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ORIGINAL PAPER

Reibergram and Oligoclonal Bands in Diagnosis of Multiple Sclerosis

Jasenko Karamehic[1], Marina Delic-Sarac[1], Djemo Subasic[1], Tomica Jukic[4], Jozo Coric[2], Mirsad Panjeta[2], Zahida Drace[1], Lamija Zecevic[1], Selma Mutevelic[1], Nejra Dzananovic[1], Nerima Grcic[1], Amila Kesmer[3]
Clinical Immunology Clinical Center of University of Sarajevo[1], Clinical Biochemistry Clinical Center University of Sarajevo[2], General Hospital "Prim.dr. Abdulah Nakaš", Sarajevo[3], Medical Faculty, Osijek University, Scientific Department of Biomedicine and Health. Osijek Croatia[4]

Introduction: In this study authors have analyzed the correlation between the IgG immunoglobulins in cerebrospinal fluid and the findings of oligoclonal bands on gel. Immunoglobulin IgG in cerebrospinal fluid (CSF) can be detected in neurological diseasses (infections and inflammatory neurological diseases and in demyelinating diseases, like multiple sclerosis (MS)). Quantitative IgG in CSF can be expressed by different formulae Reiber (Reiber and Felgenhauer 1987), Tourtellotte (Tourtellotte 1970), Schuller (Schuller and Sagar 1983) and IgG Index (Link and Tibbling 1977). In this study we used Reibergram. Qualitative CSF IgG can be measured by electrophoresis and isoelectric focusing (IEF). We used IEF for analysig CSF and seum because of its higher sensitivity. **Aims of the study:** To determine the correlation of immunoglobulins IgG positivity in CSF with the finding of oligoclonal bands on the gel. **Material and methods:**: The retrospective study based on data processed in OJ Clinical Immunology KCUS. Patients were suspicious of multiple sclerosis according to clinical findings and magnetic resonance imaging. All CSF and serum samples were processed by nephelometry, isoelectric focusing on the gel. Statistical analysis of intrathecal synthesis was also performed according to Reibergram. **Results:** Analyses were performed on 76 samples of cerebrospinal fluid and serum of patients from neurological clinic, suspected of multiple sclerosis. We received following results: 42 samples tested had type 1. 25 samples tested showed type 2. 3 samples had type 3. 5 samples had type 4. 1 sample had a fifth type. When we compare these results with values obtained by intrathecal synthesis of which is determined by Reibergram we obtained the following values: 16 samples had intrathecal synthesis of 20%–60%, 9 samples had a negative value of intrathecal synthesis of 10% or less. **Discussion** and **Conclusion**: For most patients with established MS we found intrathecal humoral response, type two, and the number and arrangement of IgG bands generally does not change during the disease, because they reflect long-term non-specific immune stimulation rather than a specific immune response that during infectious disease changes (quantitatively and qualitatively). **Keywords: oligoclonal bands, nephelometry, isoelectric focusing.**

Corresponding author: prof Jasenko Karamehic, MD, PhD. Clinical center of University of Sarajevo, Bolnicka 25, Sarajevo, B&H. E-mail: karamehic.jasenko@hotmail.com

1. INTRODUCTION

Multiple sclerosis is a chronic inflammatory disease of the central nervous system, which was thought to be a T cell mediated disorder in a long time. Lots of new study recently conducted on B cells and antibodies as well as significant participants in the pathogenesis of multiple sclerosis. Oligoclonal immunoglobulin G (IgG) bands were found in the cerebrospinal fluid but not in the serum of more than 90% of patients with multiple sclerosis, indicating intrathecal immunoglobulin (Ig) production. There is proven presence of B cells, plasma cells, complement and myelin-specific antibodies in chronic MS lesions. There is much evidence in the literature to supplement the knowledge of the humoral immune response as the basis of the pathogenesis of MS.

Today there are many approaches to study B cell reactivity with varying degrees of success, and despite the efforts are still not entirely clear what the target cell B autoreactive cells and antibodies. Since MS is characterized as a heterogeneous disease with a significant genetic basis and background, the new markers in the diagnosis of new patients and multicenter treatment strategy is needed to better control of the disease. Monitoring of the humoral im-

mune response this remains a burning issue in the analysis and MS research, where identification of target antigens and characterization of B cell repertoire, are two main objectives (1).

