Detection Methods of Immunoglobulin IgG in CSF and Serum

Marina Delic-Sarac1, Sebija Izetbegovic2, Jasenko Karametic1, Djemo Subasic1, Aida Saban3, Tomislav Jukic4, Jozo Coric5, Mirsad Panjeta5
Clinical Immunology, Clinical Center of Sarajevo University, Sarajevo, Bosnia and Herzegovina1
General Hospital “Prim. dr. Abdulah Nakaš”, Sarajevo, Bosnia and Herzegovina2
Faculty of Stomatology and Pharmacy, University of Travnik, Travnik, Bosnia and Herzegovina3
Scientific Department of Biomedicine and Health, Faculty of Medicine, University of Osijek, Osijek, Croatia4
Clinical Biochemistry, Clinical Center of Sarajevo University, Sarajevo, Bosnia and Herzegovina5

INTRODUCTION

This study represents a new approach to the extended analysis of correlation of findings of oligoclonal bands on gels and the level of intrathecal synthesis of immunoglobulin G in the central nervous system. Previous studies have shown that there is no correlation at this level as well as the number of tape or finding does not correlate with the forecast effect of therapy or patient outcome. Aims of the study: To determine the correlation of level of immunoglobulins IgG in CSF with the number of oligoclonal bands on the gel. Material and methods: The retrospective study based on data processed in Clinical Immunology Clinical Center University of Sarajevo. Patients were assumed 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 results was also performed by using SPSS statistical analysis program. Results: Analyses were performed on 254 samples of cerebrospinal fluid and serum of patients from neurological clinic, suspected of multiple sclerosis. We concluded that there is no correlation between the level of intrathecal synthesis obtained by Reibergram with the number of oligoclonal bands on gels. We think that the reason could be a small sample of patients analyzed and it leaves room for future analysis on a larger sample. 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). Key words: oligoclonal bands, nephelometry, isoelectric focusing.

Corresponding author: Marina Delic-Sarac. Clinical Immunology, Clinical center of Sarajevo University, Sarajevo, Bolnicka 25. Phone: +387 33 297 030. E-mail: marinadelicsarac@gmail.com

1. INTRODUCTION

Multiple sclerosis is a chronic inflammatory progressive multifocal, demyelinating autoimmune disease of the central nervous system, which can manifest different symptoms of the nervous system. Symptoms may occur periodically (85-90%) or slowly progressive over time (1, 2).

The cause and pathogenesis of multiple sclerosis are not known. A hereditary factor plays an important role in pathogenesis of multiple sclerosis. Some of the triggers of autoimmunity may be an infection, psychological stress and postpartum period. It occurs mostly between 20 and 40 years of age, most often between 20 and 30 years of age. The disease is twice as common among women, but men have severe clinical course. Multiple sclerosis is disease of whites, and the prevalence of the disease increases with distance from equator (3, 4).

One of the risk factors in patho-ethiology of the disease are genetic variations on the alleles DR15, DQ6, HLA. Viral infection is commonly blamed environmental factor in genetic predisposed persons. This can lead to activation of the immune system against of its own myelin, which is why multiple sclerosis is classified in the group of autoimmune diseases (5, 6, 7).

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.

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 (8, 9, 10).

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...
2. PATIENTS AND METHODS

This study included 254 patients who were patients of the Department of Neurology Clinical Center University of Sarajevo. We analyzed serum and CSF samples on the presence of oligoclonal bands.

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 (15, 16). The procedure involves isoelectrofocusing on agarose gel, and conducted semiautomatic 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 (17, 18).

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 particles 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 (19, 20). 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 (21).

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 they identify involved immunoglobulin and provide the necessity of a specific test (22, 23).

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
- Avoid concentrating of CSF

Analysis of samples is performed in two steps:

- Isoelectric focusing on agarose gel to separate the proteins of cerebrospinal fluid and serum
- Immuno fixation 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.

**Figure 1.** Number 1 and 2 Oligoclonal bands represents type 2, number 3 oligoclonal bands represents type 1.
The concentration of IgG that is 1mg/dl or above that value, 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 significance.

Classification of oligoclonal immunoglobulin G (IgG) findings:

- **Type 1**: Normal findings of diffuse-type immune precipitation 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 post infectious syndromes, multiple sclerosis, opportunistic infections, paraneoplastic syndromes, metastasis);
- **Type 4**: Two or more IgG bands in CSF and serum that are specific – mirror type (e.g., 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 at the same distance from each other with the growth of intensity toward the cathodic end. Only type 2 and type 3 indicate intrathecal synthesis of antibodies.

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 syndrome, neurosyphilis, 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, (e.g. 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 (24, 25).

3. RESULTS

At Clinical Immunology Clinical Center University of Sarajevo we analyzed 254 samples of CSF and serum which were sent from the Department of Neurology Clinical Center University of Sarajevo, from May 2011 to October 2012. Of all samples 184 were women and 70 samples were from male patients. Of the total number of 254, patients treated samples, 82 samples had present oligoclonal bands on agarose gels. Of this number, 27 were found positive male patients and 55 female patients.

We used descriptive statistical methods to obtain information about the central tendency, the mean value and the variability of the observed data. We included patients that had positive oligoclonal bands on agarose gel. Most of our patients were 39 years old. The oldest patient with positive oligoclonal bands was 66 years old and the youngest was 14 years old.

