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Cytological Analysis of CSF in Patients with Acute Schizophrenia

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Abstract - Cerebrospinal fluid (CSF) analysis is one of the most important tests in the diagnosis of central nervous system (CNS) diseases. Although CSF analysis is most commonly used in neurological pathological conditions, it also has its place in psychiatry. Studies to date have described several valuable specific cytomorphological phenomena in the cerebrospinal fluid of patients with acute schizophrenia, which indicate inflammatory or immune-mediated etiopathogenesis of the disease. Additional and long-term research is needed to confirm and standardize the importance of cytological analysis of cerebrospinal fluid in the diagnosis and etiopathogenesis of acute schizophrenia.

Key words: cerebrospinal fluid; cytology; schizophrenia

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Introduction

Cerebrospinal fluid (CSF) analysis is a method that has been applied for more than a hundred years, and has not diminished since then despite significant advances in the diagnosis of a number of conditions that have an impact on central nervous system (CNS) [1]. CSF analysis provides data on a number of acute and chronic inflammatory, dementia, oncological or hemorrhagic pathological processes in the brain that can not be obtained by available electrophysiological tests or brain imaging. The correct choice of method and interpretation of the results contribute to the diagnosis and treatment of various CNS diseases, which further emphasizes the need for the best possible CSF analysis [2-4]. Even today, cell number, total protein, and lactate concentration are the basis of any emergency CSF analysis.

Cytological analysis of the presence and number of leukocytes in the CSF effectively diagnoses a number of CNS diseases. The total and differential number of cells provides important first information about the state of the CNS and possible pathological disorders. Basic cytological examination of CSF includes counting and differentiation of CSF cells in the Fuchs-Rosenthal chamber and morphological analysis of cells. Normally, there are 0 - 5 mononuclear cells / μL in the CSF,

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and lymphocytes predominate in relation to monocytes, 70:30. CSF samples must be processed as soon as possible, within one hour of lumbar puncture, i.e. sampling. After centrifugation, the preparations are standardly stained according to May – Grünwald – Giemsa; additional staining is needed to identify microbiological agents such as bacteria or fungi. In case of tumor cells, immunocytochemical staining is applied [5].

In various neurological diseases, especially acute conditions, there is a change in the composition of the CSF, whether it is an increase in the total number of cells (pleocytosis), the emergence of new cell types (tumor cells, cells of tissue origin) or changes in functional cells (phagocytes, plasma cells).

In the case of pleocytosis, most cells are reactive cells of hematogenous origin, polymorphonuclear leukocytes (neutrophilic, eosinophilic and basophilic granulocytes/mast cells), mononuclear leukocytes (lymphocytes, plasmacytoid and plasma cells), mononuclear phagocytes (monocytes, monocytoid cells / histiocytes, macrophages, multinuclear giant cells) and erythrocytes. Cytological analysis can reveal tumor cells, cells of tissue origin and additional elements (cartilage, skin and bone marrow cells). Tissue-derived cells, such as pial and arachnoid cells, choroid epithelial cells and ependymal cells, are rarely found in normal CSF, but can be found in purulent inflammation. They do not have much diagnostic significance, but it is important not to confuse them with malignant cells [5]. In primary and metastatic tumors of the central nervous system, tumor cells can be found in the CSF. Leptomeningeal metastases and cell proliferation into CSF are detected by cytological analysis of CSF, neurological examination, and radiological examination of neuroaxis, with CSF cytology considered the gold standard in the diagnosis of leptomeningeal metastases [6].

Changes in the cellular composition of CSF have been detected in a number of CNS diseases, and cytological analysis of CSF provides valuable information on the type and course of neurological diseases.

CSF analysis in psychiatry and neurology

In various acute neurological conditions, CSF analysis is especially useful and helps diagnose and treat patients more quickly. Diagnosis includes biochemical and cytological analysis, determination of biomarkers, and microbiological evaluation [7]. By measuring the levels of various components of the CSF using relevant techniques, one can diagnose, assess severity, and predict the course and outcome of neurological conditions such as infections and subarachnoid hemorrhage, but also demyelinating or tumor conditions [8].

