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Biochemical markers of damage of the central nervous system in multiple sclerosis

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) characterised by multicentric inflammation and destruction of myelin. This is the most common cause of neurological disability in young adults. Approximately 85% of patients start the disease with a relapsing-remitting course, 80-90% of them enter a secondary progressive course within 20-25 years from the onset. The other 15% of patients have a progressive course from the onset, with or without overimposed relapses.

The primary cause of the disease is unknown. Currently, MS is considered an organ specific autoimmune disease orchestrated by autoreactive CD4 T cells, which cause a cell mediated immunological reaction. Activated autoreactive T cells selectively cross the blood brain barrier and on exposure to a putative autoantigen initiate a cell mediated inflammatory reaction. This results in a complex immunological cascade with T-cells, B-cells, macrophages and endothelial activation and the induction of cytokines and inflammatory mediators. The non-specific mediators such as reactive oxygen and nitrogen free radicals, proteases, pro-inflammatory cytokines and eicosanoids are all capable of damaging myelin and oligodendrocytes (12). Although it was formerly assumed that in the early period of the disease axons were spared from the destructive process, the recent study indicates that axonal injury in MS occurs not only in chronic MS lesions, but also in acute lesion in patients with relapsing-remitting MS. It may cause irreversible neurologic dysfunction. The mechanisms of axonal loss are uncertain, but may involve axonal degeneration secondary to demyelination, an action of inflammatory mediators and directed immune attack at axonal components (2, 14). The intensity of all this process and its resolution determine the severity and duration of the clinical symptoms – recovery after exacerbation or irreversible damage in the CNS and the increase of disability.

The clinical diagnosis of MS is given on the basis of clinical observations. The symptoms of multiple neurological lesions must be separated in space (i.e. they involve anatomically distinct regions), and time (i.e. they occur on two or more separate occasions). The third essential criterion is the exclusion of other conditions that may produce a similar clinical picture. Clinical observations are supported by other diagnostic tests: the presence of IgG oligoclonal bands in the cerebrospinal fluid (CSF) that are not found in serum, dissemination lesions in evoked potentials and dissemination, demyelination lesions in MRI. Very sensitive MRI technique showed disruption to the blood-brain-barrier (BBB), inflammatory process and demyelination plaques, but axonal loss is poorly showed. Only MRI spectroscopy with N-acetyl aspartate allow for examination of axonal damage during life and to conclude about recovery of disease. Unfortunately, MRI spectroscopy is a little available. This prompted to look for factors which might be used as markers of various pathology, especially axonal damage, during life.

Cerebrospinal fluid is a perfect material for investigating the relationships among various factors involved in different phases of the pathological process. Its analysis may give definite help in the different diagnoses, in the examined activity of the disease and in better focusing on therapeutic objectives and therapeutic tools. There are some interesting biochemical markers, which reflect the pathogenetic processes in the brain. There are normal intracellular proteins, which after cells damage leaked into CSF. The changes in levels of these proteins indicated the kind of pathology within CNS. As markers of various brain damage have been estimated so far: 1) myelin basic protein (MBP) – as a marker of white matter demyelination, 2) neuron specific enolase (NSE) – as a marker of neuronal damage, 3) tau protein, 14-3-3 protein, neurofilament protein-subunit light (NFL) – as markers of axonal damage, 4) S-100 protein, glial fibrillary acidic protein (GFAP) – as markers of astrogliosis or astroglial damage.

Human 14-3-3 protein is a highly conserved phosphoserine-binding protein that modulates interactions between components of signal transduction pathways. There is a normal intracellular protein expressed in neurons and glial cells. The 14-3-3 family consists of homo-and heterodimeric proteins in which 7 isoforms were found. As a consequence of extensive destruction of brain tissue, 14-3-3 is released into the CSF. The analysis of 14-3-3 protein in the CSF showed that the protein may be a highly specific marker for antemortem diagnosis CJD, when is used in the appropriate clinical context (8,13). An increase in the 14-3-3 level in the CSF was usually associated with acute neuronal damage.

Tau protein is an intracellular human brain phosphoprotein that binds to microtubules in the neuronal axons. Its normal function is to promote polymerisation and stability of microtubules. Tau plays an important role in the intraneuronal transport. In neurodegenerative disorders tau is the main component of neurofibrillary lesions, especially the case in Alzheimer disease. Neurodegenerative process leads to increased neuronal loss which may give rise to increased tau levels in the CSF. The tau level reflects the degree of neuronal/axonal damage and degeneration (1, 3). In MS the tau levels were significantly increased compared with controls both in RR-MS and SP-MS or PP-MS, but also were associated with the course of disease. In the CSF of patients with secondary or primary progressive MS, tau level was higher than in patients with RR-MS. In patients with RR-MS the high tau level was found in patients with exacerbations and depended on parameters of inflammatory process. That supports axonal damage in MS and indicates, that in early course of MS, the axonal pathology is related to inflammation and demyelination (5).

