The importance of autoimmune antibodies in the laboratory diagnosis of neurological disorders


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Summary

Several immune markers are used in the modern laboratory diagnosis. The aim of this study is to present the correlation between certain immune markers their role in the diagnosis of neurological diseases. Method: We processed the data of patients who had immune marker measurements at the Marmed Laboratory during 2012 (antinuclear, anti-native DNA, anticardiolipin IgG, IgM antibodies). These subjects were previously diagnosed with neurologic diseases (n=143). The determination of these antibodies was performed by ELISA (enzyme-linked immunoassay) using the StatFax3200 analyzer. PCR (polymerase chain reaction) was used in another private laboratory (Bioclinica) to identify methylene-tetrahydrofolate reductase (MTHFR) deficiency. Statistical calculations were performed using GraphPadInStat. Results: The patients’ mean age was 46.30 years+/14.53 (SD), those diagnosed with an autoimmune disease were younger than those with other diseases. Vascular pathology was often associated with neurological disorders. In a few cases genetic background was identified. We obtained positive correlation between antinuclear and anti-native DNA antibodies, both were increased especially in patients suffering from autoimmune diseases. Conclusions: Immune markers have important an role in the diagnosis and monitoring of neurological diseases. There is correlation between certain autoimmune antibodies, and patients with autoimmune diseases have more frequently pathological values compared to those with other diagnoses.

Introduction

Modern laboratory medicine uses several autoantibodies for the proper diagnosis of autoimmune diseases. Many of these conditions have neurological and cardiovascular manifestations as well. Often complex panels of autoantibodies are requested by the clinicians to facilitate differential diagnosis. The most common autoantibodies used for this purpose in Romania are the antinuclear antibodies (ANA), anti-native deoxyribonucleic antibodies (ANDA) and antiphospholipid antibodies (APLA).

Antiphospholipid syndrome (APS) is a multisystem autoimmune disease characterized by the production of APLA, which promote recurrent vascular thrombosis, and/or pregnancy complications and

miscarriage. Thrombocytopenia is frequently associated to this syndrome (Zhu et al, 2014).

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with heterogeneous clinical manifestations and disease course. The antibody titers can be very different depending on the therapy and the phase of the disease (Kronbichler et al, 2016).

The ANA belong to a large family of autoantibodies that react with antigens present in the cell nucleus and have a major role in the diagnosis of autoimmune diseases. In SLE abnormal production of antibodies can be observed, these harming the healthy tissues. The skin, the kidneys, the bone marrow, the joints, the heart and the brain could all be involved. The hematological disorders in SLE include anemia, leucopenia and thrombocytopenia. The decreased platelet count is considered to be due to a peripheral immunological mechanism of cross-reactivity between the antiplatelet antibodies, the ANA and ANDA.

The anticardiolipin antibodies belong to the group of antiphospholipid antibodies specific for anionic (negatively charged) phospholipids, components of cell membranes. The anticardiolipin antibodies are often present in people with antiphospholipid syndrome.

Two clinical forms of the antiphospholipid syndrome have been described: primary or in association with other autoimmune diseases (SLE, Sjogren’s syndrome, scleroderma, mixed connective tissue disease, rheumatoid arthritis).

Autoantibodies can be correlated in some cases with the severity of autoimmune diseases and their presence can be used as marker for certain complications. Generalized SLE activity or damage and antiphospholipid antibodies are identified as major risk factors for neuropsychiatric involvement in lupus (Fanouriakis et al, 2013).

Because SLE symptomatology is heterogeneous, its diagnosis can be a considerable challenge, especially for young clinicians with limited expertise. This is particularly true in the early stages of the disease. Furthermore, the suboptimal performance of immunological testing in patients referred for possible SLE is a serious problem. As a result, SLE remains largely a clinical diagnosis that is made after excluding alternative diagnoses. Although it is often stated that at least 95% of patients with SLE have positive ANA test using immunofluorescence screening, the sensitivity of ANA testing can be as low as 70%, especially in early phases of the disease (Bertsias et al, 2013).

Autoantibodies are also generated in systemic sclerosis, and overlap with systemic lupus erythematosus. In APS autoantibodies are found mainly against phospholipids and cell surface proteins such as β2-glycoprotein 1 and lead to an increased risk of thrombosis in the patient (Leffler et al, 2014).

Fluorescence enzyme immunoassay (FEIA) is the gold standard technique with a highly sensitivity and specificity in the detection of autoantibodies like ANA (Mocanu et al, 2015), but enzyme linked immunoassay (ELISA) is also commonly used for the measurement of different autoantibodies. Investigation of the genetic background is important especially in case of thrombophilia.

The aim of this study is to present the importance of immune markers in neurological diseases, to reveal the correlation between them and to compare the results obtained in case of different subgroups.

Materials and methods

Antinuclear antibodies, anti-native DNA antibodies, anticardiolipin IgG and IgM antibodies were determined in case of 143 patients of the Marmed Laboratory in Tîrgu Mureş during the year 2012. The subjects were divided in two subgroups: those diagnosed with an autoimmune disease and those diagnosed with other diseases. The measurement of immune markers was performed at the Marmed Laboratory using the StatFax 3200 analyzer based on ELISA method. The commercial kits used were: Quanta Lite ANA, Quanta Lite dsDNA and Org 515 Anticardiolipin IgG/IgM. PCR (polymerase chain reaction) was performed in
another private laboratory (Bioclinica) for selected cases to reveal MTHF reductase homozygous or heterozygous mutation (C677T and A1298C).