The course of disease in MS is highly variable, and while some patients remain asymptomatic long time, others rapidly became worse. The analysis shows the presence of CSF oligoclonal bands in most patients with MS. Oligoclonal bands unique to CSF speaks in favor of the immune response of a small number of antigens in the intrathecal space (2). For antibodies that have the capacity to recognize specific antigens in the CNS, it is assumed to contribute to tissue damage in patients with MS. Due to the lack of a single pathognomonic laboratory test, diagnosis of MS is traditionally based on the integration of clinical and paraclinical findings. Oligoclonal bands were found in 90 to 95% of patients with MS, they are not specific for MS and can be found in other diseases, such as CNS infections (3).

2. PATIENTS AND METHODS

This study included 76 patients who were patients of the Department of Neurology of Clinical center of University of Sarajevo (CCU). For each patient we analyzed both serum and CSF on the presence of oligoclonal bands on Hydrasys SEBIA, by isoelectric focusing in ultrathin gel. This method significantly increases the effectiveness of the procedure of proving oligoclonal IgG. Separating proteins by isoelectric focusing with the possibility of direct immunofixation in ultrathin gels allows the use of native CSF samples thus obviating the need of concentrating of CSF protein. Hydrasys 3CSF that were used in this study provide a qualitative detection, identification of oligoclonal bands in electrophoretic pattern of cerebrospinal fluid and confirmation of their immunoglobulin nature (4). The procedure involves isoelectrofocusing on agarose gel, and conducted semiautomatised method on Hydrasys system, immunofixation with anti IgG antiserum. The use of enzyme labeled anti IgG antiserum to detect only "real" oligoclonal IgG binding at increased detection sensitivity, so that the analysis can generally be carried out with concentrated liquor. Pattern of IgG immunofixation, cerebrospinal fluid and serum of the same patient are compared visually. It enable detection of binding by an oligoclonal intrathecal immunoglobulin synthesis (5, 6).

Nephelometry

Nephelometry is an optical method for measuring the intensity of light scattered by particles in the fluid that are suspended in liquid or macromolecules in solution. It is based on the capacity of dilute suspensions of microparticles to scatter the light. The initial antigen-antibody complexes were macromolecule that does not disperse light. However, these complexes have the ability to form aggregates. The size of aggregates grows within a few minutes to several hours while their ability to dissipate the light reaches the maximum. Scattered light is collected and measured in the detector at a certain angle. Unknown concentration can then be determined by measuring and comparing the responses to the standard curve. In case of excess antibody, the concentration of soluble immune complexes as measured through the intensity of scattered light is proportional to the concentration of antigen. In case of excess soluble antigen immune complex size decreases with increasing concentration of antigen, based on the measured signal is acquired by a false image of a low concentration of antigen. Nephelometry method was used to determine the value of immunoglobulin M, G and A in the cerebrospinal fluid and serum as well as the value of albumin in CSF and serum (7).

Quantitative analysis of antibodies and evaluation of findings by Reibergram

Analysis of results performed by Reibergram are essential part of the analysis of cerebrospinal fluid. In order to calculate Reibergram we must determine albumin, and immunoglobulin IgA, IgM and IgG in serum and cerebrospinal fluid. Then the calculated quotient CSF/serum and the results are included in Reibergram. This pattern can give better information than independent analysis.

Reibergram can provide the following information: evaluation of the intrathecal synthesis of immunoglobulin, the permeability of fluid/blood barrier and the clinical data and symptoms which may be important in the differential diagnosis so it is possible to determine whether that is MS or other diseases caused by opportunistic infections, neuro tuberculosis or autoimmune inflammatory diseases. So Reibergram is the best way to determine the function of blood/CSF barrier and intrathecal synthesis of immunoglobulin in neurological disorders. Many CNS disorders are associated with increased CSF protein concentration, either due to increased permeability of the blood-CSF barrier, or the synthesis of immunoglobulin, primarily immunoglobulin G within the CNS. Intrathecal synthesis of immunoglobulin is often associated with heterogenic diversity which manifests as oligoclonal binding, seen in the gamma zone of high-resolution samples. Bands that are not immunoglobulin may also be present in the gamma globulin zone, but do not have diagnostic significance. Immunofixation is the detection technique of choice since it can confirm the character of oligoclonal immunoglobulin bands thay identify involved immunoglobulin and provide the necessity of a specific test (8, 9).

