General Views on Autoantibody Tests, Allergy Tests and Immunodeficiency Tests in Dermatology

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Introduction

The reliability of a diagnostic test is determined by its specificity and sensitivity. The sensitivity of a diagnostic test is defined as the ratio between the number of true positives and the sum of true positives and false negatives. The specificity of a test is defined as the ratio between true negative results and the sum of the number of true negative results and false positive results.

Anti-nuclear antibodies (ANA, ANF)

This is used as a screening test for systemic lupus erythematosus (SLE) in which 95% of cases are positive. It is also useful for screening of non-organ specific autoimmune diseases. These are detected by indirect immunofluorescence using HEp-2 cells which has replaced rat liver as a substrate. Four patterns are seen: centromeric pattern correlates with CREST; nucleolar pattern correlates with scleroderma; while the speckled and homogenous patterns are non-specific. The most common patterns are the homogenous and speckled patterns. Titres indicate the highest dilution at which the antibody can still be detected. ANA can be detected in 80% of patients with Sjogren's syndrome and 70% of patients with rheumatoid arthritis. However, it may also be positive in a low titre (1:40) in 20% of normal individuals and the titre itself is not useful for monitoring disease activity. A titre of 1:160 is significant and would require further investigation.

Anti-DNA antibodies

These tests are done when the ANA is > 1:40. They are best detected by the Farr assay (RIA) which is more specific than ELISA. However, the ELISA technique is more sensitive and less expensive (as the latter detects low affinity antibodies which are not specific for SLE). It is therefore used as a screening test. When the ELISA test is positive, the Farr assay is used for further evaluation.

Anti-dsDNA is diagnostic and specific for SLE. However, it has a low sensitivity for SLE as only 70% of SLE cases are positive. Anti-dsDNA level correlates with disease activity and increased level precedes disease relapse by several months. Nevertheless, treatment should be modified according to the clinical picture and not the level of anti-dsDNA.

Anti-ENA antibodies (extractable nuclear antigens)

These are detected by counter-current immunoelectrophoresis (CIEP), and double immunodiffusion. ELISA and Western blot are gradually being introduced. Anti-ENA antibodies that are frequently tested include Sm, nRNP, Ro/SS-A, La/SS-B, Jo-1, Scl-70, ribosomal P, and centromere antibodies. Their titres are not useful for monitoring the disease activity. Two or more techniques are often required for sufficient sensitivity. In the immunology laboratory in the University of Hong Kong, CIEP is supplemented by immunoblot.

Anti-Sm antibody is specific for SLE but is only present in 30% cases. It has common epitopes with anti-nRNP (nuclear ribo-nucleoprotein) and is almost always present with anti-nRNP but not vice versa. Anti-nRNP is found in high titre in 90% of cases with mixed connective tissue disease. Low titre of nRNP is present in a small percentage of other connective tissue diseases (e.g. SLE).

Anti-SS-A/Ro antibody is present in 60% of primary Sjogren's syndrome and in 60% of SLE. It is also found in neonatal lupus eythematosus, congenital heart block and subacute cutaneous lupus erythematosus. The Ro antigen consists of 60 kd and 52 kd molecules. The 52 kd molecule is not detected by CIEP and requires ELISA or immunoblot. It is more closely associated with neonatal lupus erythematosus.

Anti-SS-B/La antibodies are present in 30% of Sjogren's syndrome. It is less sensitive but more specific than anti-SS-A antibodies. It is almost always associated with anti-SS-A but not the reverse. La antigen is physically associated with Ro antigen.

Anti-neutrophil cytoplasmic antibody (ANCA)

These are useful for the diagnosis of small vessel vasculitis and are detected by indirect immuno-fluorescence using human leucocytes as a substate which are processed by ethanol fixation. There are two staining patterns: cytoplasmic (cANCA) and perinuclear pattern (pANCA). The perinuclear staining pattern is an artefact of ethanol fixation. cANCA targets proteinase 3 which is tested if cANCA is positive. Anti-proteinase 3 indicates disease activity. Myeloperoxidase is targeted by pANCA and is tested if pANCA is positive. cANCA is detected in Wegener's granulomatosis, while pANCA is detected in Churg-Strauss syndrome and microscopic polyarteritis. Titres are useful in monitoring disease activity.

