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The meaning of blood and cerebrospinal fluid biomarkers in early diagnosis of Alzheimer's disease

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1. Abstract

Introduction and purpose: Alzheimer's disease (AD) belongs to the group of neurodegenerative diseases and is the leading cause of dementia worldwide. Its development

includes the impact of genetic, metabolic and environmental factors. Despite high prevalence of Alzheimer's disease and dynamic development of medical science, there is currently no clinically proven causal treatment. The proposed therapies are only symptomatic. However, there is a wide set of substances known as biomarkers that are detected in the blood or in the cerebrospinal fluid (CSF) in the stages preceding full-blown Alzheimer's disease.
Brief description of the state of knowledge: In order to obtain material for CSF examination, a lumbar puncture must be performed. It is an invasive procedure, with the risk of complications such as bleeding into the spinal cord, infection or even nerve damage. Obtaining a blood sample for testing specific indicators is less invasive and more widely available. Currently, the diagnostic significance of commonly known markers arising in the progress of the AD pathological process, such as amyloid β, tau protein and its phosphorylated form or β-secretase, is being investigated. As the knowledge on the pathogenesis of AD grows, further markers such as ubiquitin, micro RNA or plasma neurofilament light are tested.
Conclusions: There is a collection of biomarkers that can perform a diagnostic function for Alzheimer's disease. Due to the advantages of blood and plasma testing, basing the diagnosis of early forms of AD on these tests seems to be particularly interesting. However, the use of biomarkers on a global scale requires further research and test standardization, as well as the development of guidelines for their interpretation.

Key words: Alzheimer's disease; blood biomarkers; cerebrospinal fluid biomarkers; dementia

2. Introduction and purpose

The aim of the study is to describe the issues related to the diagnosis of Alzheimer's disease and to present the possibilities of improving the diagnostic process using biomarkers specific for this disease. To achieve this objective the literature available in Pubmed database was reviewed.

2.1. Epidemiology

Dementia is a condition primarily caused by Alzheimer's disease, vascular dementia (VaD) and dementia with Lewy bodies.[1] It leads to the weakening of the acquisition and remembering new information, impaired cognitive and behavioral abilities. Unlike mild cognitive impairment (MCI), dementia causes significant functional disorders in both everyday life and work.[2] In 2009, Alzheimer's Disease International (ADI), based on 154 publications, estimated that in 2030 as many as 66 million people will suffer from dementia, and in 2050 respectively 115 million. However, the data updated in 2013 indicate that the estimated number of patients will increase by 15% in 2030 and 17% in 2050, which corresponds to 75.63 million in 2030 and 135.46 million in 2050.[3] As a result of this trendency, growing number of people suffering from dementia generates increasing socio-economic costs. In 2010, approximately US$ 604 million was allocated to the treatment and care of patients worldwide, which is comparable to 1% of the GPD at that time.[4]

Of the many neurodegenerative diseases, Alzheimer's disease is the main cause of dementia worldwide.[5] It is estimated that AD may be responsible for approximately 50% - 75% of diagnosed cases.[4] The prevalence of disease in the population of people over 65 years of age ranges from 10 to 30% and the incidence of AD doubles every 10 years from 60 years of age.[6] The average duration of the disease ranges from 8 to 10 years.[7] It is estimated that over 95% of AD cases are associated with the sporadic form of the disease, with the onset occurring in the 7-8th decade of life. The early-onset genetic form of AD accounts for <1% of all cases.[7]

2.2. Pathogenesis

Already in 1906, psychiatrist and neuropathologist Aloysius Alzheimer, during the autopsy of his patient's brain, linked the presence of atypical deposits with symptoms such as progressive memory loss or confusion.[6] It is already known, that the pathology he observed was associated with excessive deposition of improperly folded amyloid-β (Aβ). The insoluble Aβ forms are deposited as plaques in the extracellular space and in the walls of blood vessels. It is formed as a result of the proteolytic degradation of amyloid precursor protein (APP), which occurs with the participation of enzymes from the γ-secretases and βs-ecretases group. The components of these enzymes include the proteins presenilin 1 (PS1; encoded by PSEN1) and presenilin 2 (PS2; encoded by PSEN2).[7] Mutations in the APP, PSEN1 and PSEN2 genes have been linked to the pathogenesis of the autosomal dominant form of AD.[8] An important role in the pathogenesis of AD is also assigned to the microtubule-associated protein tau (MAPT). It is responsible, inter alia, for regulating the process of microtubule formation and stabilization. Total tau (T-tau) in CSF is increased during the process of excessive neuronal degeneration that occurs in AD, Creutzfeldt–Jakob syndrome, frontotemporal dementia (FTD), and stroke. Whereas the level of the phosphorylated form of tau (P-tau) in the CSF during the diagnosis of AD reaches an average sensitivity of 78% and specificity of 92%.[9]