Statistical processing of the results was performed by the GraphPad InStat software (unpaired Student t test with and without Welch correction and Pearson’s correlation test). The threshold of significance was set at \( p<0.05 \). The Kolmogorov-Smirnov test was used to check the normal distribution of the obtained data.

Results and discussions

The patients’ mean age was 46.30 years +/- 14.53 (SD). Patients with autoimmune diseases were younger - average age 42.09 years +/- 13.46 (SD) - compared to those with another diagnoses – mean age 47.90 years +/- 15.31 (SD), the difference is not quite significant (\( p=0.0698 \)).

Neurological diseases often have vascular pathology as background disease. 48.3% of the studied subjects had cardiovascular disease as associated pathology. The studied patients’ diagnosis included thrombosis at a young age, autoimmune diseases, etc.

We obtained a positive statistical correlation between ANA and ANDA antibodies (\( r=0.6511, p<0.0001 \)). No correlation has been found in case of other autoantibodies present in these patients.

The average value of anticardiolipin IgG autoantibodies was 2.42 GPLU/ml +/- 1.12 (SD) – the lowest value 0.40, the highest 6.30 GPLU/ml , while the mean anticardiolipin IgM autoantibody level was 2.50 MPLU/ml +/- 1.29 (SD) – the lowest value 0.70, the highest 6.80 MPLU/ml.

In 11.4% of the tested patients with neurological diseases we observed pathological values of ANA, especially in those with autoimmune disorders and neuropathies, while 7.1% of the patients presented increased anti-native DNA antibodies. Normal ranges for ANDA are under 200 IU/ml, 0-23 IU/ml for antinuclear antibodies, 0-10 GPLU/ml for anticardiolipin IgG antibodies and 0-7 MPLU/ml for anticardiolipin IgM antibodies.

The average values of the ANA and ANDA in the two subgroups of patients are presented in Table 1.

<table>
<thead>
<tr>
<th>Type of the autoantibody</th>
<th>Patients with autoimmune diseases</th>
<th>Patients without autoimmune diseases</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native anti-DNA antibodies</td>
<td>145.74 ±138.54</td>
<td>89.89 ± 76.60</td>
<td>0.2346</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>15.25 ± 23.71</td>
<td>6.57± 4.28</td>
<td>0.0649</td>
</tr>
</tbody>
</table>

In three cases of early thrombosis MTHFR deficiency was observed (PCR method). This leads to elevated homocysteine levels, a cardiovascular risk factor enhancing thrombosis. Repeated miscarriages and neurological disorders due to brain thrombosis were also observed. All these patients with thrombophylia were 28-38 years old.

Discussion. A limitation of our study is that our research group measured only four types of autoantibodies, and the available methodology was ELISA. Enzyme immunoassays or other automated assays have a sensitivity between 70–98% for the detection of immunofluorescence-positive ANA titers \( \geq 1:160 \). ANDA - which can be detected by the Farr assay, Crithidia luciliae immunofluorescence test (CLIFT) or ELISA - are found in up to 70% of patients with SLE at some point during the course of their disease and have 95% specificity in established SLE cohorts, being a valuable diagnostic marker. The prevalence of patients
with SLE and a positive ANDA despite a negative ANA result has been reported to be under 5.5%. Early studies demonstrated that patients with a positive test for both ANA and high-avidity ANDA (by Farr assay) are at increased risk of developing SLE within a few years (Bertsias et al, 2013).

Another limitation is that we made a cross-sectional laboratory research and we didn’t have the opportunity to follow the patients and the dynamics of their autoantibodies. This is also true for the patients with thrombophilia.

According to the literature, apparently higher incidence of secondary APS was detected in ANDA positive SLE patients. Research data suggest that anti-DNA positivity cannot be considered as the only predictor of secondary APS and further studies may be necessary to detect other factors which may increase the incidence of APS in SLE patients (Gamal et al, 2013).

SLE patients with neurological manifestations have increased genetic predisposition and novel genomic approaches are expected to elucidate its pathogenesis. Animal data suggest that, in cases of disturbed blood–brain barrier, autoantibodies may cause diffuse neuropsychiatric manifestations through brain inflammation or neuronal apoptosis; only very few human data are available regarding this aspect. In neuropsychiatric SLE, advanced neuroimaging uncovers structural and metabolic abnormalities in brain regions with normal appearance on conventional magnetic resonance (Fanouriakis et al, 2013).

Unfortunately our patients had limited access to expensive genetic diagnosis, only in special, selected thrombophilia cases PCR was performed to reveal possible MTHFR mutations.

Conclusions

Immune markers have an important role in the diagnosis and monitoring of neurological diseases, especially easily available ELISA methods can be widely used for detecting different autoantibodies. Positive correlation could be observed between certain autoimmune antibodies (such as between ANA and ANDA). Although these auto-antibodies can be detected in different disorders, they have important diagnostic role in patients suffering from autoimmune diseases.

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References


