Biomarkers in the diagnosis of neurodegenerative diseases

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Abstract. Biomarkers are molecules that behave as of biological states. Ideally, they should have high sensitivity, specificity, and accuracy in reflecting the total disease burden. The review discusses the current status of biomarkers used in neurological disorders. Neurodegenerative diseases are a heterogeneous group of disorders characterized by progressive loss of structure and function of the central nervous system or peripheral nervous system. The review discusses the main biomarkers that have predictive value for describing clinical etiology, pathophysiology, and intervention strategies. Preciseness and reliability are one of important requirement for good biomarker. As a result of the analysis of literature data, it was revealed that beta-amyloid, total tau protein and its phosphorylated forms are the first biochemical biomarkers of neurodegenerative diseases measured in cerebrospinal fluid, but these markers are dependent upon invasive lumbar puncture and therefore it’s a cumbersome process for patients. Among the various biomarkers of neurodegenerative diseases, special attention is paid to miRNAs. MicroRNAs, important biomarkers in many disease states, including neurodegenerative disorders, make them promising candidates that may lead to identify new therapeutic targets. Conclusions. Biomarkers of neurological disease are present optimal amount in the cerebrospinal fluid but they are also present in blood at low levels. The data obtained reveal the predictive value of molecular diagnostics of neurodegenerative disorders and the need for its wider use.

Key words: biomarkers, neurodegeneration, amyloid, tau protein

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Introduction

A biomarker is an indicator molecule of a biological as well as pathological condition or pharmacological response to a therapeutic intervention. It can be a simple laboratory test or as complex as a pattern of genes or proteins. In real practical point of view, the biomarker would specifically and sensitively reflect a disease condition and could be used for diagnosis as well as for disease monitoring during and following therapy. Biomarkers with molecular approach can be divided into 3 broad categories [1]:

1. Biomarker that track disease progression over time and correlate with known clinical measures;
2. That detects the effect of a drug.
3. In clinical trials it behaves as surrogate endpoints.

Characteristics of a biomarker for neurologic disorders.

1. Biomarker should be minimally invasive or non-invasive and produces reproducible results.
2. There are thousands of biomarkers of various diseases including neurologic disorders, but not all of them have been validated.
3. Biomarkers in blood can provide early indicators of disease and help in understanding the pathomechanism of disease as well as determine prognosis.
4. Besides bestowing to diagnosis, biomarkers helps in the integration of diagnosis with therapy and are useful for monitoring the course of disease as well as response to treatment.
5. Some biomarkers are potential targets for discovering new drugs for neurologic disorders and are useful for clinical trials during drug development.

Historical background

Bence Jones protein in urine one of the first biomarker used in was used in mid-19th century. During the early 1960s the term «biomarker,» or biochemical biomarkers started showing its presence in the literature in connection with metabolites and biochemical abnormalities associated with several diseases. During the last decade of the 20th century, discovery of biomarkers was accelerated by mass spectrometry used for analysis of biological samples for biomarkers, applications of proteomics for molecular diagnostics, and disclosure of metabolomics for the study of biomarkers. In the blood the best known protein biomarkers are troponin (for myocardial infarction), carcinoembryonic antigen (CEA) for different types of cancer, aminotransferases such as ALT and AST (for liver diseases) and the prostate-specific antigen (PSA) for prostate cancer [2]. In year 2000 completion of sequencing of the human genome opened the way for discovery of gene biomarkers.

Since 2005, biomarkers play a major role in the field of biotechnology and biopharmaceutical industries. Now a day the term «molecular biomarkers» is commonly used to any molecular modification of a cell on DNA, RNA, and metabolite or protein level.

Of the thousands of biomarkers that have been discovered, most remain to be validated. A biomarker is valid if:

1) It can be measured in a test system with well-established performance characteristics.
2) Evidence for its clinical significance has been established.

Neurodegenerative diseases are mainly identified by progressive loss of cognitive function, dementia,
and problems with movements. It leads to the loss of structure or function of neurons, which might also, causes death of neurons [3—7]. Neurodegenerative diseases, such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Amyotrophic Lateral Sclerosis (ALS); Multiple Sclerosis (MS); Huntington’s disease (HD); MachadoJoseph disease; Amyloid Polyneuropathy. With extended life expectancy worldwide, neurological disorder increases in the coming years. Nowadays, patients are treated on the basis of symptoms, and currently no effective known drugs are available to reverse or stop the progression of the diseases. For early and predictive diagnosis of neurodegenerative diseases enormous efforts are under way [8—10]. Beta-amyloid (Aβ), total tau and its phosphorylated forms (p-tau) are the firsts biochemical biomarkers of neurodegenerative diseases measured in cerebrospinal fluid (CSF), but, these markers are dependent upon invasive lumbar puncture and therefore it’s a cumbersome process for patients [11—13], so there is a urgent need for new biomarkers in more easily accessible body fluids such as peripheral blood. Cortisol is the one of potential biomarker for neurodegenerative disease and also used for stress evaluation.

