PLIG: Immunology Form

Review of Tests

Introduction
The Lothian immunology request form is no longer generic to both primary and secondary care since the introduction of the hospital TRAK system. The majority of GPs are unfamiliar with many of the tests listed on the current form as these autoimmune conditions are rare and complex. There is concern that many tests are done inappropriately or requested by doctors then unable to interpret the results. Now that the form is only used by General Practitioners it can be redesigned to meet their needs.

The current immunology form also includes allergy testing which is an issue separate to autoimmune disease testing. There is no allergy service in Lothian and testing is controversial. This is an area that needs its own consideration and will not be discussed here.

Autoimmune diseases are diagnosed in light of clinical symptoms not test results. Testing is complex and the following issues should be considered:

- Very few tests correspond directly to diagnoses.
- Large proportions of ‘normal’ populations may test borderline positive for some tests (e.g. ANA).
- Negative results do not exclude conditions (e.g. seronegative rheumatoid arthritis)
- Many autoimmune diseases are extremely rare and require specialist input regardless of test results
- Test results alone cannot be used to guide referral and secondary care advice is easily accessible
- Many of these tests are very costly (IgG £35.27)

A brief reminder of the following statistical terms:
Sensitivity = percentage of true positives correctly identified (useful for confirming diagnosis).
Specificity = percentage of true negatives correctly identified (useful for excluding diagnosis).

Review of the current immunology form tests
Divided into 4 areas
1. Organ Specific Antibodies [pg 2-5]
2. Immunochemistry [pg 6-8]
3. Rheumatoid Arthritis and Connective Tissue Disease autoimmune serology [pg 9-14]
4. Vasculitides (Small Vessel Vasculitis) and Goodpasture’s syndrome autoimmune serology [pg 15-17]
1: ORGAN SPECIFIC ANTIBODIES

> **Thyroid Peroxidase (TPO)**

Associated with autoimmune thyroid disease

- Graves (70-90%), Hashimoto’s Thyroiditis (85-100%), Primary Atrophic Hypothyroidism (60%)\(^1\)

But also positive in other autoimmune diseases

- Pernicious anaemia (60%), Type 1 DM (20-25%), Myasthenia Gravis (15-25%)\(^1\)
- Low levels in 2-8% of normal individuals, esp. elderly and women.\(^1\)

In subclinical hypothyroidism request anti-TPO antibodies to help to determine if an autoimmune process is present and help predict risk of progression to overt hypothyroidism.\(^2\) If anti-TPO antibodies are positive then TFTs should be checked annually as patients will likely progress to overt hypothyroidism at some point in the future. There is clear guidance on refhelp.\(^2\)

*Note: TSH receptor antibodies (TRAB) are performed by biochemistry not immunology and are performed automatically by the biochemistry laboratory on all hyperthyroid bloods (associated with Graves’ disease).*

**Recommendation:** This test has a clear role in primary care.
It should be performed in all patients with newly diagnosed subclinical hypothyroidism.

> **Gastric Parietal Cell Antibodies** and **Intrinsic Factor Antibodies**

- Used in the investigation of pernicious anaemia
- Pernicious anaemia: megaloblastic anaemia secondary to B12 deficiency due to intrinsic factor deficiency – see haematology refhelp guidance.

**Intrinsic factor antibodies**

- Diagnostic for pernicious anaemia
- But only present in 50-60% cases\(^1\) (sensitive- true positives, not very specific- lots false negatives)

**Gastric parietal cell antibodies:**

- positive in >90% patients with pernicious anaemia\(^1\), but it is also positive in many other conditions H.Pylori gastritis, Addison’s, autoimmune thyroiditis, DM (if this test is negative the cause is unlikely to be pernicious anaemia)
- however it is diagnostic of **chronic autoimmune gastritis** whether or not this is due to pernicious anaemia\(^1\). (i.e. evidence of parietal cell destruction but not enough to cause intrinsic factor deficiency and anaemia)
- asymptomatic positive results should be followed up as they may progress to pernicious anaemia

*Note: this test is performed on LKS tissue which automatically also tests for anti-smooth muscle antibody and anti-mitochondrial antibody (see relevant sections). GPs should be aware that if these tests are positive they will be reported even if they were not requested.*
Recommendation: This test has a clear role in primary care. Both gastric parietal antibodies and intrinsic factor should be tested in investigation of possible pernicious anaemia. ³

> Smooth Muscle Antibodies

Associated with Type 1 Autoimmune Hepatitis (40-70%). ¹
But also positive in PBC (50%) and cryptogenic cirrhosis (28%). ¹

- The majority of patients are between 10 and 30 years of age. ⁴
- Seventy-five percent are female.
- Presentation is usually insidious. The patient may be generally unwell and jaundiced.
- Amenorrhoea is common. Epistaxis, bleeding gums and easy bruising may be other complaints.