Cytological evaluation indicates individual conditions depending on the number and type of cells in the CSF. The predominance of neutrophilic granulocytes indicates bacterial meningitis and suggests a search for intracellular bacteria. In viral and chronic infections, lymphocytes and monocytes predominate. Upon activation, lymphocytes enlarge and eventually differentiate into plasma cells. Similarly, monocytes differentiate into macrophages that clear cellular debris. Macrophages that contain fragments of erythrocytes or breakdown products of haemoglobin are called erythrophages or siderophages and indicate earlier subarachnoid hemorrhage [8].

Tumor cell detection is specific for neoplastic meningitis, although false-negative findings are common [8]. This is a highly specific (> 95%) but poorly sensitive diagnosis. However, the optimal sampling technique of ≥ 10.5 mL, repeated lumbar puncture in case of a negative first finding, taking CSF as close to the tumor as possible (when the tumor can be visualized by imaging) and rapid sample processing increase the likelihood of detecting tumor cells in CSF [9].

Detailed morphological analysis of CSF cytological samples provides valuable diagnos-

tic information and is mandatory in case of pleocytosis, suspected subarachnoid hemorrhage not shown by computed tomography, and neoplastic meningitis. In all cases, the cytological finding must be interpreted in a clinical context and as a complement to other clinical and laboratory findings [8].

Although CSF analysis is a diagnostic method most used in neurological pathological conditions, it also has its place in psychiatry. Numerous neurological diseases are accompanied by psychosyndromes. In some cases, neurological diseases are initially manifested by psychiatric symptoms. Patients with multiple sclerosis, who first experience symptoms from the spectrum of bipolar disorder, may begin their treatment with a psychiatrist with the wrong symptomatic treatment, unless a CSF analysis is performed first. Therefore, the primary goal of CSF analysis in psychiatry should be to rule out abnormal findings, such as the inflammatory cause of psychiatric symptoms, like in cases of multiple sclerosis [10].

However, in psychiatric diseases, the results of CSF analysis can be very complex, as shown by the results of different groups of authors. In one study 30% of patients with schizophrenia spectrum disorders or affective disorders had a higher albumin level and the CSF flow rate was reduced without any other abnormal CSF findings [10]. In the same group, independent of patients with elevated albumin, CSF neopterin was elevated in 30% of patients, without any other signs of humoral or cellular immune response [10,11]. The proven absence of other signs of inflammation or intoxication currently leaves these observations without diagnostic significance. A key prerequisite for a reliable interpretation of these results is the exclusion of drug-induced changes because most psychiatric patients are on multiple drug therapy [10].

In the group of psychiatric patients with autoimmune encephalitis or systemic tumors with antineuronal antibodies, the inflammatory, immune cause of their psychiatric symptoms is easier to detect by analysis of appropriate antibodies in the blood and CSF [12].

In a subset of patients with affective and schizophrenia spectrum disorders, minor nonspecific changes, such as activated monocytes or elevated CSF cytokine levels, may indicate an inflammatory condition [13].

These examples show the dilemma of psychiatric diagnosis and therapy if the analytical approach does not include appropriate CSF and blood tests. As in neurology, a complete range of CSF tests is recommended for psychiatric diagnoses. Regardless of diagnostic relevance, CSF analysis can help distinguish actual mental illnesses from the psychiatric symptoms that accompany immune responses, with a necessarily different therapeutic approach. Symptomatic treatment of psychiatric patients with bipolar spectrum disorders is erroneous if CSF analysis shows an inflammatory cause of the disorder [14].

Our knowledge of the pathogenic mechanisms leading to the development of schizophrenia is still fragmented. Much is known about the multifactorial and heterogeneous etiology or etiopathogenesis of this psychiatric disorder, but it is precisely because of this that estimates of biological parameters yield a wide range of variable outcomes that are difficult to relate. In addition to neurological developmental aberrations, there is growing evidence that relates numerous forms of progressive organic processes in the brain to the development of schizophrenia. Research using new imaging techniques in neurology has revealed the development of neuroanatomical abnormalities during this disease [15].

A number of infectious pathogens of low pathogenicity are risk factors for the development of psychosis, including schizophrenia, and autoimmune disorders. There is growing research to support the mild encephalitis (ME) hypothesis that low-level neuroinflammation (LLNI) is a key pathogenetic mechanism lead-

ing to the development of severe psychiatric disorders in a subset of patients, mostly with affective and schizophrenia spectrum disorders [16,17]. Bechter and associates analyzed albumin, immunoglobulins, and CSF-specific antibodies, and found immune and inflammatory mechanisms corresponding to LLNI in 40% of patients with treatment-resistant schizophrenia [18]. By investigating the T lymphocyte subpopulation in CSF Maxeiner and associates further supported the hypothesis of low-grade inflammation in the pathogenesis of psychiatric disorders [19].

