

CLINICAL IMMUNOLOGY & ALLERGY DIAGNOSIS GUIDE

CONTENTS

Allergy/Hypersensitivity	page 3
Other Hypersensitivity	page 4
Autoimmunity	page 5
Immunological Tests in Obstetrics/Gynaecology	page 7
Infection & Immunodeficiency	page 7
Malignancy	page 8
Neurological Disease	page 9
Renal Disease	page 9
Rheumatology/Connective Tissues Autoimmunity	page 10
Vasculitis	page 11

Clinical Synopsis

Allergy/Hypersensitivity

Brief Overview

Allergy tests may help identify which allergens suggested by the history could cause symptoms. However, the finding of an antigen specific IgE in the serum does not prove that the antigen is responsible for the symptoms under investigation, nor does it necessarily indicate that avoidance measures will help the patient. Specific IgE testing provides similar, although not identical, information to skin testing; but may be particularly valuable in assessing some groups of patients (young children, extensive eczema/dermographism, taking antihistamines, past history of anaphylaxis). Total IgE is needed to interpret the significance of the specific IgE.

Conjunctivitis

When the allergic reaction is strictly limited to the conjunctiva allergy tests are frequently negative. When conjunctivitis is part of a more generalised allergy, specific IgE and skin prick tests are usually positive for the causative allergen.

Rhinitis

Allergy tests may help distinguish allergic from vasomotor or other causes of rhinitis.

Total IgE is often in the normal range or slightly elevated.

Specific IgE may be sought to inhalant allergens: the range of allergens tested should be sensibly guided by a careful history but should generally include allergens to which most people are exposed such as cat and HDM. Investigation of seasonal rhinitis is only indicated if there is some doubt about the diagnosis or if desensitisation is being considered. The pollens involved in seasonal rhinitis or asthma are as follows: grasses (May-Sept), trees (March-May), weeds (July-Sept). There is little point in finding the exact pollen allergen unless it is intended to desensitise the patient - when skin tests are mandatory.

Asthma

Total IgE is usually raised in extrinsic asthma where specific IgE to relevant allergens is detectable. A very high total IgE may be found in allergic bronchopulmonary aspergillosis. Specific IgE should invariably be sought against the house dust mite. Often the history will suggest the appropriate animals (cats, dogs, horses etc). IgE to Aspergillus is associated with the need for closer monitoring and maybe more intensive steroid treatment. For seasonal asthma see seasonal rhinitis.

Atopic eczema

Total IgE is often markedly elevated in widespread disease and Specific IgE may be present at high level to allergens that cause no overt symptoms. Any positive specific IgE results therefore need careful interpretation. Specific IgE to house dust mite is often high and house dust mite allergy may exacerbate eczema in such patients.

Anaphylaxis

Please refer all patients for full clinical assessment; laboratory tests need careful interpretation. Blood samples for mast cell tryptase taken within 1-2 hours of the reaction will be helpful to confirm that the reaction was anaphylactic.

Anaphylaxis and anaphylactoid reactions to drugs used in anaesthesia etc.

Please refer the patient to the Allergy clinic for assessment.

Acute urticaria

Total IgE and Specific IgE may help identify the causal antigen involved in type I hypersensitivity reactions.

Chronic urticaria

Total IgE often is normal - high values should prompt further investigation.

Bronchopulmonary eosinophilia

Total IgE, specific IgE to *Aspergillus*, and *Aspergillus* precipitins should identify cases due to hypersensitivity to the fungus.

Total IgE may be raised in association with parasitic infestation.

Positive ANCA (anti-neutrophil cytoplasmic antibodies) may point to a vasculitic cause (Churg-Strauss).

Food allergy and intolerance

Evidence of specific IgE antibodies may be consistent with a diagnosis of food allergy, but, unfortunately, the presence of such antibodies does not prove clinical sensitivity. Elimination and food challenge testing may be more directed assessments. Laboratory immunology tests cannot help investigate non-allergic food intolerance. Referral to the clinical allergy service may be appropriate.

