**The Missing Antibody: The Pitfalls of ANCA Testing**

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**Presentation**

A 44-year-old Caucasian lady presented to hospital with acute shortness of breath whilst on a flight home from a holiday in Turkey. She denied having a productive cough, haemoptysis, chest or calf pain. She was asthmatic and her symptoms were improved by repeated administration of her salbutamol inhaler during the flight.

she had a past medical history of nasal polyps that had required surgical intervention two years previously.

One month previously she had been treated with a course of Amoxicillin for ‘chronic’ otitis media and was awaiting an ENT appointment. She had also developed swollen tender metacarpal-phalangeal and ankle joints and was found to have a positive rheumatoid factor. Her symptoms subsequently improved on a short course of prednisolone and she felt well enough to go on holiday.

She worked as a secretary for a law firm, never smoked and had a long-term partner. She drank a bottle of wine a week. She had no children and had never been pregnant.

**Assessment**

On presentation she had a temperature of 37.4C and a respiratory rate of 20 breaths per minute. Her heart rate was 80 beats per minute and blood pressure 125/67 mmHg. She required oxygen supplied at 10L/minute to maintain her saturations >95 %. Auscultation of her chest revealed bilateral crackles and urine dipstick test revealed 3+blood, 2+ protein and was negative for a pregnancy test. The remainder of her physical examination was unremarkable.

Initial investigations results are shown in Table 1. A CT thorax (Figure 1) demonstrated extensive bilateral patchy air space infiltrates and ground-glass changes but no evidence of embolic disease.

Given the history, elevated CRP, anaemia and diffuse alveolar infiltration, a diagnosis of pulmonary haemorrhage secondary to autoimmune /vasculitic disease, and a superimposed chest infection was made.

She was transfused with 2 units of blood and treated with intravenous Co-Amoxiclav & oral doxycycline. 500mg intravenous methylprednisolone was given over 3 days then converted to oral prednisolone at 60mg per day.

Results of viral serology and immunological testing were available on Day 3 of admission (Table 2). Repeated samples remained serologically negative for an autoimmune process.

Lung spirometry on Day 4 demonstrated a DLCOc (corrected transfer factor)and KCO (transfer coefficient) at 104% and 88% of predicted respectively.

Given the complexity of the case, a lung biopsy was undertaken on Day 12 (Figure 2). This demonstrated an organising pneumonia with evidence of pulmonary haemorrhage but no active vasculitis.

Our patient improved over the course of two weeks and was discharged with a plan to taper her dose of Prednisolone.

She was seen in rheumatology clinic 3 weeks post discharge and was found to have haematoproteinuria with a creatinine of 167 umol/l (on discharge this was 80umol/l).

Repeated ANCA serology preformed locally remained negative.

However, during this period our Immunology lab had noted a discrepancy on ANCA results of a different patient. This second patient had been transferred following positive ANCA serology at another hospital but their result was not replicated by our local methods.

Stored blood samples from our patient were sent for analysis at another hospital. Immunofluorescent screening at both sites demonstrated a c-ANCA pattern. However though IgG to proteinase 3 was <2 U/ml at our site, this was measured at 44 U/ml at the other site.

Given her renal impairment and active urinary sediment, our patient underwent a renal biopsy (Figure 3) that demonstrated pauci-immune cresenteric glomerular changes consistent with vasculitis.

**Diagnosis**

Granulomatosis with polyangitis (GPA), formerly known as Wegners Granulomatosis, is one of the ANCA-associated vasculitides (AAV) typically presenting with upper and lower respiratory tract granulomas and pauci immune glomerulonephritis. The incidence and prevalence of GPA in the United Kingdom is estimated at 10.2 and 250 cases per million per year respectively 1, presents most commonly from 35-55 years of age andhas a male to female ratio of 1.2: 1 2.

Since the 1980’s (ANCAs) have been used as a serological marker to aid diagnosis of ANCA-associated vasculitides (AAV) 3. Most laboratories worldwide use indirect immunoflourescence microscopy (IIF) to screen for ANCA. This technique mixes patients serum with neutrophils. The presence and pattern of any binding by ANCA can then be revealed by subsequent fluorescent staining. A cytoplasmic (c-) ANCA staining pattern is typically associated with IgG antibodies to proteinase 3 (PR3) [Figure 4] and a perinuclear (p-) pattern to myeloperoxidase (MPO).

Though across studies the specificity of IIF is high (95-100%) the positive predictive value is only 73%. Significant variability in results occur due to differences in slide preparation and operator experience. Co-existing inflammatory conditions also cause a high frequency of false positive results 3

Thus in 2003, it was recommended that initial IIF screening for ANCA should be confirmed with a solid phase assay 4.

Solid phase immunoassays, based on the binding of the antigens PR3 or MPO to a solid phase (e.g. the wells of a microtitre plate), allows for quantification of IgG against PR3 and MPO.

However the clinically significant epitopes of the target antigens can become hidden in the process of antibody binding on the ELISA 5 leading to under-reporting.

Newer assays now use an antibody which binds to a distinct “non-functional” epitope of the antigen to ‘anchor’ or ‘capture’ the molecule in the support medium. This allows for increased assay specificity and sensitivitiy despite antigen orientation and results can be obtained between 2 and 4 hours.

However variation in antigens, techniques and a lack of international reference materials means significant variation in results continue to exist.

In addition every patient will also make a slightly different IgG antibody to a particular antigen and that the epitopes of the antigen to which a patient responds may change during the course of a disease or treatment.

A study comparing the different ELISA techniques in healthy controls and patients with known GPA showed high specificity (>98%) for all methods but sensitivity varied from 72% with capture ELISA to 96% with anchor ELISA5.

International Reference preparations for IgG anti MPO is prepared and for IgG anti PR3 is currently in final evaluation but the full impact of their introduction will take some years. Other components of the ELISA based techniques will also need to be harmonised (e.g. detailed definition of the antigen or epitope being used).

Never the less, the discrepancies seen in these assays poses a problem for aiding the diagnosis of GPA, particulalry in patients with complex or atypical presentations.

**Management**

Our patient was catagorized as having generalized systemic vasculitis and was commenced on a course of remission induction with Rituximab infusions and Prednisolone according to the EUVAS vasculitis protocol (Reference 6)

Twelve months following her initial presentation she has had no further episodes of pulmonary haemorrhage and her creatinine is 127 umol/L (eGFR of 40 ml/min/BSA). . Her prednisolone dose has been subsequently tapered with the introduction of Mycophenolate mofetil (2g per day) to maintain remission. (Reference 6)

This case highlights the potential pitfalls of ANCA testing in the diagnosis of a patient with a GPA. Despite improvements in ELISA assay sensitivity and specificity, high inter-method variation exists.

It is important that clinicians appreciate the limitations of these tests and that no single assay will show 100% clinical specificity or sensitivity.

This case highlights the need for good communication with the laboratory when results are surprising or inconsistent with the clinical picture and that the diagnosis of AAV is a clinical and not a laboratory one. This latter point is reflected by the American College of Rheumatology Criteria used to identify GPA patients for clinical trials, which does not include ANCA results at all (reference 4)

**References**

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