Examination may reveal:

- spider naevi are almost invariable - usually on the face, neck or arms
- striae on the abdominal wall, usually the lateral aspect
- acne and hirsutism
- splenomegaly - in the absence of portal hypertension
- lymphadenopathy
- hepatomegaly - in early disease; the liver then gradually shrinks
- ascites, oedema, hepatic encephalopathy - late features

- 25% percent of cases present as an acute hepatitis
- About 20% of patients run an anicteric course. ⁴

Blood results

- LFTs: raised bilirubin, raised transaminases (x10), (ALP normal or slightly raised) ⁴
- raised ESR, normocytic anaemia, prolonged prothrombin
- raised AFP (in 1/3 cases) ⁴

Anti-SMA can be diagnostic of type 1 autoimmune hepatitis, especially if ANA also positive (ANA positive in 80% cases)

> Anti-Mitochondrial Antibodies

Used in diagnosis of Primary Biliary Cirrhosis (90%) ¹
Also can be positive in secondary Sjogren’s syndrome, autoimmune thyroid disease, SLE, polymyositis

Primary Biliary Cirrhosis: cholestatic liver disease classically affecting middle aged women

- Range of reported prevalence rates in the UK (12.9 per 100,000 - 30/million)
- Female predominance (95% cases are women)
- Aged 30-65yrs
- 50% cases are asymptomatic with incidental finding of hepatomegaly or raised ALP
- Signs: hyperpigmentation, xanthelasma, xanthomata, hepatomegaly (70%), splenomegaly (35%), signs of cirrhosis; spider naevi, dupuytrens, limb wasting, ascites, oedema, palmar erythema
- Bloods: markedly raised ALP, [raised transaminases and bilirubin are late findings]
- Elevated HDL, low albumin, prolonged prothrombin are late signs associated with cirrhosis

In the lab there are 9 patterns of AMA (M1-M9). M2 most useful. If any AMA pattern is found results are divided into Non-M2 and M2 titres. M2 type AMAs are found in 90% PBC and considered diagnostic.

In PBC, SMA is raised in 50% cases, ANA (20%), IgM (80%).

Note: both smooth muscle antibody and anti-mitochondrial antibody tests are performed on LKS tissue which automatically also tests gastric parietal cell antibody (see relevant sections). GPs should be aware that if any of these tests are positive they will be reported even if they were not requested.

Recommendation: Both SMA and AMA tests have a clear role in primary care. Immunology testing can be used as part of investigation of persistently abnormal LFTs. If performed it should include SMA, AMA and ANA.

>Pancreatic Islet Cell Antibodies

Used in the diagnosis and management of Type 1 Diabetes

- Previously testing for pancreatic islet cell (PIC) antibodies
- But combined Anti-GAD and Anti-IA-2 are more sensitive and replacing PIC testing
- Anti IA-2 highly sensitive for type 1 DM. Antibody titres at diagnosis can be used to predict slow or rapid progression to beta cell failure
- Anti-GAD found in type 1 and 2 diabetics. Presence in type 2 diabetics is suggestive of progression to insulin dependency
- Positive anti-GAD in gestational diabetes is highly predictive of progression to type 1 diabetes postnatally

NICE and SIGN guidance does not support using antibody testing to routinely diagnose diabetes but they can possibly be used in specialist circumstances to distinguish type 1 from type 2 diabetes in rare cases of uncertainty. Type 1 diabetes is diagnosed in primary care by blood glucose levels and ketonuria and GPs will refer patients to secondary care for full assessment. Progression to insulin in type 2 diabetes is done on clinical grounds not immunology tests.
While there are known antibody markers of prediction in high risk subjects, there is no evidence for effective methods of prevention of type 1 diabetes. Screening for pre-type 1 diabetes is not recommended in either the general population or in high risk children and young people.  