Other Hypersensitivity

Farmer's lung

Hypersensitivity to the spores of thermophilic actinomyces may be the cause of acute disease 4-8 hours after exposure (cough, dyspnoea, malaise & fever) or chronic symptoms with progressive dyspnoea and fatigue.

Precipitins to thermophilic actinomyces (performed in virology) (Farmer's lung) indicate exposure but are not invariably associated with disease. The

diagnosis is made by a combination of clinical features, X-ray and lung function tests.

Bird fancier's disease

The symptoms are similar to farmer's lung but more commonly are of the chronic type.

Precipitins to avian proteins (performed in virology) provide good evidence of the cause of the symptoms.

Coeliac disease (Gluten sensitive enteropathy)

The immune response in GSE is directed towards epitopes formed between tissue transglutaminase and gliadin (the alcohol soluble fraction of gluten). IgA antibodies to tissue transglutaminase (antibodies to endomysium) are found in active disease, and can be used to monitor compliance with treatment. Similar antibodies are seen in dermatitis herpetiformis. We may have to measure IgA levels to ensure that IgA deficiency (particularly common in these patients) is not causing a false negative result.

Autoimmunity

Screen

Requests for "autoimmune screen" are not acceptable. Requests should be for specific autoantibodies as indicated by the history.

Thyroid goitre / nodule, hypo / hyperthyroidism

The level of antibodies to thyroid peroxidase is closely related to the degree of lymphocytic infiltration in the thyroid. Levels are raised in autoimmune thyroiditis (90% of hypo-, >60% of hyper-) and also post-viral and post-partum thyroiditis. They are far less often raised in thyroid neoplasia/nodules/cysts, but their presence does not exclude these conditions.

Adrenal failure and gonadal failure

In the UK Addison's disease is most often due to autoimmunity; the presence of antibodies to adrenal cortex strongly indicates an autoimmune cause. There may also be antibodies to steroid producing cells of ovary and testis. A small proportion of cases of premature menopause are due to autoimmune oophoritis. Some of these patients also have adrenal failure - the same tests are done for both.

Liver autoimmunity: Autoimmune hepatitis and Primary biliary cirrhosis

PBC and AIH are associated with characteristic autoantibodies that are helpful in classifying the hepatitis and separating autoimmune AIH from the other forms. Patterns may include antibodies to smooth muscle and/or nuclei, liver/kidney microsome antibodies in LKM-positive autoimmune hepatitis and antibodies to mitochondria in PBC. Presence of autoantibodies does not exclude a viral cause for the hepatitis.

Because of the overlap between the various different forms of hepatitis it is usually best to test for all the types of autoantibody - AMA, SMA, LKM and ANA. The profound disturbance in immune regulation, and in the normal processing of gut derived antigens will lead to characteristic changes in the levels of IgG, IgA and IgM. Serum electrophoresis may reveal lack of alpha 1 antitrypsin if this is associated with the cirrhosis.

Primary sclerosing cholangitis has no definitive serological markers, but may be associated with ANCA (anti-neutrophil cytoplasmic antibodies) or ANA or SMA.

Polyendocrine autoimmunity

- Type 1: usually presents under 10 yrs old, m=f, hypoparathyroidism, adrenal failure and candidiasis - maybe also hepatitis, alopecia, delayed puberty, etc.
- Type 2: adolescent/early adult, f>m, Addison's + thyroid failure, type 1 DM - maybe gonadal failure, vitiligo.
- Type 3: older, f>>m, autoimmune thyroiditis together with DM, gastric autoimmunity (GPC, anti-IF) - maybe other such as myasthenia

Ab to adrenal cortex, ovary, testis, thyroid microsomes, GPC, islet cells, (+ ANF, SMA, AchR etc if indicated). The spectrum of results may help confirm the diagnosis.

Diabetes (insulin-dependent)

Type 1 Diabetes is associated with characteristic Autoantibodies tested under the umbrella term "islet cell Autoantibodies". In most cases the clinical presentation of Type 1 diabetes is sufficiently classical that auto-antibody measurement is not required. However it is now estimated that 10% of Type 2 (maturity onset) diabetes is actually autoimmune in nature and progress to be insulin requiring. Therefore, measurement of islet cell antibodies is clinically indicated (i) when presentation is atypical and (ii) when apparent Type 2 diabetes is proving difficult to manage by diet and medication alone or is atypical (eg insulin requiring).