Although oligoclonal binding is not specific for MS, it is often used as additional information. Criteria for the detection of oligoclonal CSF binding are:

- Isoelectric focusing is the most sensitive technique for detecting oligoclonal binding,
- Oligoclonal immunoglobulin G (IgG) must be identified by specific serum,

To confirm the intrathecal synthesis of IgG, serum and CSF of the patient must be simultaneously analyzed in order to detect differences in the distribution of IgG

In addition to identifying the concentration of IgG in CSF and serum should be adjusted to the same level of dilution of samples

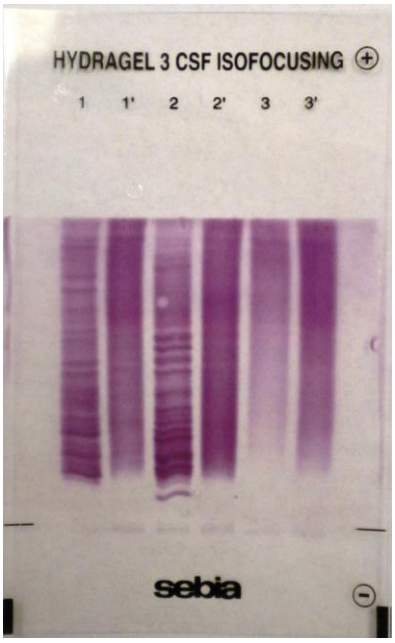
2.1. Avoid concentrating of CSF

Analysis of samples is performed in two steps:

- Izofocusing on agarose gel to separate the proteins of cerebrospinal fluid and serum

- Immunofixation with enzyme (peroxidase) labeled with anti IgG antiserum to detect IgG oligoclonal bands and to show the differences, or lack of them, in the distribution of IgG in CSF and serum.

The concentration of IgG that is 1mg/dl or above that valuie, and recognition of its distribution, can be detected without concentrating of the sample fluid. The method of isoelectric focusing is possible to prove a series of strips of IgG in CSF and in serum. Certain patterns of IgG oligoclonal band have a differential diagnostic



Picture 1. 1 and 2 Oligoclonal bands type 2, 3 oligoclonal bands type 1

significance.

Classification of oligoclonal immunoglobulin G (IgG) findings:

- Type 1: Normal findings of diffuse-type imunoprecipitation IgG in CSF and serum;
- Type 2: Two or more bands of IgG in CSF only (the most common finding in MS);
- Type 3: Multiple IgG bands in CSF and some of them in serum (infection and postinfectious syndromes, multiple sclerosis, opportunistic infections, paraneoplastic syndromes, matastasis);
- Type 4: Two or more IgG bands in CSF and serum that are specific – mirror type (eg, inflammatory neuropathy, Guillain-Barre syndrome, a neurological complications of systemic diseases such as collagen diseases, vasculitis, or malignancies);
- Type 5: Three to five identical IgG bands in CSF and serum specific distribution of bands looks like a ladder. The zones are the same distance from each other with the growth of intensity toward the cathodic end.

Only type 2 and type 3 indicating intrathecal synthesis of antibodies (10, 11).

In acute infectious inflammatory diseases of the central nervous system, high titers of specific antibodies in serum and cerebrospinal fluid are expected. Intrathecal synthesis of antibodies can be demonstrated in a range of neurological disorders, from acute and chronic infections of the central nervous system and autoimmune diseases such as multiple sclerosis, or neoplastic syndroma, neurolues, brain trauma, neurodegenerative diseases and many others, but also in healthy individuals. Intrathecal synthesis of antibodies can indicate a variety of disorders:

- Acute inflammatory disease of the central nervous system with an increased number of cells and disruption of barrier (albumin quotient is increased);
- Residual immunological activity that are in correlation with CNS infections in the past, which is not relevant to present clinical symptoms, usually manifest with normal number of cells, normal barrier function and low titers of serum IgM;
- Chronic inflammation of the CNS autoimmune type mostly, (eg MS) reflects in oligoclonal immune response.

The cerebrospinal fluid and serum of each patient were analyzed and immunoglobulins IgA, IgM and albumin, and on that basis is determined by the quotient (CSF/serum) that was added to Reibergram (12).

