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ORIGINAL ARTICLE

Isoelectric Focusing of Cerebrospinal Fluid Significance in Differential Diagnosis of Multiple Sclerosis

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Abstract

Background/Aim: Isoelectric focusing (IEF) of cerebrospinal fluid (CSF) is the gold standard for detecting intrathecal oligoclonal immunoglobulin G (IgG). Besides its diagnostic and predictive significance, the IEF method also has differential diagnostic impact in multiple sclerosis (MS). The goal of the research was to analyse the influence of IEF on the differential diagnosis of MS, as well as in neurology in general.

Methods: Research included 418 participants treated in the Neurology Clinic of the University Clinical Centre of the Republic of Srpska. Among them, 177 were suffering of MS. The control group, divided into major disease categories, consisted of 241 patients. The following were analysed for participants: demographic and clinical characteristics, IEF and cytobiochemical findings of CSF. Based on IEF findings, participants with oligoclonal bands (OB) were separated from those without OB. The findings of IEF in different disease categories and their differential diagnostic significance were analysed.

Results: In the examined cohort using the IEF method, intrathecal synthesis of oligoclonal IgG was evident only in inflammatory diseases, primarily multiple sclerosis and was absent in any non-inflammatory diseases. This indicated high sensitivity of the method for MS patients (96.6 %) and very high specificity for CNS inflammatory diseases (100 %).

Conclusion: IEF is a highly specific for CNS inflammatory diseases, indicating the differential diagnostic significance of oligoclonal IgG in MS, as well as in neurology in general.

Key words: Isoelectric focusing; Multiple sclerosis; Diagnosis, differential.

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Introduction

Detection of oligoclonal immunoglobulin G (IgG) is one of the most useful markers in cerebrospinal fluid (CSF) in diagnostic, differential-diagnostic and prognostic terms in multiple sclerosis (MS).[1] The goal of future research, apart from defining surrogate markers of demyelination, remyelination, neuroaxonal loss, neural repair and regeneration, is to investigate CSF as thoroughly as other bodily fluids.[2]

Isoelectric focusing (IEF) is recommended as the "gold standard" for the detection of oligoclonal bands (OB). Recent studies indicate a high sensitivity of the IEF for diagnosing MS (95 %) and research excluding central nervous system (CNS) infections has shown very high specificity of the IEF method up to 99.5 %.[3, 4]

The IEF method for detecting OB IgG has a differ-

ential diagnostic value for MS, as OB can also be detected in other inflammatory CNS diseases.[5, 6] There is a very high prevalence of OB in CSF in subacute sclerosing panencephalitis (SSPE), neurosyphilis and lymphocytic meningoradiculitis, where it reaches up to 70 %, in patients infected with human immunodeficiency virus (HIV) from 12 % to 77 % and in viral meningitis from 30-40 %. In patients with neuroborreliosis, OB in CSF is almost always present.[7]

Oligoclonal IgG is present in about 30 % of patients with immune-mediated inflammatory CNS diseases such as systemic lupus erythematosus (SLE) with CNS involvement, neurosarcoidosis and acute inflammatory demyelinating polyradiculoneuropathy (AIDP). In patients with paraneoplastic syndrome oligoclonal IgG occurs in up to 50-100 % of patients.[8]

In non-inflammatory neurological patients, the presence of OB in CSF is not expected. However, they can be found (5-10 %) in cerebrovascular diseases (CVD), brain tumours, lumbar radicular syndrome, polyneuropathies, subarachnoid haemorrhage, Parkinsonism, myasthenia gravis (MG) and amyotrophic lateral sclerosis (ALS). This usually does not lead to diagnostic errors, as these diseases have a different clinical course from MS and can be defined by other diagnostic tests.[9, 10]

The goal of the research was to demonstrate the differential diagnostic impact of IEF in MS and neurology in general.

Methods

This was a retrospective and partly prospective cohort study. The study included participants who had a lumbar puncture and then the CSF and serum were simultaneously analysed using the IEF method by one neurologist at the Neuroimmunology Laboratory of University Clinical Centre, Banja Luka, over a period of 4 years.

The identification of IgG OB in CSF and serum was performed, according to the recommended Criteria for CSF analysis, by the standardised IEF method on agarose gel with protein transfer to a nitrocellulose membrane, immunofixation and immunoperoxidase staining. The Helena Biosciences SAS IgG IEF kits on the IEF apparatus (*LKB*) which consists of three parts: Multiphor-II, power supply EPS3501XL i MultiTemp-III was used.[3, 7]

The material was analysed and IEF was interpreted with the presence of provisional diagnostic assumptions. All patients were categorised according to the final discharge diagnoses based on the International Classification of the World Health Organization and then grouped into 17 major categories according to diagnoses. The group of MS patients was separated from the group of patients with other diseases and the diagnosis was made based on Mc Donald's criteria, which were revised in 2017.[2, 11] The Expanded Disability Status Scale (EDSS) score was used to determine functional disability.[12] For all patients, demographic data, clinical parameters, IEF and cytobiochemistry of CSF were analysed.