**Cortisol**

Cortisol is a steroid hormone that is mainly produced by the adrenal glands (cortex region), and by a complex network of neuroendocrine cascade (coordinated from the brain via a signaling system) known as the hypothalamic pituitary adrenal (HPA) axis. The HPA axis is a key player by which the brain can exert control over physiological activity, which it does in normal everyday activity and also in response to stress. Cortisol crosses the blood–brain barrier easily; owing to its lipophilic character [14]. Binding of cortisol receptors which are present on the most of the bodily cell to specific intracellular receptors which affects multiple and diverse systems, ranging from regulation of the metabolic, immune, cardiovascular and cognitive systems [15]. This important function makes cortisol a crucial hormone to protect overall health and well-being. When these receptors are activated bind to «hormone response elements» in the DNA and regulate the transcription of target genes [16].

Cortisol also increases blood pressure, blood sugar levels, and has an immunosuppressive action. Hydrocortisone (synthetic cortisol) as an antagonist used in the treatment of allergies and inflammation as well as substitute supplementation in cortisol production deficiencies. Cortisol is metabolized by the 11-beta hydroxysteroid dehydrogenase system (11-beta HSD). Any alteration in 11-beta HSD 1 has been suggested to play a pivotal role in the pathogenesis of obesity, hypertension, and insulin resistance which ultimately lead to osteoporosis, digestive problems, hormone imbalances, cancer, heart diseases and diabetes.

Apart from cortisol, changes in the levels and activities of neurotrophic factors have been observed, such as brain-derived neurotrophic factor (BDNF).

**Brain-Derived Neurotrophic Factor**

BDNF is the a secretory protein, dimeric growth factor present in most human tissues, including neurons where it helps to support their survival and encourage neuro- and synaptogenesis and it that help to build new brain cells and it keeps your existing brain cells strong [17]. BDNF belongs to the member of the neurotrophin family (growth factors) along with nerve growth factor (NGF); neurotrophins-3 (NT-3), NT4/5 and NT-6. It is synthesized in the endoplasmic reticulum (ER) as a 32—35 kDa precursor protein (pro BDNF) that then moves to the Golgi apparatus and trans-Golgi network (TGN) where pro-BDNF is sorted by vesicles and transported into post-synaptic dendrites. The terminal domain of pro-BDNF is cleaved by a distinct protein convertase enzyme to form 13 kDa biologically active mature BDNF (mBDNF) [18, 19].

BDNF is involved in the function and survival of cholinergic neurons in the basal forebrain [20, 21]. In whole blood, serum, or plasma have reported significantly lower BDNF levels in patients with major depression [22—23], schizophrenia [24], bipolar disorders [25], autism spectrum disorders or mild cognitive impairment (MCI) [26, 27]. It has been described in a number of other neurodegenerative
disorders, including Huntington disease, Alzheimer disease and Parkinson disease. BDNF is stored in platelets, and their concentration in plasma and serum may not be an accurate value because of platelet activation and degranulation. Moreover, most of the earlier studies have used conventional enzyme-linked immunosorbent assays (ELISAs) for the estimation of BDNF levels in CSF in neurodegeneration disorder and their concentrations found below the linear range of the assay, raising doubt as to the validity and accuracy of the reported disease differences [28, 29]. The normal range of mean plasma BDNF values was ~92.5 pg/ml (8.0—927.0 pg/ml). It was higher in women, and decreased with advancing age in both genders [30]. It is a physical reaction to the issues that confront us. Whilst our brain continues to perceive these situations as threats, stimulating an onslaught of biochemical reactions inside us, we have the ability to halt it.