S 100 protein is an acidic calcium-binding protein consisting of a heterodimer of two isomeric subunits, alpha and beta, with molecular weight of 10.4 kDa and 10.5 kDa, respectively. S-100b (beta, beta-S-100) is present in glial cells, in addition, S-100a (alpha, alpha-S-100) is mainly found in glial cells except in Schwann cells. The protein serves as a marker of astroglial cells damage (4). In patients with MS only several percent had elevated CSF S-100 level, but if compared patients with different form of the disease, the higher level S-100 was found in patients with progressive form of MS and during clinical relapse (6, 10).

Neurofilament protein: The neurofilament is a major structural protein of axons, that maintains the axonal compartment and determines the axonal calibre. Neurofilament consists of the three subunits (heavy – 200 kD, NFH; medium – 150 kD, NFM; light – 68 kD; NFL). The light subunit of the neurofilament protein (NFL) is the essential component of the neurofilament core. The NFL in CSF is used as a marker of axonal damage (7). In the CSF of patients with RR-MS the level NFL was significantly increased compared with healthy controls. The high NFL concentrations were significantly associated with clinical exacerbations and low concentrations with clinically stable periods. Moreover, there were found correlations between the NFL concentration in CSF and clinical outcome – a disability of patients, exacerbation rate and time from the start of the previous exacerbation to the time of the

lumbar puncture. These results suggest that axonal damage occurs during RR-MS and it contributes to appearance of clinical outcome and the disability (9).

Myelin basic protein (MBP): Its concentration in cerebrospinal fluid is commonly used as a biochemical marker of demyelination in patients with multiple sclerosis. The concentration of myelin basic protein in CSF of patients with MS significantly correlated with acute exacerbations, with the Kurtzke EDSS scale and the inflammation parameters in the CSF, but the MBP value was not related with NSE and S-100 levels (6). MBP is a diagnostic indicator of myelin breakdown in the central nervous system and may be used as a marker of MS disease activity (10).

Neuron-specific enolase (NSE) is a glycolytic enzyme and a soluble protein of neurons mainly located in neuronal and neuroendocrine cell bodies, but also present in axons. NSE is a useful CSF marker of neuronal damage in acute neuronal diseases. In cross-sectional studies CSF and NSE, levels are generally not raised in patients with MS (6).

Glial fibrillary acidic protein (GFAP) is the major structural protein of the glial intermediate filament of astrocytes. Concentrations of CSF GFAP are increased in conditions associated with astrocytosis, neurodestruction and inflammation. In CSF of patients with MS, concentrations of GFAP are increased in a varying proportion (9-39%). In longitudinal study (24 months) the GFAP level increased over the study period and correlated with the clinical deficit scores (11).

None of these potential markers is specific, but when are used with the conventional diagnostic work-up and in the appropriate clinical context, they may be of diagnostic value. Markers of axonal damage, gliosis and demyelination need to be developed and investigated in well-designed longitudinal studies. The sampling of body fluids should be as frequent as practically possible. The most important are examinations of the markers of axonal degeneration, because they indicate irreversible damage. Patients with high levels of axonal damage markers should be treated more actively or longer. CSF examination and other diagnostic methods should not be considered as alternative tools, or in competition, but should be used together, to take the maximum advantage of their individual possibilities.

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SUMMARY

The examination of biochemical markers of the damage of the central nervous system may be the excellent complement of the neuroimaging methods. There are factors in the cerebrospinal fluid which indicate the damage of the precise structures of the CNS. In this paper there are described the main markers which are used in diagnostic multiple sclerosis: myelin basic protein (MBP) as a marker of demyelination of the white matter, neuron specific enolase (NSE) as a marker of neuronal damage, tau protein, 14-3-3 protein, neurofilament protein-subunit light (NFL) as markers of axonal damage and S-100 protein and glial fibrillary acidic protein as markers of astroglial damage.

Biochemiczne markery uszkodzenia ośrodkowego układu nerwowego w stwardnieniu rozsianym

Ocena biochemicznych markerów uszkodzenia struktur ośrodkowego układu nerwowego (OUN) może być doskonalym uzupełnieniem badań neuroobrazujących. Określono substancje, których wzrost lub pojawienie się w płynie mózgowo-rdzeniowym wskazuje na uszkodzenie określonych struktur OUN. W pracy przedstawiono główne markery używane w diagnostyce stwardnienia rozsianego - podstawowe białko mieliny jako marker demielinizacji, specyficzna neuronalna enolase jako marker uszkodzenia neuronów, białko tau, 14-3-3 oraz lekka podjednostka neurofilamentu jako markery uszkodzeń aksonalnych oraz białko S-100 i kwaśne włókienkowe białko glejowe jako markery uszkodzenia astrocytów. Omówiono zastosowanie tych badań u chorych na stwardnienie rozsiane.