Abstract

The heterogeneity of antiphospholipid antibodies (APA) may be reflected not only in the specificity of different antigens but also in their avidity. APA avidity appears to be clinically useful as a valuable additional characteristic. APAs that are not included in the laboratory criteria for antiphospholipid syndrome (APS), the, so-called non-criteria antibodies, have been detected in patients with seronegative antiphospholipid syndrome. Antiphosphatidylethanolamine (aPE) antibodies represent one type of APA directed against neutral phospholipids, phosphatidylethanolamines.

The main objectives of this dissertation were:

1) to compare several modifications of ELISA (enzyme-linked immuno sorbent assay) with different chaotropic reagents for the determination of avidity of anticardiolipin antibodies (ACLA),

2) to investigate the relationship between ACLA IgG avidity and their levels,

3) to evaluate the levels and avidity of aPE as an example of non-criteria antibodies in several groups of patients and compare them with conventional APAs.

To compare different methods for determining the avidity of ACLA IgG, we tested a modified ELISA method using different concentrations of urea and sodium chloride as chaotropic reagents and different serum dilutions. We analyzed the levels and avidity of ACLA and aPE in the serum of patients with thrombosis and immunologically related diseases. In some patients, changes in APA and avidity levels were long-term monitored.

In the methodological part of the study, we demonstrated that the ELISA method for determination of avidity using a single dilution of serum in the presence of a fixed concentration of the chaotropic agent is suitable for determination of ACLA IgG avidity and is in good agreement with more challenging procedures.

The results of the clinical part of the study showed that the ACLA avidities did not differ between the groups of patients classified according to the ACLA levels. IgG/IgM aPEs were significantly higher in the group of patients with venous thromboembolism than in patients with nonthrombotic internal disorders. IgG/IgM aPEs elevated above our cutoff values were found in 10.8% of patients with venous thromboembolism and as the only APA in 6.5%. Levels of aPE IgG levels higher than our cutoff values were found in 29% of patients with immunologically mediated diseases with other positive APAs. Avidities of aPE IgG ranging from low to high levels were found in patients from both cohorts. The avidities of aPE IgG differed from those of ACLA IgG. ACLA and aPE IgG levels and avidities did not change significantly during follow-up.

The results of our investigation extended the knowledge of the avidity of APA and some non-criteria APAs both methodologically and clinically. ACLA avidity does not seem to be related to ACLA levels, but is one of the stable characteristics. Furthermore, we found that aPE may be related to venous thromboembolism and may be part of the APA repertoire in immunologically mediated diseases.

Keywords: Antiphospholipid antibodies, anticardiolipin antibodies, antiphosphatidylethanolamine antibodies, antiphospholipid syndrome, ELISA, avidity, chaotropic agents, urea, sodium chloride, thromboembolism, immunologically mediated diseases.