**N-acetylaspartate**

N-acetylaspartate (NAA) level is a neuronal marker or biomarker of functional integrity and vitality in neurons, thus its concentration correlates with neuronal density and neuronal function [31—33]. NAA which is synthesized from aspartate and acetyl-coenzyme A in neurons play an important role in biochemical features of CNS metabolism. NAA has two primary roles, as a facilitator of energy metabolism in neuronal mitochondria [34] and a source of acetate for fatty acid and steroid synthesis necessary for axonal myelination by oligodendrocytes [35, 36]. NAA moves from neurons to the cytoplasm of oligodendrocytes, where apartoacylase (ASPA) cleaves the acetate moiety for the synthesis of fatty acid and steroid and this fatty acids and steroids acts as building blocks for myelin lipid synthesis. The fatal leukodystrophy Canavan disease is caused by mutation in the gene for ASPA, for which there is currently no effective treatment. Acetylation of l-aspartic acid leads to the formation of NAA, which is present in optimal concentrations (more than 10mM) in mammalian brain and particularly in neurons. It has role in the Neuronal osmolyte that is involved in fluid balance in the brain. Source of acetate for lipid and myelin synthesis in oligodendrocytes, the glial cells those myelinate neuronal axons.

Daily turnover of NAA is regulated through an intercompartmental cycle involving extracellular fluids among neurons, oligodendrocytes, and astrocytes [37]. Evidence suggests a continuous NAA efflux from neurons to blood circulation, and in physiological conditions, low serum NAA level relates to its rapid glomerular filtration in the kidneys [38, 39]. A slightly decreased NAA level in the brain is a normal part of aging, particularly in older men [40, 41]. In contrast, pathological decreases shows in the brain of patients with Alzheimer disease, Parkinson disease, and multiple sclerosis (MS) by in vivo proton (1 H)—magnetic resonance spectroscopy or by postmortem histopathological evidence [42—43]. Low NAA level was found in cortical brain regions of patients with ALS [44—47].

**Serum Amyloid P-Component**

Serum Amyloid P-Component (SAP, PTX2) is a member of the pentraxin family 25kDa homopentameric discoid arrangement of five non–covalently bound subunits glycoprotein first identified as the pentagonal constituent of in vivo pathological deposits called «amyloid» similar to C-reactive protein (PTX1), it is secreted by the liver and a found in plasma at a concentration of approximately 31 mg/L [48]. SAP is a highly conserved an acute phase protein molecule and it is a precursor of amyloid component P which is found in basement membrane and associated with amyloid deposits that may play an important role in innate immunity modulates immunologic responses, inhibits elastase, in human SAP and CRP share 66 % homology and the gene for SAP is located on chromosome number 1. One of the unique properties of SAP that it binds both to amyloid plaques and to tau tangles, stabilizing and protecting them against the body’s normal clearance mechanism for abnormal protein deposits. The structure of SAP (and CRP) pentamers is a flat disk with a hole in the middle [49, 50], containing two Ca²⁺ atoms bound to it, and the pentamer thus has 10 Ca²⁺ atoms on one
side of the disk. Ca\(^{++}\) helps in the binding of a variety of molecules including apoptotic debris, bacterial polysaccharides, amyloid deposits, and bacterial toxins [51, 52]. Phagocytic cells such as monocytes and macrophages then bind the SAP, CRP and engulf the debris or other material the pentraxin has bound [53]. Further very interesting results provides a research of amyloid precursor protein (APP).

**Beta Amyloid**

Beta Amyloid is a peptide of 36—43 amino acids produced by β- and γ-secretases of APP. In normal physiological condition, more than 90 % of Aβ is in the form of Aβ\(_{40}\) which is soluble while less than 5 % is generated as the longer form of Aβ\(_{42}\) which is insoluble; it appears to be the main constituent of amyloid plaques in the brains of Alzheimer’s disease patients. The most important isoforms are Aβ-40 and Aβ-42; the smaller form is produced by cleavage that takes place in the endoplasmic reticulum, while the longer form is produced by cleavage in the trans-Golgi network. Aβ-42 is the more fibrillogenic and therefore associated with disease states and thought to be especially toxic. Aβ aggregation is considered to be the primary reason for the neurotoxicity in the classic view, and Aβ oligomers are the most neurotoxic form [54].

Beta-amyloid is a small piece of a larger protein called «amyloid precursor protein» primarily present in central nervous system, but it is also expressed in peripheral tissues such as in circulating cells is a type 1 membrane glycoprotein that plays an important role in biological activities, including neuronal development, signaling, intracellular transport, and other aspects of neuronal homeostasis. APP consists of a single membrane-spanning domain, an extracellular glycosylated N-terminus is long and a shorter cytoplasmic C-terminus. It is one of three members of a larger gene family in humans that protein clump together to form plaques in the Alzheimer’s brain that collect between neurons and disrupt cell function. The APP isoforms can be detected in platelets membrane. The intact 150 kDa weight APP is divided into two forms after platelet activation [55]. The ratio of forms with molecular weight 120—130 kDa and of 110 kDa weight are called «platelet APP isoform ratio,» and in AD and mild cognitive impairment (MCI) its ratio decreases not in other dementias [56, 57]. The next candidate biomarker is galanine.

