [38] in another combination, T-tau and P-tau 396/404 had a sensitivity of 96 and 100% specificity [39].

The T-tau protein in CSF reflects the neuronal and axonal degeneration, so the higher values are recorded in stokes, i.e. Creutzfeld-Jacob disease [40].

In order to differentiate between AD and other forms of dementia, the p-tau /Aβ42 or t-tau/Aβ42 ratio is used, with a sensitivity of up to 92% and a specificity of up to 86%. The ratio between Aβ42 and p-tau is significantly lower in AD patients compared to those with vascular dementia [41].

According to the report issued by the *Working Group on Biomarkers in AD*, demonstrating the validity of a biomarker if the sensitivity and specificity are greater than 80%, this study showed that the discrimination between AD and vascular dementia can be made based on the ratio between A42 and p-tau(20). This ratio also helps distinguishing between AD and FTD, PD, but it is not as clear in the case of *dementia* with Lewy bodies and vascular dementia [42].

Another utility of the mentioned biomarkers is to predict the conversion of MCI to AD. Thus, low Aβ42 and increased t-tau or p-tau have also shown their accuracy in the distinction between normal aging and AD (> 85%) and have a good predictive value in predicting MCI conversion to AD (> 90%) [43]. Another role of biomarkers may be to establish thedynamics of NFT development in the preclinical stage. Thus, p-tau growth at S262 or S181 appears early in NFT development, and S396 level rise occurs in advanced stages [44].

*Links between the biomarkers and clinical features in AD*

In 1984, NINCDS-ADRDA criteria have been issued presenting the criteria for Probable AD dementia, Possible AD dementia, Probable or possible AD dementia with evidence of the AD pathophysiological process [45]. These criteria aimed at the dementia syndrome containing the classical clinical features of AD as well as the exclusion of other causes of non-degenerative dementia.

Regarding the Possible AD dementia with evidence of the AD pathophysiological process, the review of the clinical criteria took into account the measurement of the following biomarkers: Aβ42 low in the CSF, increased tau in the CSF (tau and p-tau), MRI showing cortical atrophy in the medial, basal, temporal lateral and medial parietal cortex, PET showing the decreased capture of fluoro-deoxy-glucose in the temporo-parietal cortex [46].

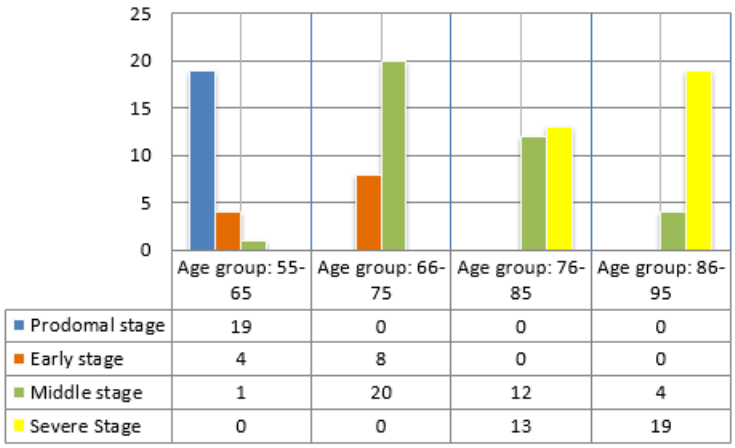
The guideline recommended by The National Institute on Aging and Alzheimer's Association (NIA-AA) makes a very important assumption about the preclinical stages of AD. In stage 1, asymptomatic amyloidosis is evident by increased PET Aβ ligand binding and low CSF $Ab_{1-42}$ levels. Stage 2 includes neurodegeneration evidenced by neuronal dysfunction based on neuroimaging (PET, MRI) high CSF tau or p-tau concentrations cortical thinning and hippocampal atrophy (MRI). Stage 3 is evidenced by amyloidosis, neurodegeneration, and subtle cognitive decline as evidenced by mild change from baseline cognitive function that does not meet criteria for MCI [47].

## Experimental part

We performed a retrospective study of 100 patients admitted to the Gheorghe Preda Psychiatric Hospital of Sibiu with the diagnosis of Mild Cognitive Impairment and Alzheimer's Dementia. The diagnosis was established according to the Diagnostic and Statistical Manual Disorders, 4$^{th}$ edition (DSM IV) criteria and the severity of the condition was determined by the Mini Mental State Examination (MMSE). Of the total of 100 patients studied, 52% were males and 48% were females, 54% of the patients were rural and 46% were urban.

## Results and discussions

It can be seen that of the majority of patients in the age group of 55-65 years, most patients are in the prodromal




| | Age group: 55-65 | Age group: 66-75 | Age group: 76-85 | Age group: 86-95 |
|---|---|---|---|---|
| Prodomal stage | 19 | 0 | 0 | 0 |
| Early stage | 4 | 8 | 0 | 0 |
| Middle stage | 1 | 20 | 12 | 4 |
| Severe Stage | 0 | 0 | 13 | 19 |