The entire study included the analysis of 418 patients. Of these, 177 MS patients constituted the experimental group and the control group, divided into major categories, comprised 241 patients. The study was approved by the local ethics committee and participants voluntarily entered the study, which they previously confirmed by signing the informed consent.

By applying the IEF method simultaneously on CSF and serum for all participants, the results were classified into five groups:

- IEF 1 - normal finding,
- IEF 2 - OB only in CSF,
- IEF 3 - OB in CSF and serum, more in CSF,
- IEF 4 - OB symmetrical in CSF and serum,
- IEF 5 - paraprotein pattern.[4,5]

The participants with IEF findings 2 and 3 were separated from those with findings 1, 4 and 5 and two groups were formed:

- Group with intrathecal synthesis of OB (IEF 2 and IEF 3)
- Group without intrathecal synthesis of OB (IEF 1, IEF 4 and IEF 5).

Statistical analysis was performed using SPSS software, version 21. Descriptive and analytical statistics methods were used in the results analysis. Analysis of variance (ANOVA) was used to evaluate differences with continuous variables and the χ^2 test was used for categorical variables. Statistical significance was determined at the 0.05 level. Results are presented in tables and figure.

Results

Of the total 418 patients, MS patients numbered 177 and the control group comprised 241 participants. In the MS patient group, there were twice as many women as men. At the onset of the disease, 72.3 % of patients were younger than 40 years, while 27.7 % were 40 or older.

The significance of differences between the age at the onset of the disease according to various variables was examined. The analysis showed that there was no statistically significant difference between the gender of MS patients according to the age at the onset of the disease, but there was a statistically significant difference between the age at the onset of the disease and the course of the disease ($p < 0.001$). Patients who were younger at the onset of the disease more often had relapsing-remitting (RR) MS, while patients with secondary-progressive (SP) and primary-progressive (PP) MS more often developed the disease at an older age. There was a statistically significant difference between the EDSS of MS patients and the age at the onset of the disease ($p < 0.001$). The EDSS was higher in patients whose disease started later (after the age of 40).

There was a significant difference between mono- and poli-regionality of the first symptoms of the disease and the age at the onset of the disease ($p = 0.023$). In patients who developed the disease at a younger age, the first symptoms more often appeared as mono-regional compared to those who developed the disease at an older age.

In the control group, the largest percentage of patients had CVD (29 %), while the smallest percentages (0.8 % each) had infectious diseases, motor neuron diseases, syndromes of unclear origin and others without verified diseases (Table 1). In the category of syndromes of unclear origin, there were 2 patients, one of whom was diagnosed with paraneoplastic syndrome during the study, while the other patient remained etiologically unresolved with a brainstem syndrome. In the group of others, there were 2 participants with no verified disease.

Examining the gender distribution of patients in the control group classified according to the disease groups they belong to; it was observed that there was no statistically significant difference ($p = 0.09$) between the gender of patients and the disease groups. Examining the age distribution of patients in the control group revealed that the oldest patients were those with motor neuron disease (56.0 years ± 2.8) and the youngest were those categorised in the group of patients with paroxysmal disorders (27.8 years ± 8.3).

Table 1: Division of control group patients according to disease categories

Disease groups	N	%
Infective diseases	2	0.8
Immunological diseases	9	3.7
Disseminated demyelinating disease	10	4.1
Neoplasms	15	6.2
Neuromyopathies	29	12.0
Bone and joint diseases	32	13.3
Cerebrovascular diseases	70	29.0
Degenerative diseases	5	2.1
Motor neuron disease	2	0.8
Paroxysmal disorders	11	4.6
Headaches	24	10.0
Traumas and consequences	5	2.1
Syndromes of unclear origin	2	0.8
Congenital diseases	10	4.1
Metabolic diseases	4	1.7
Psychiatric diseases	9	3.7
Others (without verified disease)	2	0.8
TOTAL	**241**	**100.0**

According to the IEF findings, the following patient distribution was observed: IEF 1 was found in patients with the following disease groups: motor neuron disease (MND), paroxysmal disorders, headaches, trauma, congenital, metabolic and psychiatric diseases and patients without a verified disease. IEF 2, besides 158 MS patients, was found in one patient with an infectious disease, two patients with immunological diseases, one with disseminated demyelinating disease (DDD) and one with paraneoplastic syndrome. IEF 3, besides 13 MS patients, was found in three other patients, two with immunological diseases and one with another DDD. IEF 4 was equally found in neuromuscular diseases (acute polyradiculoneuritis, chronic inflammatory demyelinating polyneuropathy (CIDP) and mononeuritis multiplex) and CVD and to a significantly lesser extent in demyelinating, degenerative, bone-joint and immunological diseases. IEF 5 was found in only one patient who has monoclonal gammopathy associated with CIDP (Figure 1).