**Galanin**

Galanin is a 29- or 30-amino acid neuroendocrine peptide, isolated in 1983 Tatemoto with colleagues [58], found in both the central and peripheral nervous systems and shows a number of physiological effects, acting mainly as an inhibitory, hyper-polarizing neuromodulator by Merchenthaler et al (1993) [59]. The sequence of amino acid in galanin is highly conserved (almost 90 % among species), indicating the importance of the molecule, it has its N-terminal glycine and its C-terminal alanine. The N-terminal end of galanin is crucial for its biological activity and the first 15 amino acids are conserved in all species (the tuna fish being the exception; [60]). The C-terminus is believed to primarily serve as a protector against proteolytic attacks [61, 62]. Galanin has number of important biological role in the body, such as regulation of food intake, metabolism and reproduction, nociception, intestinal contraction and secretion, and in nervous system it response to injury. This type of action is controlled by several galanin receptor subtypes.GALR 1, GALR 2, and GALR 3; they are mostly expressed in gastric and intestinal smooth muscle cells, in the pancreas, and in the CNS [63]. In the CNS, galanin release several neurotransmitters. The ability of galanin to inhibit acetylcholine release together with the observation of hyper innervation of galanin fibers in the Alzheimer’s disease patients suggests a possible role for galanin in this disorder. Galanin is an inhibitory, hyperpolarizing neuropeptide that inhibits neurotransmitter release. The galanin receptor is often co-localized with classical neurotransmitters such as acetylcholine, dopamine, serotonin and norepinephrine and also with other neuromodulators such as Vasoactive Intestinal Peptide, Neuropeptide Y and Substance P. Besides proteins, microRNAs (miRNAs) have also demonstrated their potential as non-invasive biomarkers from blood and
serum for a wide variety of human pathologies [64]. In many disease states altered expression of miRNA, including neurodegeneration, and increasing relevance of miRNAs in biofluids in different pathologies has prompted the study of their possible application as neurodegenerative diseases biomarkers aim to identify new therapeutic targets.

Circulating miRNAs as biomarkers of nervous system diseases

During evolutionary process miRNAs remain conserved, and their expression may be constitutive or spatially and temporally regulated. Increasing efforts to identify the specific targets of miRNAs lead to speculate that miRNAs can regulate more than of human genes. Specific miRNA subsets were expressed in specific brain area and in neuronal and glial cell subtypes [65]. In the transcriptional networks of the human brain miRNAs play important roles, and in many pathological conditions changes in brain-specific miRNA expression occurs, depression and epilepsy among them. Recently, several groups have proposed the use of microRNAs (miRNAs) circulating in plasma or serum for ND detection [66—67]. miRNAs are small molecules (~22 nucleotides) that play important roles in gene regulation by binding to complementary regions of messenger transcripts and repressing their translation or regulating their degradation [68, 69]. Sequence complementarity analysis shows an individual miRNA can regulate more than 100 messenger RNAs (mRNAs), and an mRNA thus behaves as potential biomarkers for multiple cellular processes. Over 2000 miRNAs have been discovered in human cells to date, and many of these miRNAs are specific to or overexpressed in certain organs, tissues, and cells [70—87]. Some miRNAs, including those that are cell-specific, can be enriched in particular cellular compartments, such as neurites and synapses [88—94]. miRNAs can be secreted or excreted into the extracellular space [95—98] and are detectable in plasma and serum [99].

**Conclusion**

In the serum and plasma circulating miRNAs reveal stability and are able to cross the blood-brain barrier, thus provide great potential as non-invasive and quantitative biomarkers. To register the full importance of miRNA biomarkers for nervous system diseases, tools are required for the routine analysis of miRNAs from clinical samples.

In the coming years, the need of blood based new biomarker to support the diagnosis of different brain disorders and to help detect progression and response to therapies.

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в оптимальном количестве в спинномозговой жидкости, но также присутствуют в крови в небольших количествах. Полученные данные выявляют прогностическую ценность молекулярной диагностики нейродегенеративных расстройств и необходимость более широкого ее использования.

**Ключевые слова**: биомаркеры, нейродегенерация, амилоид, тау белок

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