**Recommendation:** These tests have no clear role in diagnosing or managing diabetes in primary care.

> **Adrenal Cortex Antibodies**

Used in the diagnosis of Addison’s disease
- positive in 75% of patients with Addison’s disease at the time of diagnosis
- also found in type 1 diabetes and Autoimmune polyendocrinopathies but very rarely positive in ‘normal’ individuals

However unless there is a clear cause then Addison’s disease is assumed to be autoimmune in origin (it is autoimmune in 70-90% of cases)
- Addison’s disease is rare. (1 per 10,000 people, currently 8400 people in UK)
- 60% of people with Addison’s have an autoimmune polyendocrine syndrome

Diagnosis is often missed because it is rare and has non-specific symptoms
- Fatigue, hyperpigmentation, weight loss, N+V, abdominal pain, muscle weakness/cramps, postural hypotension, headache, low grade pyrexia, polydipsia, polyuria, delayed puberty
- If suspected perform serum cortisol (8-9am) but low sensitivity due to diurnal variation, and U+Es- low sodium and high potassium
- But Addison’s is diagnosed by SynACTHen test which is usually done in secondary care.

This is a ‘send away’ test performed in Sheffield which reflects the infrequency of testing.

**Recommendation:** This test has no clear role in diagnosing Addison’s disease in primary care.

> **Ovary and Testis Steroid Cell Antibodies**

Associated with autoimmune gonadal failure [premature ovarian failure 30-70%, testicular failure- very rare condition], Addison’s disease (18%) and autoimmune polyglandular syndrome (APGS)-60-80%

All of these conditions are very rare and unlikely to be diagnosed in primary care. This is also a ‘send away’ test performed in Sheffield which again reflects the infrequency of testing.

**Recommendation:** These tests have no clear role in primary care.
2: IMMUNOCHEMISTRY

> Complement

C1 Esterase Inhibitor (C1-INH) deficiency is associated with Hereditary Angioedema (HA).
- usually starts in childhood
- recurrent episodes of angioedema and/or abdominal pain
- may involve the larynx - can be fatal
- diagnosis suggested by recurrent episodes of non-urticarial swelling unresponsive to antihistamines, positive family history, recurrent episodes of unexplained abdominal pain and vomiting, episode of laryngeal oedema, onset in childhood
- Steroids and anti-histamines are not effective in attacks
- investigation involves C4 levels, C1-INH levels and C1 function if available
- Results dictate subtype - type I, II, or III
- If diagnosed also need to perform FBC, U+Es, LFTs, lipids, blood borne virus testing, urinalysis and liver/spleen USS as likely to need blood products if future episodes (plasma derived C1-INH shortens attack and acts in 30-60 mins - untreated attacks usually last 2-5 days)
- If complement testing negative also consider if HA is; drug induced (ACEI), allergic, acquired, idiopathic

Issues regarding testing
There are issues about whether this is a condition likely to be diagnosed in primary care. There is no adult allergy or anaphylaxis service in Edinburgh. We need to consider where these patients would be referred - possibly dermatology. However C1 testing may be useful if recurrent episodes as could guide management in future, particularly if there is a life threatening episode.

Complement C3 can be used to monitor disease activity in SLE.

Recommendation: This test should not normally be initiated by primary care. Its role in diagnosis and management is uncertain and as such it may be more appropriately used in secondary care.

> Immunoglobulin Deficiency

Classified depending on area of immune system affected: B cell, T cell, phagocytes and complement
- 5 isotypes of immunoglobulins: IgG, IgA, IgE, IgM, IgD
- Predominant class is IgG (73% of total)

IgG deficiency
- 4 subtypes: IgG1, IgG2, IgG3, IgG4
- IgG deficiency divided into selective (associated with normal levels of IgA/E/D/M) and deficiency associated with inadequate levels of other immunoglobulins
- IgG4 deficiency is found in 20% ‘normal’ patients and is rarely clinically significant
IgG2 deficiency is the most common deficiency. It can be associated with IgG4 and IgA deficiency. It is associated with recurrent infections.  

The classification of IgG subclass deficiencies as primary immunodeficiency is controversial. IgG subclasses 2 standard deviations below the age matched ‘normals’ are considered deficient. But they may be asymptomatic and have no clinical consequences. Selective deficiency can occur where there is a normal or high total IgG (e.g. in SLE, EBV, HIV).