Anti-skin antibodies

The antibodies detected in pemphigus vulgaris target the intercellular substance and are present in 90% of patients. Titres correlate to disease activity and antibodies can be transferred through the placenta resulting in neonatal disease. In bullous pemphigoid, IgG binds to the target antigens of molecular weight 180kd and 230 kd at the dermo-epidermal junction. Titres known are not correlate to disease activity. Complement is activated resulting in inflammation and clinical disease.

C1 esterase inhibitor, C3 and C4

C1 esterase deficiency may be congenital (which is rare), or secondary, such as in SLE or lymphoproliferative disorders. Deficiency of C1 esterase inhibitor results in excess activation of the complement system via the classical pathway, generating a kinin-like substance leading to angiodema. Different complement abnormalities occur depending on whether activation occurs via the classical or alternative pathway: low level of C4 occurs when complement is activated by the classical pathway, while C3 is reduced when either the classical or alternative pathway is activated. In the laboratory, C3, C4 and C1 esterase inhibitor are detected by nephelometry. Both C3 and C4 are useful in monitoring SLE.

Immunoglobulin E (IgE)

Total IgE is raised in atopy, but may be normal in atopic eczema. Allergen specific IgE is useful in situations where the history of atopy suggests specific allergens (e.g. food allergies). They can be detected by skin prick tests which are cheaper; or by blood tests (RAST-radioallergosorbent test) which are more useful in suspected food allergies. However, in practice, the results may not correlate with the clinical picture.

Tests of immunodeficiency

Clinically, a history and/or family history of recurrent infection may be suggestive of immunodeficiency. The type of infection may indicate the defect in the immune system, e.g. recurrent fungal, viral or protozoal infection in defective cell-mediated immunity; recurrent systemic bacterial infection occurs in phagocytic disorders; recurrent bacterial pneumonia occurs in hypogammaglobinaemia; and recurrent neisserial infection in C6-9 complement deficiency.

Screening tests can be divided into those which evaluate the T and B cell populations, immunoglobulins and the complement system. Immunization is a good method of testing the antibody response (eg. pneumovax, tetanus, diphtheria), but in immunodeficient patients live vaccines are contra-indicated due to the risk of transmission of disease. IgG subclasses can also be evaluated by radial immunodiffusion or ELISA. In some cases, normal level of IgG occurs and the defect is due to functional deficiency. Deficiency of one IgG subclass may occur in normal individuals. At the moment, there is no clinical application for detecting IgA subclass.

T-cell immunity can be investigated by assessing the response to mitogens (phythaemagglutinin) and delayed type hypersensitivity (DTH) to various antigens. Proliferation of T-cells may be tested by 3H-thymidine incorporation, while neutrophil and monocyte phagocytic function may be assessed by the nitro-blue tetrazolium reduction test which assesses the integrity of the respiratory burst. Other aspects of neutrophil phagocytic function that can be assessed include killing of micro-organisms, ingestion and opsonization, expression of adhesion molecules CD11/CD18 and chemotaxis (Boyden chamber technique, agarose gel technique).

Complement function can be assessed by measuring CH50. This evaluates the haemolytic activity

of serum. Sensitised sheep erythrocytes are used as the substrate for assessing the classical pathway while rabbit erythrocytes are the substrate for assessing the alternative pathway. These are indicated when hereditary complement deficiency is suspected.

Learning points:

Allergen specific IgE testing is indicated only if the clinical history is suggestive, but false negative results are not uncommon and clinical correlation is often difficult. The practical value is therefore limited in the management of atopic patients.