Pemphigus / pemphigoid

Blistering skin conditions may involve autoimmunity - antibodies are found to the epidermal intercellular "cement" in pemphigus, and to the epidermal basement membrane in pemphigoid. The pemphigus-like pattern is also seen in some patients with leprosy, burns, penicillin rashes, SLE, MG with thymoma, dermatomycoses, erythema multiforme etc - that of pemphigoid in herpes gestationis and epidermolysis bullosa acquisita. The appropriate investigation is anti skin antibodies.

Dermatitis herpetiformis

Though the diagnosis of DH is based on the appearance of the rash and IgA at the dermo-epidermal junction in the dermal papillae in biopsies, the presence of IgA antibodies to endomyseum may point to the association of

DH with gluten sensitivity - (see Coeliac disease). This disease cannot be diagnosed with anti skin antibody testing.

Immunological Tests in Obstetrics/Gynaecology

Recurrent foetal loss

Mid-trimester fetal loss may be due to thrombosis of placental vessels in the anti-phospholipid syndrome (primary or associated with SLE). The appropriate investigation is anti cardiolipin antibodies both IgG and IgM

Gonadal failure

This may be due to autoimmune damage to the gonads in males or females associated with antibodies to ovary, testis and adrenal cortex. This is if found with adrenocortical failure in autoimmune polyendocrinopathy type I.

Infection & Immunodeficiency

Brief Details

Please phone to discuss the investigation of recurrent unusual infection. Five basic arms of defence can be considered in the fight against infection. These are represented by non-specific resistance (local barriers, neutrophils, complement), and specific resistance (B cells-antibodies; T cells- cell mediated immunity).

Recurrent infections at one site might suggest localised defects (eg recurrent pneumonia and cystic fibrosis).

Appropriate responses to persistent infection may include a neutrophil leucocytosis and hypergammaglobulinaemia, together with raised levels of acute phase reactants (CRP, complement components).

The nature of the organism responsible for recurrent infection may give valuable clues into the possible type of deficiency.

Secondary causes of immunodeficiency are more common than primary causes. Low levels of immunoglobulins might reflect decreased production (eg lymphoproliferative disorders, drugs) or increased losses (nephrotic syndrome).

Screening tests for primary immunodeficiency, if appropriate, must include neutrophil count and morphology, lymphocyte count, serum immunoglobulins, and CH50 (broad test of classical pathway function). Further tests should be directed towards the suspected arm of defence considered deficient, and

might include tests of neutrophil function, assessments of functional antibodies to specific organisms already encountered (eg after immunisation),

flow cytometric analysis of the different populations of T and B lymphocytes, and the measurement of other complement components.

CD4 monitoring in patients with AIDS gives information about the progress of the disease.

Malignancy

Lymphoproliferative disorders

The demonstration of paraproteins by electrophoresis is one of the criteria required for a diagnosis of multiple myeloma. Their quantification, the presence of free urinary light chains, the degree of associated immunosuppression of other immunoglobulins, and the excessive proliferation of plasma cells in the bone marrow are all laboratory indicators of a malignant paraproteinaemia. Serum levels of b2-microglobulin are useful indicators of prognosis, partly reflecting the degree of renal damage (and are raised in other causes of renal failure, malignancies, and some autoimmune disorders).

Serum Free Light Chains

This test is for the quantitation of serum free light chains, both kappa and lambda.

The result will also include the Kappa: Lambda ratio.

The early identification of plasma cell dyscrasias is key to their treatment and management. "Intact" myeloma is readily detected in conventional serum electrophoresis. However, as always there are caveats to such a statement. Other forms of myeloma, light chain disease and non-secretory disease, do not exhibit monoclonal immunoglobulin in serum detectable by electrophoresis. In the case of light chain disease urine electrophoresis is the conventional diagnostic. Accurate quantitation of urinary free light chains is not without problems and hence is not an ideal tumour marker. SFLC measurement is able to detect abnormal concentrations in both of these conditions. Also difficult to detect by serum electrophoresis are cases of AL amyloidosis, where this assay has been shown to be of particular use (Reference 1).