Primary Immunodeficiency

- Usually single inherited gene disorders, (80% of pts <20yrs old at diagnosis)
- Incidence 1 /10,000
- Antibody deficiency syndromes: hypogammaglobulinaemia, thymoma, x-linked agammaglobulinaemia, Di George syndrome, severe combined immunodeficiency disease

Secondary immunodeficiency

- Lymphoreticular malignancy (CLL, multiple myeloma),
- Viruses (HIV),
- Drugs (steroids, cytotoxic drugs)
- Nutritional (vit A, zinc, selenium)
- Metabolic (liver and kidney failure)
- Trauma (major surgery)
- Protein loss (nephrotic syndrome, protein losing enteropathy)

Primary and secondary immunodeficiencies present with

- Recurrent infections, especially respiratory
- Severe, persistent, recurrent bacterial infection
- Infections which lead to complications, e.g. bronchitis -> pneumonia, bronchiectasis and respiratory failure
- Opportunistic infections: pneumocystis carinii, CMV, resistant thrush, oral ulcers, warts
- GI symptoms: diarrhoea, malabsorption, failure to thrive
- Haematological abnormalities: leucopaenia, thrombocytopenia, haemolytic anaemia
- Neurological problems: seizures, encephalitis
- Autoimmune disease: arthralgia, vasculitis

Clinical findings

- Look ill, cachexia, skin changes
- Eyes inflamed
- Chronic ENT infections
- Bibasal crepitations
- Hepatomegaly, splenomegaly
- Children: delayed development

Screening tests in primary care

- IgG, IgA, IgM, ESR
- CXR, appropriate swabs
Issues regarding testing

- There is no clinical immunology service in Edinburgh. Guidance on interpreting results can come from the immunology laboratory but they will not be able to offer clinical assessment or management. If GPs are concerned and patient warrant further investigation possible approach would be to refer to specialities such as ENT (chronic sinusitis), respiratory, paediatrics or infectious disease.
- Expensive Test: currently costs £35.27 for test.
- Guidance from the Oxford handbook of clinical and laboratory investigations: There are no absolute indications for testing as significant immunodeficiency can occur in the presence of normal subclasses and conversely complete genetic absence of a subclass may be completely asymptomatic. Measurement is usually performed on patients as part of work up for recurrent infections but would usually be done in secondary care (e.g. respiratory for recurrent chest infections). ¹²

Recommendation: This test should not routinely be initiated by primary care. Its role in diagnosis and management is uncertain and as such it may be more appropriately used in secondary care.
>Rheumatoid Arthritis (RA)
- clinically look/feel/move approach to joint examination
- characteristic joints – bilateral symmetrical polyarthritis commonly affecting MCP joints, wrist and PIPJ
- If clinically suspicious -> consider urgent referral to early arthritis clinic [see refhelp for details]
- Bloods: anti-CCP, ESR (but anti-CCP can be negative in seronegative RA)
  Consider ANA if also signs/symptoms suggestive of connective tissue disorder
- XR hands/wrists/feet
- Remember to multiply ASSIGN score by x1.5 if the patient has two out of the following three: seropositive, rheumatoid arthritis for over 10 years or extra-articular involvement

Anti-CCP positive in 70% patients with RA and can be present up to 10yrs before diagnosis. Table below taken from talk by Dr Helen Harris. 

<table>
<thead>
<tr>
<th>Rheumatoid arthritis</th>
<th>Sensitivity of +ve CCP result</th>
<th>Specificity of +ve CCP result</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months</td>
<td>45-65%</td>
<td>95-98%</td>
</tr>
<tr>
<td>Established RA</td>
<td>70-80%</td>
<td>95-98%</td>
</tr>
</tbody>
</table>

Also rarely found in Sjogren’s (suggests progression to future RA), Juvenile Idiopathic Arthritis and a subgroup of patients with Type 1 autoimmune hepatitis.

The use of anti-CCP is well established and routinely used in primary care although results as always should be interpreted with clinical findings.

Recommendation: anti-CCP testing has a clear role in primary care. Anti-CCP should be requested only when there is a clinical suspicion of inflammatory arthritis. Patients found to have clinical features suggestive of synovitis should be referred up to the clinic irrespective of their anti-CCP result.