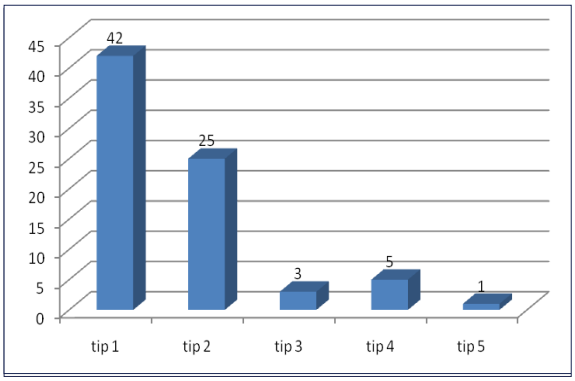


Figure 1. Results for analised patients devided in type 1 to type 5

3. RESULTS

We tested 76 samples of cerebrospinal fluid and serum of patients with suspected MS and got the following:

- 42 tested samples had type 1,
- 25 tested samples had type 2
- 3 samples had type 3,
- 5 samples had type 4,
- 1 sample had a fifth type

When we compared results of **25 patients with type 2**, with values obtained by intrathecal synthesis determined by Reibergram we obtained the following values:

- 16 samples had intrathecal synthesis of 20%–60%;
- 5 samples had intrathecal synthesis of 10%;
- 4 samples had intrathecal synthesis less than 10%.

Patients	Intrathecal synthesis	OCB
16	> 10%	Type 2
5	10%	Type 2
4	Less than 10%	Type 2

Table 1. Number of patients with quantitative and qualitative value of IgG

If the value of intrathecal synthesis by Reibergram was 20% and higher than 60% we usually had more than 10 IgG oligoclonal bands, and if the type 2 intrathecal synthesis was 10% by Reibergram we had one to two IgG bands (13, 14).

4. DISCUSSION

Detection of oligoclonal IgG band in CSF is most sensitive biochemical evidence of intrathecal synthesis of antibodies, but is also nonspecific. Oligoclonal IgG bands can be demonstrated

with different frequency in the range of chronic and subacute inflammatory diseases of the central nervous system of different etiology for example 95% of patients with multiple sclerosis and 100% of patients with subacute sclerosing panencephalitis (SSPE (15, 16).

Determination of IgG oligoclonal band by isoelectric focusing is more sensitive laboratory method for determination of intrathecal IgG synthesis than quantitative analysis. Therefore, in suspected subacute and chronic inflammation of the CNS is necessary to determine oligoclonal IgG bands regardless of the negative results obtained by quantitative analysis of antibodies and evaluation of findings in Reibergram (17, 18, 19). Intrathecal humoral type 2 response is most common pattern in MS found with bands in pH region 8-10, and the number and arrangement of IgG bands is generally not change during the disease, because they reflect long-term non-specific immune stimulation rather than a specific immune response that is changes in infectious disease (quantitatively and qualitatively).

For about 25% of patients with MS oligoclonal IgG bands can be detected also in serum: a intrathecal systemic response (type 3).

Different types of oligoclonal IgG response can be found in CNS infections:

- Only intrathecal response (type 2),
- Intrathecal systemic response (type 3),
- Only systemic response (type 4)

Type 2 and Type 3 we found mainly in cases where infectious germs penetrate the CNS and initiated long-term humoral response (subacute and chronic infections). Systemic inflammatory diseases such as autoimmune connective tissue disease, vasculytis, inflammatory neuropathy, or neoplastic disease usually triggered only systemic humoral response (type 4), which manifests with identical bands in CSF and serum. Unlike MS, in neurosarcoidosis or neurolupus, corticosteroid therapy can influence the number and intensity of the IgG bands in CSF (11). Increase of the bands number in liquor at immunocompromised people with congenital or hypogammaglobulinemia usually indicates the development of opportunistic infections. Finding bands only in CSF or in CSF and serum, does not mean monoclonality, but indicates the high specificity of the immune response (20, 21, 22).

5. CONCLUSION

In this study we noticed strong correlation between quantitative abnormal IgG CSF expressed in Reibergram and visible oligoclonal bands. Elevated IgG presented in Reibergram is highly predictive of oligoclonal bands. Quantitatively elevated IgG presented in Reibergram, without oligoclonal responce in some patients might indicate an unspecific responce, due to altered blood brain barrier in certain neurological disorders (23).

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