Anticardiolipin antibodies in patients with autoimmune diseases: Isotype distribution and clinical associations


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SUMMARY A prospective study of IgG and IgM isotypes of anticardiolipin antibodies (aCL) was performed in a series of 167 patients with various autoimmune diseases, including rheumatic and nonrheumatic disorders, and in a group of 100 healthy blood donors. The IgG aCL serum was regarded as positive if a binding index (BI) greater than 2.85 (3.77 SD) was detected and a BI greater than 4.07 (3.90 SD) was defined as positive for IgM aCL. Forty patients (24%) were found to be positive for IgG and/or IgM aCL. IgG aCL were detected in 23% of patients with systemic lupus erythematosus (SLE), in 9% with idiopathic thrombocytopenic purpura, in 7% with progressive systemic sclerosis, and in 6% with dermatomyositis-polymyositis. IgM aCL were present in 43% patients with primary biliary cirrhosis, in 33% with rheumatoid arthritis, in 22% with SLE, and in 8% with giant-cell arteritis. IgG aCL were found to have a significant association with thrombosis and thrombocytopenia, and IgM and aCL with haemolytic anaemia and neutropenia, in SLE but not in the other autoimmune diseases. The identification of these differences in the aCL isotype associations, depending on the autoimmune disorder, may improve the clinical usefulness of these tests.

Key words: Anticardiolipin Antibodies, Antiphospholipid Antibodies, Autoimmune Diseases, Systemic Lupus Erythematosus, Thrombosis, Thrombocytopenia, Haemolytic Anaemia.

Several recent studies found that patients with antiphospholipid antibodies (aPL) are prone to repeated episodes of venous and/or arterial thrombosis (1-11), recurrent fetal loss (6-8,11-15), thrombocytopenia (2-6,8,9,11,15,16), haemolytic anaemia (17), and neurological events such as cerebrovascular accidents (18), seizures, migraine (19), and chorea (20). Among the diversity of aPL, anticardiolipin antibodies (aCL) received greater attention due to their sensitive, reproducible and reliable detection by radioimmunoassay (3) or ELISA method (21-25).

Systemic lupus erythematosus (SLE) is the autoimmune disease where aCL were first reported. Subsequently, they have been found in a variety of other autoimmune disorders, haematologic and nonhaematologic neoplasms, infections, drug hypersensitivity reactions, and pregnancy. In addition, in several cases, there has been no known underlying association and they may be present as an isolated idiopathic phenomenon (14,21-25).
Despite these reports, prospective studies in large series of patients with autoimmune diseases are very few (14, 21, 24). On the other hand, most previous studies appear to have relatively low or variable cut-off levels for aCL positivity and a small number of normal controls. In the present report, we have studied a large cohort of unselected patients with various autoimmune diseases, including rheumatic and nonrheumatic disorders, and a large group of healthy blood donors. In addition, a previously standardized ELISA method has been used to detect aCL, and the cut-off level for positivity has been calculated using statistical analysis. Thus, the purpose of this study was to determine the frequency and isotype distribution of aCL in 167 consecutive patients with different autoimmune diseases seen at our center, and in 100 healthy blood donors. We have also examined the relationship between aCL and different clinical manifestations and biological parameters.

**PATIENTS AND METHODS**

**Patients and controls**

Clinical and laboratory features of 167 consecutive and unselected patients (125 females and 42 males) with different autoimmune diseases were prospectively studied during the years 1986-87. Sixty patients had been diagnosed as SLE, 24 had giant cell arteritis (GCA), 23 idiopathic thrombocytopenic purpura (ITP), 16 dermatomyositis-polymyositis (DM-PM), 16 primary biliary cirrhosis (PBC), 15 rheumatoid arthritis (RA), and 13 progressive systemic sclerosis (PSS). All diagnoses were determined according to established or proposed criteria (26-32). The normal control group consisted of 100 age and sex-matched healthy blood donors from the Blood Bank of the Hospital Clinic. All of them showed normal coagulation assays and negative serological tests for syphilis.

**aCL enzyme-linked immunosorbent assay (ELISA)**

aCL were measured by an ELISA method as described by Loizou et al. (24) and Gharavi et al. (25) with minor modifications of our own. Briefly, the flatbottomed wells of microtiter plates (Nunc, Denmark) were coated with 30 ul/well of cardiolipin (Sigma) suspended in ethanol at a concentration of 50 µg/ml and left to dry overnight at 4°C. The plates were treated for nonspecific binding of immunoglobulins by incubation with 110 ul of 10% fetal calf serum (FCS, Flow) in phosphate-buffered saline (PBS, pH = 7.2) solution for 2 hr. The wells were then washed four times with 120 ul of PBS. To assay for the presence of aCL in a patient’s serum, 100 ul of 1:100 dilution of the serum in PBS-FCS solution were added in triplicate to the test wells and 100 ul of PBS-FCS was added to the blank control wells. The plates were incubated for 1 hr at room temperature. After washing with PBS, the first antibody (100 ul), a goat anti-human IgG or IgM (Tago Inc.) diluted 1 :4000, was added to each well and incubated for 1 hr at 37°C. The plates were washed again with PBS and 100 ul of alkaline phosphatase-conjugated secondary antibody (rabbit anti-goat IgG, Sigma), diluted 1:1000 in PBS-FCS, were added to each well. The plates were then placed in a humidifier incubator at 25°C for 1 hr. They were washed with diethanolamine buffer (DEA, pH = 9.8), and P-nitrophenyl phosphate (1 mg/1 ml), prepared in DEA immediately before use, was added (100 ul) to each well. The plates were incubated in the dark at room temperature for 1 hr. The reaction was stopped by addition of 3 M NaOH (50 ul) to all wells and the optical absorbance (OA) was read at 405 nm on an ELISA microplate reader (Organon). Results were expressed as binding index (BI) calculated from OA values as follows:

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BI = \frac{OA \text{(test samples)} - OA \text{(blank)}}{OA \text{(referred normal pool)} - OA \text{(blank)}}
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