> CONNECTIVE TISSUE DISEASE (CTD)

Tests:
- ANA (Anti-nuclear Antibodies),
- Anti dsDNA (Anti-double stranded DNA)
- Anti-ENA (Anti-Extractable Nuclear Antigens)
  *Includes antibodies to: histones, RNP, Ro, La, Sm, Scl70, Jo1
- ACA (Anti-cardiolipin).

Reviewed in relation to specific CTD diseases GPs might be expected to be aware of (CTDs listed in Oxford Handbook of GP).
>Systemic Lupus Erythematosus (SLE)

More common in black populations and women.

Diagnosing SLE can be difficult as it presents with numerous non-specific symptoms. Classification criteria require four of the following to be present (these criteria were developed to classify lupus in clinical trials and not for diagnosis):

- Malar rash
- Discoid lesions
- Photosensitivity
- Oral ulcers
- Arthritis
- Serositis
- Renal disorder
- Haematological disorder
- Immunological disorder
- Positive ANA

Features

General: fatigue, fever, malaise, anorexia, weight loss
Cutaneous: mouth ulcers, diffuse alopecia, malar rash, photosensitivity, discoid lesions, nasal ulcers, vaginal ulcers, Raynaud’s
MSK: arthralgia, myalgia
Haem: leucopaenia, lymphopaenia, thrombocytopaenia
Renal: nephritis, proteinuria/haematuria and casts, nephrotic syndrome
CNS: headache, seizures, aseptic meningitis, CVA, myelopathy, demyelinating syndrome (Guillane-Barre), movement disorders, anxiety, psychosis
Pulmonary: pleurisy, pneumonitis, pulmonary haemorrhage, pulm hypertension
CVS: pericarditis, myocarditis, endocarditis
GI: abdominal pain, nausea, vomiting, diarrhoea (all in up to 50%), mesenteric vasculitis ->acute abdomen, aseptic peritonitis, bowel obstruction, hepatitis, sclerosing cholangitis, pancreatitis, ascites

Investigations

FBC, U+Es, LFTs, CRP (usually not elevated; high CRP would suggest infection and sometimes serositis in a lupus patient)
Urinalysis

Antibodies

ANA (90-95%)¹
Anti-dsDNA (60-80%)¹ can be useful for assessing disease activity and usually rises during a flare

Anti-ENA (just done once- not for monitoring),
   ->Anti-Ro, Anti La and antiphospholipid antibodies if considering pregnancy¹
   ->Anti-RNP associated with mixed CTD¹
C3 C4 used for monitoring disease activity (levels fall with disease activity due to complement consumption)¹
>**Sjogren’s syndrome**

**Symptoms:** sicca symptoms (dry eyes and mouth)

**Primary vs secondary:** associated with SLE, RA, SS, PBC

**Primary:** features similar to mild SLE (arthritis, photosensitivity, fatigue, alopecia)

**Antibodies:**
- High total IgG
- ANA (70%), Anti-CCP (if positive suggestive of future RA)
- Anti-ENA [anti-Ro (60-70%), anti-La (50-60%)]

>**Antiphospholipid Syndrome**

**Cause of recurrent thrombosis, stroke/TIA and recurrent miscarriage.** May be associated with SLE.

**May find:** thrombocytopaenia, haemolytic anaemia, livedo reticularis (skin appearance), cutaneous vasculitis (when associated with SLE)

**Antibodies:**
- Anticardiolipin Antibody (ACA) (in 90%) (in 90%)
- Lupus anticoagulant (in 20%-but assoc high risk thrombosis)
  - lupus test done by haematology not immunology (2 green tubes and 1 brown)

**Antibody tests need to be positive on 2 separate occasions 6 wks apart**

**Testing should be done prior to starting anticoagulation in patients with a DVT.**

>**Raynaud’s Disease/Phenomenon**

**More common in younger people and in women**

**Episodic cold induced vasospasm**

**Primary vs secondary**

**Secondary:** Occupation (outdoors/fishing/vibration tools/chemicals- vinyl chloride)
  - Proximal vascular occlusion (examine CVS)
  - Drugs: beta blockers, contraceptives migraine therapy, bleomycin
  - Autoimmune rheumatic disease: symptoms arthritis /SLE/Sjogren’s/Systemic Sclerosis (95% pts with SS have Raynaud’s)

**Primary**

If no evidence of additional features no further investigation is necessary.