Another example of often undetected neurological inflammation is limbic encephalitis, which in the prodromal stage is associated with various psychiatric syndromes. The diagnosis is made on the basis of neurological symptoms, magnetic resonance imaging, or most often by detecting CNS-specific antibodies. Early limbic encephalitis is associated with the process of low-grade inflammation and the ME hypothesis [17]. Gultekin and associates described paraneoplastic limbic encephalitis (PLE), a rare disorder characterized by personality changes, irritability, depression, epileptic seizures, memory loss, and sometimes dementia. In a study with 50 patients with PLE, these symptoms preceded the diagnosis of cancer in 60% of cases. In diagnosing PLE, CSF analysis helps in two ways; cytological finding negative for malignant cells in combination with the absence of meningeal enlargement on magnetic resonance helps to exclude the existence of leptomeningeal metastases, while the detection of inflammatory abnormalities (pleocytosis, intrathecal IgG synthesis) supports the diagnosis of inflammatory or immune-mediated neurological disorder [20]. The analysis of CSF in these studies has proven to be a useful diagnostic method in the differential diagnosis of psychiatric disorders.

CSF cell morphology in patients with acute schizophrenia

A group of Finnish authors (Nikkilä and associates) analyzed the proportion of CD4+ and CD8+ T lymphocytes in the CSF of patients with acute schizophrenia and compared it with the proportion of T cells in peripheral blood. Given the low total number of cells in the CSF, lymphocyte phenotyping was carried out with the 3-layer indirect immunoperoxidase technique on air dried cell smears, and the same method was used for peripheral blood samples. Compared to the control group, schizophrenic patients had both abnormally high and abnormally low incidence of CD4+ and/or CD8+ lymphocytes. In patients with advanced disease, a significantly increased proportion of CD8+ lymphocytes were found, which led to the conclusion that most schizophrenic patients had an abnormal distribution of T lymphocytes in the CSF. The changes detected in the CSF were not reflected in the values of T lymphocytes in the peripheral blood. These results prompted the authors to further investigate cytological disorders in the CSF of patients with schizophrenia [21].

Nikkilä and associates continued the study by analyzing cytomorphological changes in the CSF cells of patients with acute schizophrenia and comparing it with the control group without psychiatric disorders or CNS diseases. Analysis of cytological preparations stained according to May-Grünwald-Giemsa revealed a significant difference in the cytological profile of samples between the studied groups. Morphological changes of lymphocytes, as well as an increased proportion of monocytes/macrophages in the CSF of patients with acute schizophrenia were found, but without a significantly increased total number of cells [15].

The most striking finding was a significantly increased frequency of lymphoid cells showing morphological features of activation/stimulation and a decreased proportion

of normal small lymphocytes. The cytological finding of schizophrenic patients in the initial phase of hospitalization was clearly different from that in the samples of the healthy control group. Differential mononuclear cell numbers, as well as lymphoid cell morphology were significantly altered in schizophrenic patients ($p < 0.001$, Mann-Whitney U test). In contrast to the CSF of the control group, where most cells were small lymphocytes, in the CSF of schizophrenic patients mostly enlarged cells and cells with basophilic cytoplasm, twisted nucleus and irregular nuclear membrane, expressed nucleoli and scattered chromatin were found. These morphological features are characteristic of stimulated or immunoactivated lymphocytes that share some structural features with atypical cells, called P cells [15].

P cells or P lymphocytes, stimulated atypical lymphocytes, have been observed in the blood of schizophrenic patients and some of their relatives. The basic morphological criteria for the identification of P lymphocytes were described by Hirata-Hibi and Hayashi who cite basophilia of the cytoplasm and nucleus with a fine chromatin structure as the main features of the cell. The cytoplasm of P cells often contains vacuoles, and the shape of the nucleus is often irregular, with one or more visible nucleoli. These cells could be an experimental laboratory indicator of the course of schizophrenia [22]. The appearance of cells of the described morphology on both sides of the blood-brain barrier may be the result of lymphocyte stimulation either in the CNS or in the blood. The high incidence of activated lymphocytes in the CSF indicates CNS as the primary source, while cells observed in the bloodstream could be the result of cells passing from one body compartment to another. Activation of lymphocytes is known to increase their motility and induce increased expression of various surface receptors, including adhesion molecules required for transendothelial migration [23]. Muller and associates described enhanced receptor expression of CD4+ and CD8+ T lymphocyte adhesion molecules in schizophrenic patients [24].