The half-life of SFLCs is measured in hours unlike intact immunoglobulin, measured in days. This allows a rapid indication of response to therapeutic intervention. This in turn may reduce the need to continue expensive drug regimes, which have less than the desired efficacy.

Neurological Disease

Myasthenia

Impaired neurotransmission in Myasthenia Gravis (MG) is caused by the presence of antibodies to the acetylcholine receptor. Associated autoantibodies to skeletal muscle and thyroid microsomes are sometimes

found. Though antibodies to acetylcholine receptor (AChR) are always present they are detectable in only 90%. They may be undetectable in 40% of patients with ocular myasthenia. Antibodies to striated muscle are present in 30% of patients with MG - and 60% of these will also have thymoma, this test is insufficiently reliable to help in management and is no longer performed.

Motor neuropathy

Anti-ganglioside GM1 antibodies are present in 80% of patients with pure motor weakness with evidence of multifocal conduction block. Low titre AGA are present in some sensorimotor neuropathies, SLE, other autoimmune disease and normal controls, rare, even by comparison with MG, but increasingly seen in paraneoplastic syndrome as well. Stiff man syndrome, Axial stiffness and rigidity associated with Autoantibodies to glutamic acid decarboxylase. Screening test is GAD antibodies/islet cell antibodies.

Renal Disease

Brief Overview

Antigen-antibody reactions are responsible for many causes of glomerulonephritis. Screens aimed at identifying humorally mediated renal disease should include measurements of serum immunoglobulins and complement components (C3, C4). CH50 may help detect primary defects of complement; it need only be tested once for each patient. Other investigations may address the underlying cause (eg ANA, rheumatoid factor in autoimmune disease, ANCA in systemic vasculitis, cryoglobulins in mixed cryoglobulinaemia).

Serum C3 levels are low in some forms of membranoproliferative glomerulonephritis, reflecting the presence of the circulating autoantibody C3 nephritic factor which binds and activates C3 convertase. Serum IgA levels may be raised in IgA nephropathies including Henoch Schönlein purpura. p-ANCA associated glomerulonephritis is the common form of necrotising crescentic glomerulonephritis, reflecting different vasculitic causes. The combination of renal and lung involvement may suggest Goodpasture's syndrome due to the presence of antibodies to the glomerular basement membrane (GBM). Serum immunoglobulins may be low in poorly selective proteinuric forms of the nephrotic syndrome (focal glomerulonephritis).

Rheumatology/Connective Tissue Autoimmunity

Screening

It should usually be possible to perform more directed investigations, but for second line investigation of PUO or high ESR/CRP it may be helpful to consider systemic autoimmune disease by screening for antinuclear antibodies (ANA), rheumatoid factor (RF), and raised immunoglobulins. If a positive ANA is found, we would investigate the specificity of the antibody binding further, as described below. Low autoantibody titres are usually not significant.

Although rheumatoid factor is often taken as an indicator of RA it is raised in 15% of the population without RA following chronic inflammation or infection and is not raised in 15% of adult RA, 95% of juvenile RA. Anti-CCP is now an essential first line investigation in patients suspected of having rheumatoid arthritis (RA). Unlike rheumatoid factor anti-CCP is found only in patients with RA. Rheumatoid factor is not specific for RA as it is found in patients with other autoimmune and infective disorders. Anti-CCP is said to be present early in the disease progression and as such offers the opportunity for early therapeutic intervention. There is some evidence to suggest that those patients presenting with moderate to high levels of anti-CCP are at greater risk of early erosive disease.

SLE

Criteria for the diagnosis of SLE include antinuclear antibodies (ANA), antibodies to ds-DNA, antibodies to extractable nuclear antigens (ENA) (particularly antibody to the Sm antigen), and anticardiolipin antibodies. The pattern of antinuclear antibody staining and the presence of particular groups of serum antibodies may be associated with different clinical patterns of disease activity.