Those patients who have positive **autoantibodies (esp. ANA) and evidence of microvasculopathy in nailfold capillaries** are likely to go on to develop CTD (10% transition).

*this is a very specific feature highly suggestive of CTD.

**Antibodies**
- ANA – used for its negative predictive value. If negative unlikely to have other CTD or progression to CTD and therefore can be reassured

**CKS**—based on 5 review articles ‘All pts with Raynaud’s should have ANA checked routinely’
Systemic Sclerosis

Systemic sclerosis is part of the spectrum of scleroderma and scleroderma-like disorders. Consisting of:

- Diffuse Cutaneous Systemic Sclerosis
- Limited Cutaneous Systemic Sclerosis
- Overlap syndromes
- Systemic sclerosis sine scleroderma (systemic features without skin involvement)

Diffuse Cutaneous Systemic Sclerosis

Sx: Raynaud’s, skin thickening, renal/cardiac/pulmonary (fibrosis), gastrointestinal (particularly oesophageal disease), articular and muscular damage

Antibodies:

- ANA (55-75%),
- Anti-ENA [RNP (20%), Scl70 (15-20%), nucleolar (50-60%)]

Limited Cutaneous Systemic Sclerosis (previously referred to as CREST)

Sx: sclerodactyly, Raynaud’s, digital ulceration, microstomia, telangiectasia, oesophageal symptoms, small bowel malabsorption, pulmonary hypertension

Antibodies:

- ANA (55-75%),
- Anti-ENA [RNP (20%), Scl70 (15-20%), centromere (60% CREST), nucleolar (50-60%)]
- ACA (60%)

Dermatomyositis/Polymyositis

Polymyositis and dermatomyositis are characterised by acute and chronic inflammation of striated muscle. In dermatomyositis there is an accompanying dermatitis.

- RARE 5-10 cases per 100,000 population
- Can be associated with malignancy
- Proximal muscle weakness (but muscle bulk preservation distinguishes it from limb girdle dystrophy).
- Myositis: muscle tenderness, proximal and symmetrical muscle weakness, difficulty rising from a chair. If severe -> dysphagia, dysphonia, respiratory failure
- Skin features (dermatomyositis): heliotrope rash, Gottron’s papules, periungual telangiectasia, cuticular hypertrophy with punctate infarcts, poikiloderma, calcinosis cutis
- Muscle biopsy is diagnostic
- Bloods: Creatine Kinase elevated
- ESR/CRP may be elevated
- ANA (30-40%),
- Anti-ENA [Jo1 (20-25%)]

Clarifying Terminology

- Undifferentiated CTD - Pt has features seen in CTD but does not meet criteria for a defined CTD
- Overlap syndrome - Pt meets criteria for 2 or more CTDs (‘rhupus’=SLE+RA)
- Mixed CTD - Overlap with RA, SLE, scleroderma and myositis with antibodies to U1RNP
This table shows the **relative percentage sera positive antibodies in CTD** (immunology handbook) \(^1\)

<table>
<thead>
<tr>
<th>Antibodies to</th>
<th>SLE</th>
<th>Drug induced lupus</th>
<th>Mixed CTD</th>
<th>Sjogren’s syndrome</th>
<th>Progressive systemic sclerosis</th>
<th>Dermato/poly myositis</th>
<th>Rheumatoid arthritis</th>
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<tr>
<td>Nuclei</td>
<td>90-95</td>
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<td>DsDNA</td>
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<td>ENA – Histones</td>
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<td>5-10</td>
<td>20</td>
<td>20</td>
<td>15-20</td>
<td>30</td>
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</tbody>
</table>

*LCSS: limited cutaneous systemic sclerosis

ANA is present in many connective tissue disorders. Results are given as either negative, positive, borderline (1/80) or strongly positive (1/640). ANA particularly in low titres can be found in the normal population, especially the elderly.\(^1\) If ANA is positive in addition to the titre the pattern of staining is reported. GPs should be aware that with certain ANA staining patterns the laboratory will perform reflex testing.