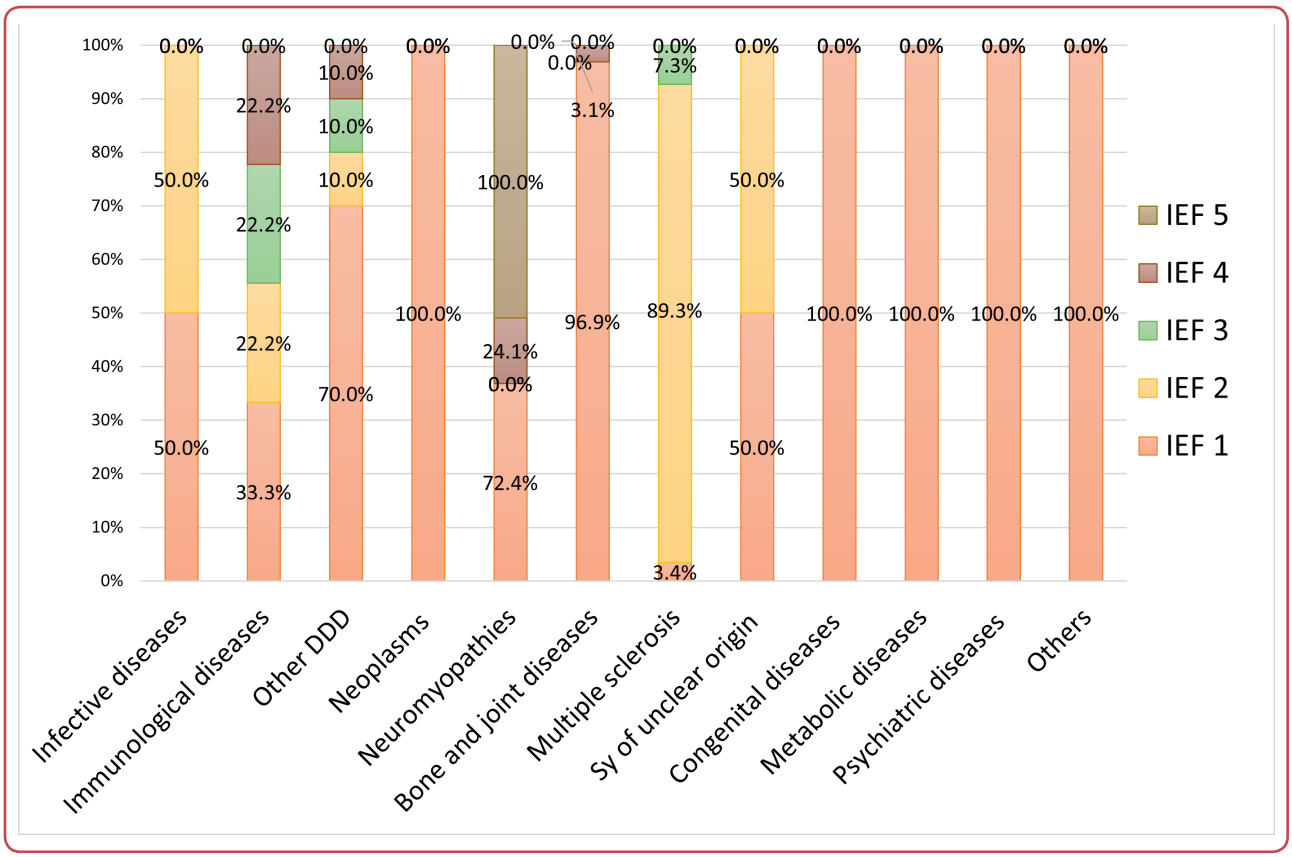

Figure 1: Distribution of patients according to IEF findings

IEF 1. normal findings; IEF 2. presence of oligoclonal bands (OB) in cerebrospinal fluid (CSF), without OB in serum - intrathecal synthesis; IEF 3. presence of more OB in CSF than in serum - local and systemic immune activation; IEF 4. identical number and pattern of OB in CSF and serum - systemic immune activation. IEF 5; Monoclonal IgG, three to five identical OB, with intensity decreasing from cathode to anode; DDD: disseminated demyelinating disease;