The polymorphism related to gene encoding apolipoprotein E (APOE) contributes significantly to the development of AD.[10] According to the conducted studies, one APOE4 allele increases the risk of developing AD threefold, while with two alleles the risk increases twelve times.[7] The APOE2 allele, on the other hand, has a protective effect, which results in a delay in the development of disease symptoms and reduced Aβ plaque deposition.[11] However, many studies conducted over the past decades has shown that the pathogenesis of AD is much more complex and its multifactoral nature is the subject of many studies.[12]

3. Description of the state of knowledge

3.1. Cerebrospinal fluid biomarkers

The main function of the tau protein is to maintain microtubule stability and regulation of intercellular trafficking. However there are neurological conditions which are characterised by disorders in tau protein functioning which leads to neurofibrillary tangles pathology (NFTs).[13] The concentration of tau protein in Creutzfeld–Jakob disease is about 10 – 50 times higher compared to the level in patients with AD.[14] Nonetheless AD is the most frequent tauopathy. Pathological changes in the structure of tau protein in brain may be observed years before the oneset of symptoms which proves relevance of clinical research on tau proteins.[13] The use of various methods such as the Enzyme-Linked Immunosorbent Assay (ELISA) method, polyclonal antibodies and monoclonal antibodies allowed detect a significant increase in tau protein in the cerebrospinal fluid of AD patients.[15,16] Test based on monoclonal antibodies revealed an increase in the level of tau protein by approximately 200-300% in comparison with people without dementia. Tau protein tests have differed AD patients and non-demented patients with 81% sensitivity and with a specificity of 91%.[15] Research has shown assorted ways of tau protein dysfunction development e.g. post-translational alterations, cytoskeletal disorders and damage of protein degradation mechanisms.[13] Deposition of tau aggregates begins in entorhinal cortex and hippocampus then spreads to other regions.[14,17] Posttranslational alterations include hyperphosphorylation, acetylation, N-glycosylation and truncation. This processes interfere tau–microtubule binding and promote impaired tau folding. Therapies targeting in one of this modifications have potential to prevent tau protein aggregations and to normalize its functioning which may withhold disease development.[13] Tau protein phosphorylation affects its tubule binding ability and interfere microtubule synthesis promotion.[18] Hypherphosphorylation comprises one of the first disorders in AD development.[13] P–tau 181 and P–tau 231 are hyperphosphorylated forms of tau protein, which could be detected in cerebrospinal fluid.[19] It has been shown that the specificity of P-tau is

higher than that of total tau (T-tau). The correct level of P–tau in the cerebrospinal fluid is observed in psychiatric (depression) as well as neurological diseases e.g. amyotrophic lateral sclerosis.[20] The other frequent disorders which affects AD patients is the higher level of acetylation. It leads to neurodegeneration in many ways as well as tau pathology – acetylation inducts tau cleavage.[13]

β-amyloid is the most common protein in AD pathology. It comes from the cleavage of proteolytic β-amyloid precursor protein (APP) which is excreted to cerebrospinal fluid. No alterations in total level of Aβ in AD patients comparing to patients without dementia, were detected in ELISA method.[15] Nonetheless further research have provided significant information about two isoforms: Aβ40 and Aβ42 which is dominant and the more toxic one.[20] There were used various methods such as ELISA or Western blot to affirm the lower level of Aβ42 in AD patients' cerebrospinal fluid. It is presumably caused by deposition of Aβ in senile plaques. Autopsy studies showed a significant correlation between the decrease of Aβ42 in the cerebrospinal fluid and an increased amount of senile plaques in the neocortex and hippocampus. Examination of the Aβ42 level in the cerebrospinal fluid allows for the differentiation of AD from the physiological aging process with a sensitivity of 86% and a specificity of 89%.[15] The normal level of Aβ42 in the cerebrospinal fluid is observed in patients with schizophrenia, depression, and in patients with progressive supranuclear palsy, and slightly decreased in patients with frontotemporal dementia and vascular dementia.[20] However, no changes in the level of Aβ40 are observed. The consequence of this phenomenon is an increase in the Aβ42 / Aβ40 ratio or a decrease in Aβ40 / Aβ42.[15] Increasing the sensitivity of the ELISA assay for Aβ40 and Aβ42 enables the detection and quantification of Aβ in human blood.