- ANA Speckled -> ENA screen
- ANA Homogenous Nucleolar -> dsDNA
- ANA nuclear dots -> anti-mitochondrial antibody

ANA testing should be undertaken when there is a clinical suspicion of a CTD. Positive predictive value of ANA testing is around 11%. With a higher pretest probability on an underlying CTD this value increases.

The other autoantibodies should be used as an aid to diagnosis rather than to exclude or diagnose clinical conditions. It is questionable whether these should be primary care or secondary care based tests. The Rheumatology talk at the Lister suggested only ANA should be requested but would it be appropriate to request other autoantibodies if there is sufficient clinical indications? A study in Italy in 2004 used a protocol to try to rationalise requests for antibody assays.\(^1\) They used ANA as first stage testing and only went on to further testing if ANA was positive. If clinicians wanted other autoantibodies (second stage testing) they were requested to use tick boxes to indicate other clinical information. This led to a significant reduction in requests and they found that 90.5% of patients positive
for second level tests had clinical confirmation of rheumatic disease. The clinical information on their protocol is below;

- Systemic inflammatory signs
- Typical rash
- Erythema
- Photosensitivity
- Raynaud’s phenomenon
- Arthritis
- Myalgia
- Xeropthathalmia
- Oral ulcers
- Xerostomia
- Pleuritis/pericarditis
- Interstitial lung disease
- Psychosis
- Elevated serum CK
- Proteinuria
- Cellular casts
- Haemolytic anaemia
- Leucopenia
- Thrombocytopenia
- Lymphopaenia

**Recommendation:** ANA testing may have a role in primary care in patient in whom a connective tissue disease is suspected clinically.

**Recommendation:** ds DNA, anti-ENA and ACA should not routinely be initiated by primary care. These tests require specialist clinical experience to interpret. They could be considered as second line tests if ANA is positive with appropriate secondary care guidance.
4. VASCULITIDES AND GOODPASTURE’S SYNDROME

> **Vasculitides**

The vasculitides are divided into large, medium and small vessel vasculitis.

**Large**: no immunology tests
- Giant cell arteritis
- Takayasu vasculitis

**Medium**: no immunology tests
- Polyarteritis nodosa
- Kawasaki disease

**Small**: ANCA

> **Small/Medium Vessel Vasculitis**

Early non-specific symptoms: fever, malaise, arthralgia, myalgia, weight loss.
Differential diagnosis: primary or secondary causes

<table>
<thead>
<tr>
<th>Primary small vessel vasculitis</th>
<th>Secondary small vessel vasculitis</th>
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<td>Wegener’s granulomatosis*</td>
<td>Rheumatoid arthritis</td>
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<td>Microscopic polyangiitis*</td>
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<td>Churg Strauss*</td>
<td>Sjogren’s syndrome</td>
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<td>Cryoglobulinaemic vasculitis</td>
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*ANCA associated

**Essential investigations**: inflammatory markers, full blood count, renal function and urinalysis to look for microscopic haematuria and proteinuria.

> **Wegener’s Granulomatosis (WG) now known as granulomatosis with polyangiitis (GPA)**

**Incidence**: rare 8.4 per million annually

**Features**
- Triad of upper and lower airways plus renal disease (limited forms are recognised but may progress to generalised disease)
- Granulomatous inflammation involving respiratory tract
- Necrotising vasculitis affecting small to medium sized vessels of glomerulus
Clinical symptoms & signs

- **Upper respiratory tract**: sinusitis – nasal crusting, bleeding, obstruction and collapse of nasal bridge, serous otitis media, tracheal stenosis.
- **Lung disease**: cough, haemoptysis, dyspnoea, pulmonary haemorrhage, lung nodules
- **Renal disease**: blood, protein, casts in urine, renal failure
- **Other features**: purpuric rashes, nail fold infarcts, conjunctival haemorrhages, scleritis, uveitis, keratitis, proptosis or ocular muscle paralysis due to retro-orbital inflammation.