Fig.1. Patients' repartition according to age and stage of disease

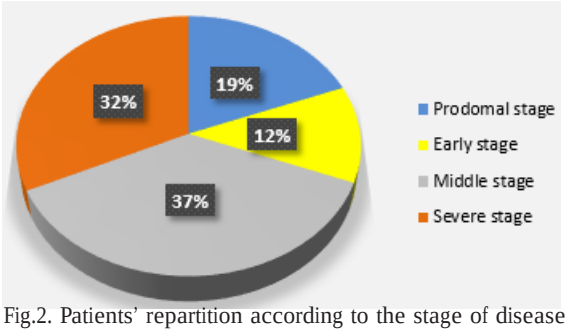



Fig.2. Patients' repartition according to the stage of disease

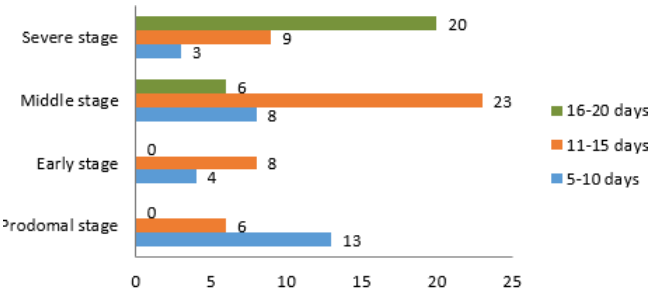



Fig.3. Patients' distribution according to the number of hospitalization days and stage of disease

phase (MCI); In the age group of 66-75 years, most patients are in the middle stage; In the 76-85 age group, most of them are in the middle to severe stage and in the age group of 86-85 years, most patients are in a severe stage.

It can be seen that out of a total of 100 patients, most are in the middle (37%) and severe (32%) stages.

Our study reveals that there are an increased number of patients with moderate and severe Alzheimer's Dementia, and also an increased number of days of hospitalization in these patients.

Moderate dementia is associated with loss of ability to perform daily activities and requiring patient placement in a care center, which requires very high costs for society. In this respect, much research focused on one hand, on finding biomarkers to reveal the conversion of MCI to AD and the progression of mild dementia into moderate dementia.

In mild cognitive impairment, a cognitive disorder that is considered an intermediary stage between normal aging and AD, values of the biomarkers in CSF detectable in AD Aβ42, tau and p-tau were found at intermediate levels between the values found in AD cases and in control cases [48].

Overlapping values were found in patients with MCI versus those with AD, two thirds of those with MCI having the same biomarker profile as AD patients, one third of them having normal values [49].

The increase in protein t-tau is very high in disorders with intense neurodegeneration, as in Creutzfeldt-Jakob disease. However, most cross-sectional studies do not describe any association between tau protein levels and the stage of dementia. However, most longitudinal studies have focused on the distinction between AD and control cases or cases with MCI, based on changes in tau protein.

In a study aiming at the relationship between tau protein levels in CSF and the time the person gets in a care center (NHP), it has been highlighted that elevated levels of tau protein (> 900 ng / L) have been associated with the increased rate of conversion to moderate dementia, both from the preclinical stage and from the MCI stage [50] (fig. 4).

This study reveals an association between the rapid decline of the disease and the high levels of tau protein in the CSF, more than in the case of Aβ42 or p-tau, which could indicate in the future, its selection as a predictive marker for an aggressive neurodegeneration.

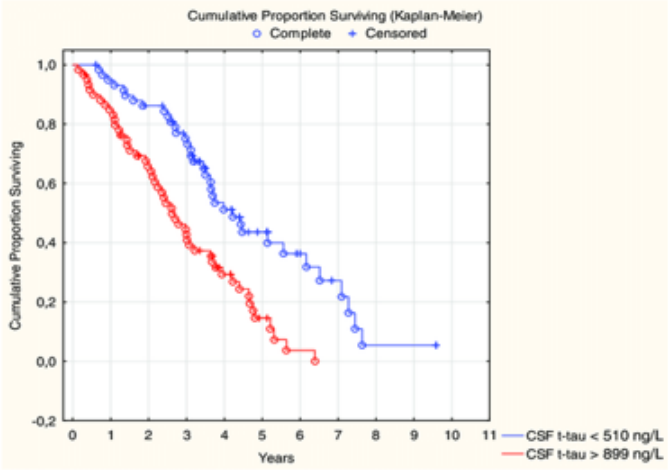



Fig. 4. CSF t-tau associated with the highest risk of being institutionalized [50]

## Conclusions

Although Aβ42, tau and p-tau measurements in the CSF can be performed in the clinical setting in the United States and some European countries, still, they are not part of the clinical criteria of AD according to DSM IV. Other diagnostic systems such as NINCDS- ADRDA and NIA-AA included biomarkers measurement in both AD and MCI diagnosis and of the preclinical stage. The area of biomarkers research now extends to their identification and measurement in plasma as well, which can facilitate even more the investigation of the biomarkers.

The medical objectives in the AD are to detect the disease at an early stage, both from the point of view of clinical symptoms and with the use of scientifically

validated biomarkers. Our retrospective study revealed that in a certain period of time, of the patients hospitalized with AD, most patients and who had the most days of hospitalization were patients in severe stage, due to the needs of multidisciplinary care and the multiple possibilities offered by medical staff [51]. So that, visual impairments, hearing loss, osteoarthritis [52], clinical conditions like urinary tract infections, pneumonia, or heart failure or sentinel events (e.g., eating difficulties, recurrent infections, hip fracture, stroke) influence severly the quality of life and prognostication [53].

At present, AD research, in addition to finding therapeutic remedies in the clinical stages of AD, also aims at primary and secondary prevention strategies, including the detection of biomarkers in the pre-clinical stage of AD.

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