We determined the values of albumin and IgG in CSF and serum with nephelometry method for the patients suspected to multiple sclerosis. After evaluation of the albumin and immunoglobulin we completed quotient and listed received values in Reibergram. The method of isoelectric focusing is determined by the presence of intrathecal synthesis of oligoclonal bands on agarose gel.

We analyzed 254 patients and in 174 patients, findings on the gel was negative, while in 80 samples we found oligoclonal bands on the gel. In each patient we observed and compared values of immunoglobulins IgG, IgA and IgG oligoclonal bands on gel.

Next, we sort our patients into three groups:

- **First group** which included patients with IgG synthesis by Reibergram in the 0-10% (negative intrathecal synthesis) and no oligoclonal bands on gel.
- **Second group** where patients had 10-30% synthesis by Reibergram and from 2 to 7 oligoclonal bands on gel.
- **Third group** which included patients with IgG synthesis by Reibergram from 31-80% and 8 to 15 oligoclonal bands on gel.
In SPSS program for statistical analysis of data we included values of immunoglobulin synthesis and the presence of oligoclonal bands on gel. We analyzed linear correlation of these values and obtained following: Pearson’s correlation coefficient is 0.671, which means that correlation is high. Significance is 0.000 (p<0.0005).

This is a significant correlation so we accept null hypothesis and the alternative hypothesis is rejected. The second null hypothesis we set up so that the number of oligoclonal bands obtained by separation on the gel correlates with the level of intrathecal synthesis of immunoglobulins IgG shown by Reibergram while the second alternative hypothesis is that the number of oligoclonal bands does not correlate with the level of intrathecal synthesis of immunoglobulins IgG shown by Reibergram.

We divided patients in the third group into two groups: One (three) and group two (three). In group one (three) are patients with intrathecal synthesis from 31% to 40% by Reibergram and number of oligoclonal bands on the gel are 7 to 10. In the group two (three) were patients with value of intrathecal synthesis from 41-80% by Reibergram ant more than 10 oligoclonal bands on gel.

Pearson correlation coefficient is 0.331 and the correlation is moderate. Significance p = 0.074> 0.05 means that there is no correlation.

From this it follows that the other rejects the null hypothesis because the significance of> 0.05, and there is no correlation between the number of oligoclonal bands we obtained by separation of the gel correlated with the level of intrathecal synthesis of immunoglobulins IgG shown by Reibergram.

We explained this result in a way, that the sample was relatively small (30 patients analyzed) because we divided all patients into separated groups and in the end we could analyze only 30 patients per group. This leaves room for future analysis of whether a larger sample of patients can get higher cor-

4. DISCUSSION

At Clinical Immunology Clinical Center University of Sarajevo we analyzed 254 samples of CSF and serum samples of patients with multiple sclerosis in period from May 2011 to October 2012. We gain the results which suggests that women is more like to be affected similar to other autoimmune disease in which is still the dominant share of the female population. Multiple sclerosis usually affects people between 20 and 50 year of age. Most of our patients were 39 years old.

Concentration of albumin quotient CSF / serum was recognized as a suitable method for the determination of individual variables in the function of the blood / CSF barrier and was introduced as it a convenient reference for most sensitive evaluation of other plasma proteins in the cerebrospinal fluid, due to the reduction of individual variables. Reibergram allows direct visual interpretation of results and diagnosis of functional disorders in the blood / CSF barrier and the presence of intrathecal immunoglobulin synthesis using CSF / serum concentration ratio of albumin. Method of isoelectric focusing determined the presence of oligoclonal IgG bands. When we analyzed the results obtained in an article published in the Journal of the Neurological Sciences 2001 by authors H. Reiber and JB Peter we have seen if we do not separate oligoclonal bands in the cerebrospinal fluid that is one of the strong evidence against the diagnosis of multiple sclerosis. Due to the high sensitivity of oligoclonal bands in the cerebrospinal fluid and the high specificity of the relevant clinical conditions, testing of oligoclonal bands in cerebrospinal fluid for the presence of IgG is intensively recommended to support the diagnosis of multiple sclerosis.

We found that the correlation coefficient is 0.671, which means that the correlation is high. Significance was 0.000 (p<0.0005). After that we divided our patients into two groups: one (three) and group two (three). In group one (three) patients with intrathecal synthesis by Reibergram was 31% to 40% and there was from 7 to 10 oligoclonal bands on gel. In group two (three), value of intrathecal synthesis according to Reibergram was 41-80% and there was more than 10 oligoclonal bands on gel.

Once we have the value of intrathecal immunoglobulin synthesis of IgG and albumin quotient included in Reibergram and split into two new groups, the values were analyzed by the statistical program SPSS linear correlation, and we got that the Pearson correlation coefficient is 0.331, which means that the correlation is moderate. Significance p = 0.074> 0.05 means that there is no correlation.

This result can be explained with the relatively small sample (30 patients analyzed) because we split all patients of the third group into two groups so that we could analyze only 30 patients per group.

REFERENCES

3. Posner CB, Paty D, Scheinberg D. Albumin quotient included in Reibergram and split into two new groups, the values were analyzed by the statistical program SPSS linear correlation, and we got that the Pearson correlation coefficient is 0.331, which means that the correlation is moderate. Significance p = 0.074> 0.05 means that there is no correlation. This result can be explained with the relatively small sample (30 patients analyzed) because we split all patients of the third group into two groups so that we could analyze only 30 patients per group.
4. DISCUSSION

At Clinical Immunology Clinical Center University of Sarajevo we analyzed 254 samples of CSF and serum