Using the IEF method, the presence of local IgG synthesis within the CNS was detected in 179 patients (Table 2). All patients with intrathecal synthesis of oligoclonal IgG belong to the group of inflammatory CNS diseases. The largest number of patients with intrathecal synthesis of IgG bands was in the MS group (96.6 %), of which 158 with IEF 2 and 13 with IEF 3. Eight patients exclusively belonged to the group of inflammatory diseases, indicating that IEF is highly specific for inflammatory diseases and very sensitive for MS. OB, besides MS, were detected in other DDD, CNS infectious diseases, immune-mediated nervous system diseases and paraneoplastic syndrome. Of the eight patients with detected intrathecal synthesis of IgG bands, three had myelitis transversalis (classified as other DDD diseases), two had cerebral vasculitis and one had CNS lupus (classified as immune diseases), one had neuroborreliosis and one had paraneoplastic syndrome.

Table 2: Distribution of participants according intrathecal synthesis of oligoclonal IgG

Disease group	Diagnosis	N
DDD	Multiple sclerosis	171
Other DDD	Transverse myelitis	3
Immunological diseases	Cerebral vasculitis	2
	CNS lupus	1
Infectious diseases	Neuroborreliosis	1
Tumours	Paraneoplastic syndrome	1

DDD: disseminated demyelinating disease; CNS: central nervous system;

Discussion

In presented research, all patients with intrathecal synthesis of OB had a defined inflammatory disease, with the largest number being from the group of MS patients. In no participant with a non-inflammatory CNS disease was intrathecal synthesis of oligoclonal IgG confirmed using the IEF method, which supports the very high spec-

ificity of OB detected by the IEF method for inflammatory CNS diseases.

From the above, it can be seen that in study, the frequency of false-positive results obtained by isoelectric focusing was 0 %, as in the large and significant studies by McLean and Drulović and in numerous recent studies.[6, 9] OB were primarily detected in MS and subsequently in other demyelinating, infectious, immune-mediated and other inflammatory CNS diseases. It can be concluded that IEF is 100 % specific for inflammatory diseases.

The findings of research, as well as all major and recent studies, support the very high specificity of OB detected by the IEF method for inflammatory CNS diseases, primarily MS. The high sensitivity and specificity have established the IEF method as the gold standard for detecting oligoclonal bands.[3]

In presented study, IEF did not show local synthesis in any patient with CVD, Parkinson's disease and other extrapyramidal disorders, congenital neurological disorders, idiopathic epilepsy, metabolic disorders, CNS trauma, ALS, GBS, vertebrogenic radiculopathies, spondylotic myelopathies, polyneuropathies, myopathies and psychiatric disorders. Similar results were described in the studies by Drulović and McLean, as well as in more recent research.[6, 13]

The discovery of local synthesis in any of the mentioned diagnostic categories would suggest the suspicion of the presence of an inflammatory CNS disease. This has been described in the example of the presence of infectious vasculitis in patients with acute cerebral infarction. Such a finding may also indicate the presence of an associated disease characterised by an immune response within the CNS.[13-15]

Summarising the results of numerous recent studies has indicated the presence of OB, in addition to MS, in cerebral lupus, Sjögren's syndrome, neurosarcoidosis, paraneoplastic syndrome, Behcet's disease, cerebral angiitis and CNS infections.[16-18]

In this study identical OB in CSF and serum in any MS patient was not found, as in most studies.[19, 20] Such a finding in MS patients should actually prompt a reconsideration of the diagnosis. This is supported by the fact that identical OB in serum and CSF are often found in diseases that are very significant in the differential diagnosis of MS (eg, connective tissue disease or spondylotic myelopathies).[21] The IEF finding indicating both systemic and intrathecal immune activity does not have additional diagnostic significance compared to the finding of intrathecal synthesis of OB alone. However, the fact that some MS patients exhibit both intrathecal and systemic immune activity can contribute to new pathogenetic considerations of the disease itself.[22]

Conclusion

Isoelectric focusing of cerebrospinal fluid, besides having diagnostic and predictive significance, also holds exceptional differential diagnostic importance in MS. Research results indicate that intrathecal synthesis of IgG is present only in patients with inflammatory diseases, predominantly in MS, which highlights its differential diagnostic significance in MS as well as in other neurological diseases.

Ethics

The study was approved by the Ethics Committee of the University Clinical Centre of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina, decision No: 01-19-552-2/24, dated 26 December 2024.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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