The obtained results of tests of total Aβ concentration in plasma in patients with AD in comparison with the control group do not allow the use of this method of tests for differential diagnosis.[21,22] However, it has been shown that the observation of the dynamics of changes in the plasma levels of Aβ40 and Aβ42 may allow the determination of the risk of Alzheimer's disease development. Total Aβ and Aβ42 levels are elevated in the preclinical-asymptomatic phase[22,23], while the concentration of Aβ40 does not increase.[23] As the disease progresses and the formation of amyloid deposits, the Aβ42 level often decreases to normal, parallel to the decline in the Aβ42 level in the cerebrospinal fluid. This makes the Aβ42/Aβ40 index a potential biomarker for the selective deposition of Aβ42 in amyloid plaques that could be used to determine the early risk of MCI conversion to Alzheimer's disease.[23]

Beta-secretase 1 (BACE1) is a transmembrane aspartyl protease encoded by a gene on chromosome 21q22. It is one of the two key enzymes involved in the proteolysis of the AβPP protein, the result of which is the formation of the β-amyloid protein.[20] This pathway is widely regarded as the primary pathogenic mechanism in Alzheimer's disease.[24] According to studies, elevated levels of BACE1 in the CSF are noticeable in the later stages of sporadic Alzheimer's disease. This is due to the association of the BACE1 level with the level of amyloidogenesis and the intensity of axon degradation.[20] Determining the concentration of β-secretase in the CSF is possible thanks to the use of ELISA and Western-Blot tests, however, the examination of the level of BACE1 activity deserves special attention, which in the future may turn out to be a particularly valuable parameter in the diagnosis of Alzheimer's disease and other dementia diseases. It is suspected that the hyperactivity and the correlating increased BACE1 levels are due to the overproduction of β-secretase by over-stimulated neurons/glial cells, especially in the early stages, and a subsequent effect combined with gradual neurodegradation.[25] Studies on substances targeted at blocking β-secretase activity show that it plays an important role in the pathogenesis of the disease, and BACE1 inhibitors have a therapeutic potential in the treatment of AD.[26] Additionally, noticing a sudden and significant increase in the level of β-secretase in the cerebrospinal fluid in people with mild

cognitive impairment may indicate a predisposition to transition to Alzheimer's disease.[27] The studies of different centers show discrepancies, on the one hand they show a decrease in β-secretase activity in patients with MCI evolved into Alzheimer's disease, and on the other hand, they show increased β-secretase activity in patients with AD and those suffering from Creutzfeldt-Jackob disease.[28] Usefulness, sensitivity and specificity in the case of this biomarker require more detailed research, undoubtedly it looks promising, but its accuracy has still not been clearly determined, and the method itself entails high costs. The solution to the problem is the standardization of the antibodies involved in the research.[25]

Ubiquitin is a small molecule protein that increases in levels in people with AD. The increase in the level of ubiquitin in patients with Alzheimer's disease correlates with the level of neurofibrillary changes. It is most effectively detected in patients' CSF.[15] Ubiquitin-proteasome system (UPS), which includes ubiquitin, helps maintain overall protostasis in eukaryotic cells and is responsible for short-term protein degradation. This system is involved in numerous cellular mechanisms, in the brain cells its most important function is participation in the synaptic function. Ubiquitin pathologies in UPS can lead to neurodegenerative diseases based on the accumulation of toxic proteins. Research shows that an increased amount of ubiquitinated elements is directly related to AD. Like previous biomarkers, ELISA is used to detect it in CSF, but it is not a standard test, and its diagnostic importance is negligible.[29]

3.2. Blood biomarkers

The detection of biomarkers in the blood is more problematic than in the CSF, due to the fact that only a small proportion of the protein which is located in the CSF passes into the bloodstream. Although blood measurement would be much more affordable, both because it would increase the availability of screening tests and allow for multiple sampling, this method is characterized by a lower sensitivity. Large amounts of proteins such as albumin and IgG bring a high risk of interference in analytical methods. In addition, brain-derived proteins that are released into the bloodstream can undergo a number of processes, including degradation by proteases, metabolism in the liver, and removal by the kidneys. The advantages of conducting diagnostic tests on blood-derived materials are the reason for carrying out numerous studies on the development of ultra-sensitive tests.[30]