> Microscopic polyangiitis (MPA)

- Necrotizing vasculitis of small and medium sized vessels of glomerulus and pulmonary capillaries
- No granuloma are seen and disease of the upper respiratory tract is uncommon

> Churg Strauss Syndrome (CSS)

- Necrotizing vasculitis affecting small to medium-sized vessels
- Eosinophil rise and granulomatous inflammation involving the respiratory tract
- Often associated with asthma

ANCA testing

ANCA is measured by indirect immunofluorescence (IIF) and/or ELISA screen. IIF is reported as negative/borderline/positive/strongly positive and the following ANCA staining patterns are reported: pANCA, c-ANCA, atypical p-ANCA, atypical c-ANCA or atypical ANCA. ELISA will identify PR3-ANCA which are auto-antibodies to proteinase 3 or MPO-ANCA auto-antibodies to myeloperoxidase.

C-ANCA is not equivalent to PR3-ANCA and p-ANCA is not equivalent to MPO-ANCA. P and c-ANCA are staining patterns which are positive to a variety of antigens, e.g. only 50% of positive c-ANCA is due to PR3-ANCA and only approx 25% of p-ANCA are due to autoantibodies to MPO.

Wegener’s Granulomatosis (WG) is associated with PR3-ANCA. Microscopic Polyangiitis (MPA) and Churg Strauss Syndrome (CSS) are associated with MPO-ANCA. However there is no absolute specificity and cross over may occur. In addition 10-20% of patients with WG or MPA and 45-50% of CSS have negative ANCA results. Levels of ANCA can also fluctuate and may be negative before and during a disease exacerbation.

If IIF and ELISA results are combined, the presence of p-ANCA and anti-MPO has 99% specificity for the diagnosis of primary systemic vasculitis, as does the combination of c-ANCA and anti-PR3. But this ‘dual testing’ has cost implications. At present our RIE laboratory recommends ELISA testing in the first instance as presence of PR3/MPO-ANCA has more clinical significance. But if needed IIF can be specifically requested as occasionally individuals can be negative in ELISA testing and positive with IIF.
In 1999 there was an international consensus statement on testing and reporting of ANCA\(^1\). They advised it should be used for the following clinical indications:

- Glomerulonephritis, especially RPGN (Rapid Progressive Glomerulonephritis)
- Pulmonary Haemorrhage, especially pulmonary renal syndrome
- Cutaneous vasculitis with systemic features
- Multiple lung nodules
- Chronic destructive disease of upper airways
- Long standing sinusitis or otitis
- Subglottic tracheal stenosis
- Mononeuritis multiplex or other peripheral neuropathy
- Retro-orbital mass

**Recommendation:** ANCA testing should not routinely be initiated by primary care. ANCA associated vasculitis is very rare. GPs who suspect vasculitis should discuss their patient promptly with secondary care.

**Goodpasture’s Syndrome and anti-Glomerular Basement Membrane Antibodies**

Rapidly progressive diffuse glomerulonephritis with or without pulmonary haemorrhage

**Incidence:** Rare, 1 case per million per year in white European populations (rarer than this in most other races\(^2\))\(^\text{23}\) and 1-2% of all cases of rapidly progressive glomerulonephritis\(^2\)\(^\text{24}\).

**Presentation:** young men
Initial symptoms vague – fever, nausea, vomiting, weight loss, chest pain, anaemia, arthralgia.
Usually presents late – pulmonary haemorrhage, respiratory failure, haematuria or acute renal failure.
If a GP is suspecting this urgent discussion with specialist is required due to risk of renal failure and pulmonary haemorrhage.

**Diagnosis:** ANCA (15-30%) and anti-glomerular basement membrane antibody (found in >90%) \(^1\) and kidney biopsy.

**Recommendation:** This test has no clear role in primary care. This is a rare and serious medical condition. If a GP suspects Goodpasture’s Syndrome they should arrange for urgent renal assessment.
Summary

Tests which have a clear role in primary care
- Thyroid peroxidase antibodies
- Intrinsic factor antibodies
- Gastric parietal cell antibodies
- Smooth muscle antibodies
- Mitochondria antibodies
- Anti-CCP
- ANA (when done in patients with a higher clinical suspicion of a CTD)

Tests which have no clear role in Primary care
- Pancreatic Islet cells (anti-GAD/anti IA-2)
- Adrenal cortex
- Testis Steroid cells
- Ovary Steroid Cells
- Glomerular Basement Membrane

Tests which should not routinely be initiated from Primary care (but could be specifically requested with appropriate clinical indication)
- Complement
- C1 esterase inhibitor
- IgG subclasses
- Anti dsDNA
- Anti-ENA
- ACA
- ANCA
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