Other typical immunological findings include raised serum IgG, and low serum complement levels (C3, C4, CH50), as well as the presence of rheumatoid factor, and other autoantibodies. C4 levels (and ESR) are of some help in monitoring disease activity.

Sjögren's syndrome

There may be considerable overlap with other autoimmune disorders, including SLE. Characteristically antinuclear antibodies and antibodies to extractable nuclear antigens (particularly antibodies to Ro or SSA, and antibodies to La or SSB) are found. Rheumatoid factor and raised immunoglobulins (particularly IgG1 subclass) may be found.

Scleroderma / systemic sclerosis

The pattern of antinuclear antibody may help define this group (e.g. presence of anti-centromere antibody associated with the CREST syndrome). Other antibodies to extractable nuclear antigens (particularly antibodies to Scl70) may be found.

Polymyositis / dermatomyositis

Antinuclear antibodies are common and antibodies to extractable nuclear antigens (particularly antibodies to Jo-1) are seen in >30% patients, especially those with pulmonary fibrosis.

Mixed connective tissue disease

The presence of autoantibodies to antinuclear antibodies and to extractable nuclear antigens (particularly antibodies to RNP), without other lupus markers, may support this clinical diagnosis.

Primary antiphospholipid antibody syndrome

Recurrent thrombosis (or fetal loss) may be associated with antibodies to phospholipids including cardiolipin. Related antiphospholipid antibodies include the lupus anticoagulant. Cardiolipin antibodies may be found in other autoimmune disorders, particularly SLE. Coagulation investigations (ordered from haematology) are also useful in diagnosis.

Vasculitis

Brief Overview

The term vasculitis refers to inflammation of blood vessels, and represents a heterogeneous group of clinical disorders. Immunopathological mechanisms may be involved in primary (eg Wegener's) and secondary vasculitides (eg infection, neoplasia, connective tissue disease, cryoglobulinaemia).

Small vessel (hypersensitivity) vasculitis

Infection, drugs, foreign proteins (as examples) may be causal factors in a vasculitis affecting predominantly the skin. Immunological findings may sometimes include raised ESR/CRP, depressed levels of complement factors suggesting consumption, and the presence of antinuclear antibodies and rheumatoid factor (low titre). General screening tests for vasculitis should also include serum immunoglobulins, and ANCA (see below).

If there is evidence for more extensive visceral involvement, one of the primary systemic vasculitides may be involved (see below). Alternatively, the vasculitis may be secondary to autoimmune disease (eg SLE, chronic active hepatitis - see earlier section), neoplasia (eg lymphoma), cryoglobulinaemia (these are immunoglobulins that form precipitates in the cold).

Primary systemic vasculitides

Some forms of systemic vasculitis are strongly associated with circulating antibodies to neutrophil cytoplasmic antigens (ANCA). In Wegener's Granulomatosis, (WG) (lung, renal) there is a diffuse cytoplasmic pattern (c-ANCA), as well as polyclonal elevations of IgG, IgA, IgE, and raised CRP. ANCA's with a perinuclear pattern (p-ANCA) are seen in some patients with polyarteritis nodosa, (PAN) (weight loss, musculoskeletal, renal), and Churg-Strauss ("asthma", eosinophilia, hypocomplementemia, raised IgE). Both types of ANCA may be seen in microscopic polyarteritis, (MPA) (clinical

overlap between classic PAN and WG). Atypical forms of ANCA reactivity may be seen in association with Henoch-Schönlein Purpura (sometimes IgA raised), Kawasaki's syndrome, and in other autoimmune disorders (eg SLE, ulcerative colitis).

Hereditary angio-oedema

Recurrent abdominal pain and/or deep subcutaneous swellings without urticaria (particularly occurring after minor trauma), often with family history, may indicate HAE. C4 and C1 inhibitor will be low. Uncommonly there may be normal C1INH level with defective function. If C4 is very low without other explanation and C1INH normal, C1INH function will be measured.

Acquired C1INH deficiency

Consumption/inactivation of C1INH may occur in SLE and lymphoproliferative disease. This may lead to episodes of angio-oedema as with the inherited form. C1q is low in acquired C1INH deficiency but usually normal in HAE.