Amyloid beta (Aβ) is a peptide of from 38 to 43 amino AIDS and is a major component of senilic plaques found in Alzheimer's disease.[31] The amyloid plaques are derived from a fragment of the Aβ precursos protein – APP. The transmembrane protein is encoded by the gene on chromosome 21, as a result of splicing alternative isoforms are being created, including APP 695 (consisting of 695 amino acid residues). The enzymatic fragmentation of APP by β-secretase and γ-secretase leads to the release of several forms of Aβ peptides. There are variants of the full length Aβ, which contain aspartic acid at position N and shortened forms that, according to a studies, play a role in the pathogenesis of Alzheimer's disease. Shortened fragments include Aβ11–42 and Aβ17–42, which are also present in amyloid plaque and preamyloid lesions in Down's syndrome.[32] The concentration of Aβ in the plasma depends on its secretion from platelets and other extra-brain tissues. Recent studies have demonstrated a very important causality between plasma Aβ concentration and cerebral β-amyloidosis as measured by mass spectrometry.[33] Despite the presence of APP throughout the organism, Aβ is produced mainly in the brain, which makes the cerebrospinal fluid appear suitable for its detection. However, it should be taken into account that CSF sampling is relatively invasive. Many studies indicate that Ab may be a promising marker in the early diagnosis of AD due to the fact that its level increases long before clinical manifestation of disease symptoms.[34] Taking into account high invasiveness of the above method, it would be more preferable to test the plasma for changes in Aβ level. However, according to the observations made by J.B. Toledo et al., the measurements of plasma Aβ40 and Aβ42 levels

do not show any significant value as a prognostic factor in Alzheimer's disease. The disadvantage of plasma testing is that, despite its low invasiveness, the Aβ measurements in it appear to be of limited value as a prognostic factor, in contrast to the CSF measurements.[35]

Plasma tau levels are significantly lower than CSF as the molecules do not enter the circulation due to the presence of the blood-brain barrier. In AD patients, levels of both T-tau and P-tau are higher than in healthy patients.[36] T-tau contains all six isoforms of tau regardless of the phosphorylation state, while P-tau has a post-translational modification at threonine 181.[30] The research of Fiandaca et al. from 2015 showed that increased levels of P-tau are detected in blood-borne exosomes that come from neurons. This concentration is measured by an immunochemical test in which exosomes are isolated from the serum, then washed and lysed. It has been shown that changes in the levels of P-S396-tau and P-T181-tau measured in exosomes by the above-mentioned method may precede the development of symptomatic Alzheimer's disease by up to 10 years.[37]

MicroRNA (miRNA) are non-coding RNA strand fragments that can be used as biomarkers for the diagnosis of damage to the central nervous system (CNS).[38] MiRNAs calibrate post-transcriptional gene expression, which is base-pairing with the target mRNA. Moreover, they are stable under unfavorable conditions, such as inappropriate pH or high temperature. Y. Zhang et al., in their meta-analysis demonstrated the effectiveness of miRNA as a biomarker in the diagnosis of people suffering from Alzheimer's disease. 770 AD patients and 664 controls were observed, the diagnostic tests proved to be heterogeneous, therefore a subgroup analysis was undertaken to clarify the discrepancy. Better miRNA results were observed in blood samples and in Caucasians, as evidenced by the achievement of the Area Under ROC Curve (AUC) respectively 0.92 and 0.93. According to this study, when comparing PET and CSF diagnosis, peripheral blood testing for miRNA is more economical and non-invasive. However, the overwhelming majority of studies assess the value of miRNAs as a biomarker for distinguishing between sick and healthy individuals, and the diagnostic value for early AD is unreliable, and requires further research. Currently, specific miRNA: miRNA-455-3p and miRNA-125b show favorable diagnostic targeting, which makes it possible to use single forms of miRNA in the early diagnosis of AD.[39]