Increased lymphocyte adhesion may also explain the formation of macrophage rosettes and activated lymphocytes in the CSF. In addition to the aberrant lymphocyte profile, an increase in the proportion of monocytes/macrophages was observed in the CSF of patients with acute schizophrenia. Morphological analysis of monocytes revealed pleomorphic stages of maturation, from juvenile mononuclear phagocytes with a compact nucleus and homogeneous cytoplasm with slightly small acidophilic granules, to mature macrophages of irregularly shaped, renal or lobulated nuclei and abundant malignant cytoplasm. Lipophages with larger cytoplasmic vacuoles, which are a typical finding in CSF after acute brain trauma, were few [25]. The presence of a higher proportion of macrophages in the CSF may be a reflection of subchronic or chronic brain tissue destruction and may be associated with radiologically proven decreased brain volume in patients with schizophrenia [25].

Cytomorphologically, occasional rosettes i.e., aggregates of lymphocytes and macrophages, could be only the simple clusters, but macrophages being the antigen-presenting cells, such intercellular binding may also indicate a functional immunostimulatory interaction [26].

Most macrophages in the CSF are thought to be of microglial origin and microglia are the main source of macrophages that accumulate in CNS lesions. In addition to phagocytic ability, microglia cells contribute to the CNS immune network by expressing HLA-DR molecules and with the role of antigen-presenting cells [27,28]. In tissue cultures, the accumulation of T lymphocytes around the microglia and the initiation of a mixed lymphocyte response, and the activation of T lymphocytes cause the production of proinflammatory cytokines by macrophage lineage cells [29].

The dominance of macrophages in the CSF of psychotic patients observed in a study by Nikkile and associates may indicate activation and/or mobilization of microglial cells and link abnormalities in T lymphocyte distribution and elevated levels of proinflammatory cytokines in the CSF [25].

Follow-up of these patients showed no change in lymphocyte morphology during neuroleptic administration, but the follow-up period was too short and the number of patients too small to make a definitive conclusion about the consistency of CSF cytological abnormalities [15]. Normalization of macrophage morphology during conventional therapy was observed in several patients [25]. It is unlikely that the effect of acute hospitalization and the use of neuroleptics a few days before lumbar puncture affected morphological changes in CSF cells [15]. Previous studies have shown that short-term antipsychotic therapy does not lead to morphological aberrations of lymphocytes cells with altered morphology [30,31].

Cytological analysis of CSF is an indispensable and accessible method, crucial in the process of diagnosing numerous inflammatory, infectious, oncological and hemorrhagic pathological conditions in the CNS, and whose application in acute psychiatric diseases is important not only in excluding differential diagnoses but also in clarifying inflammatory and immune etiopathogenesis of the psychiatric disease and its targeted treatment. Studies to date have described several valuable specific cytomorphological phenomena in the CSF of patients with acute schizophrenia, which indicate inflammatory or immune-mediated etiopathogenesis of the disease. Additional and long-term research is needed to confirm and standardize the importance of cytological analysis of CSF in the diagnosis and etiopathogenesis of acute schizophrenia, as well as to verify changes in the cytomorphological findings of CSF in monitoring treatment responses.

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Conflicts of interest

None to declare.

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Citološka analiza cerebrospinalnog likvora u oboljelih od akutne shizofrenije

Sažetak- Analiza likvora jedna je od najvažnijih pretraga u dijagnostici bolesti središnjeg živčanog sustava (SŽS). Iako se analiza likvora najčešće primjenjuje u neurološkim patološkim stanjima, svoje mjesto ima i u psihijatriji. Dosadašnje studije opisuju nekoliko vrijednih specifičnih citomorfoloških fenomena u likvoru bolesnika s akutnom shizofrenijom, koji ukazuju na upalnu, odnosno imunosno posredovanu etiopatogenezu bolesti. Potrebna su dodatna i dugoročna istraživanja koja bi potvrdila i standardizirala značaj citološke analize likvora u postavljanju dijagnoze i etiopatogenze akutne shizofrenije.

Ključne riječi: cerebrospinalni likvor; citologija, shizofrenija