According to the meta-analysis made by Y. Wu et al., there is a correlation between the level of serum lipids and the risk of developing Alzheimer's disease. Meta-analysis using the Comprehensive Meta-analysis (CMA) software showed an association between LDL-C, total cholesterol (TC) and the risk of AD. Blood samples from patients from Asia were used for the analysis.[40] LDL is a low-density lipoprotein that consists of proteins and lipids including cholesterol, phospholipids and triglycerides.[41] Increased levels of LDL-C and TC may affect the metabolism of amyloid precursor protein in neurons, and consequently the deposition of amyloid plaques is intensified, leading to the development of AD. During the above-mentioned study, an increased risk of AD was observed in people with increased levels of LDL-C and TC. Performing a lipid profile as a biomarker in the diagnosis of Alzheimer's disease may be of great benefit, considering imbalance in cholesterol homeostasis as predictive factor.[40]

BACE1-AS, a long non-coding RNA, is an antisense BACE1 transcript that significantly influences BACE1 mRNA expression, affirming the formation of Aβ.[42] S.N. Fotuhi et al. compared the plasma levels of BACE1-AS in AD patients and in healthy subjects, the diagnostic efficacy of BACE1-AS was assessed by real-time PCR and its intensity as a biomarker by analyzing the ROC curve. Low levels of BACE1-AS were observed in the state preceding the development of Alzheimer's disease, but high in AD patients compared to healthy people. The analysis of the ROC curve allowed for the distinction between pre-disease and healthy people, suffering from AD advancement from healthy people, and the group of pre-AD patients from the advanced stage of the disease. The usefulness of BACE1-

AS in plasma as a prognostic and diagnostic biomarker in Alzheimer's disease has been demonstrated.[43]

Plasma neurofilament light (NfL) is a structural protein found inside axons. At the moment of axon damage, NfL penetrates into body fluids. Elevated levels are detected not only in AD but also in other neurodegenerative diseases. This biomarker is elevated in both sporadic and familial AD. In the family form, elevated NfL levels can be observed even 10 years before the first symptoms of the disease. In sporadic form, the increase is observable later. During the aging process, there is also a physiological increase in NfL in CSF of about 3% per year.[44] The concentration of NfL in serum and plasma correlates with the concentration of NfL in CSF (the correlation coefficient ranges from 0.75 to 0.97). Most CSF measurements were reconstituted in blond.[33] Much more sensitive than analytical test standard ELISA or Meso Scale Diagnostics (MSD) determining NFL method has a single molecule array (Simoa).[30] Recent reports suggest that plasma NfL can be successfully used as a non-invasive marker reflecting neurodegeneration and treatment outcomes in patients with AD.[45]

lL-4 is one of the anti-inflammatory cytokines that is supposed to neutralize the pro-inflammatory effect by inhibiting the secretion of IL-1β, IL-6 and TNF-α by activated monocytes. It has been shown that IL-4 levels in blood is closely associated with cognitive decline in AD. There was a significant increase in blood levels of Il-4 in patients with rapid cognitive impairment.[46]

Heat shock protein 90 kDa (Hsp90) is one of the erythrocyte proteins. The physiological role of this cytoplasmic protein includes response to oxidative stress that arises in the cell as a result of disease progression or cell aging. It is involved in cell signaling pathways and, moreover, helps to remove misfolded proteins. A growing body of evidence suggests that Hsp90 inhibitors may be effective in the treatment of AD. Research indicates that the level of Hsp90 in AD patients is higher than in control groups, which may mean that Hsp90 may have the potential as a diagnostic biomarker in AD.[47]

4. Summary

Aβ is a promising biomarker emerging before the clinical manifestations of AD when examining the CSF, unfortunately it is a highly invasive method, while plasma testing for Aβ is of limited value as a prognostic factor. Plasma BACE1-AS testing is promising because it can differentiate healthy individuals from those in the preclinical stage of AD and from those with advanced symptomatic AD. In the early diagnosis of AD, a blood test for miRNA-455-3p and miRNA-125b levels shows diagnostic potential. P-tau and its specific variants seem to have a greater diagnostic importance than the level of T-tau, both when the blood sample and CSF are tested. The use of blood-derived biomarkers is advantageous for clinical use because blood tests are widely available, the collection is minimally invasive, and is economically viable. However, due to the specificity of Alzheimer's disease, the detection of markers in blood and plasma is difficult due to the fact that not all CSF proteins pass freely in unchanged form and concentration into the bloodstream. Properly early diagnosis of the disease with the use of the above-mentioned biomarkers makes it possible to quickly introduce both pharmacological and non-pharmacological therapy. This can be crucial in delaying the progression of the disease and alleviating its symptoms. However, in order to use the full diagnostic potential of both those tested in the blood and CSF markers, the methods of their measurements should be standardized and unified patterns of interpretation of their results should be established.

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