World Journal of Immunology

Online Submissions: http://www.wjgnet.com/esps/
wji@wjgnet.com
doi:10.5411/wji.v3.i2.18

World J Immunol 2013 July 27; 3(2): 18-30
ISSN 2219-2824 (online)

REVIEW

Role of the clinical immunology laboratory in disease monitoring

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Received: February 22, 2013 Revised: April 24, 2013
Accepted: May 16, 2013
Published online: July 27, 2013

Abstract

Immunological investigations provide useful information to guide diagnosis of several disorders. Many such tests are also commonly repeated at intervals, in an effort to facilitate disease monitoring. In general however, immunology test results are often slow to alter. Furthermore, audit activity has indicated that repeated testing accounts for a substantial workload in many immunology services, which may waste resources and compromise the efficient completion of necessary tests. Consequently, the need and appropriate minimum interval between repeated testing requires critical evaluation. In this review, the clinical utility of repeated performance of several common immunology investigations has been evaluated, based upon published evidence. In some cases (*e.g.*, paraprotein quantification, or measurement of anti-glomerular basement membrane antibodies), repeated testing provides vital clinical information and can be justified on a frequent and individualized basis. However, many other investigations provided by immunology services provide less valuable information when used to aid disease monitoring rather than diagnosis. It is hoped that the data summarized here will facilitate a more evidence-based approach to repeated testing. Such information may also assist with the local implementation of demand management strategies based upon setting of minimum retesting intervals for these investigations.

Key words: Clinical immunology; Immune monitoring; Retest; Test interval

Core tip: Immunological investigations provide useful information to guide diagnosis of several disorders. Many such tests are also commonly repeated at intervals, in an effort to facilitate disease monitoring. Here, the evidence underlying the need and appropriate minimum interval between repeated testing has been critically evaluated.

Maher J. Role of the clinical immunology laboratory in disease monitoring. *World J Immunol* 2013; 3(2): 18-30 Available from: URL: http://www.wjgnet.com/2219-2824/full/v3/i2/18.htm DOI: http://dx.doi.org/10.5411/wji.v3.i2.18

INTRODUCTION

The clinical immunology laboratory serves an important role in the diagnosis of several disorders. Equally, periodic immunological testing can contribute to the monitoring of disease status. However, such tests are expensive, relatively slow to undergo change and are commonly repeated. In two recently published audits, up to 30% of tests were unnecessarily repeated within a 3-mo time window[1,2]. Most clinical immunology laboratories experience a continued year on year increase in test requests. For example, combined data from two large National

Health Service Trusts has indicated that requests for serological screening for coeliac disease had doubled over a 5-year interval[3]. Consequently, as part of their demand management strategy, some services have imposed minimum retesting intervals in order to reduce misuse and unnecessary investigation. However, the evidence base for the implementation of such protocols is scattered throughout several publications and is variable in quality. In this review, the role of several immunology tests in disease monitoring has been examined. The overall goal was to provide an evidence base to support the need for repeated testing and, where possible, to provide guidance on intervals that may be appropriate between retesting.

RHEUMATOLOGY AND NEPHROLOGY

Anti-nuclear antibody

Testing for anti-nuclear antibodies (ANA) plays an important role the diagnosis of several rheumatological and other conditions. However, the major limitation of ANA testing is the high frequency of false positive results. Up to 30% of healthy individuals have ANA at a serum dilution (titre) of 1:40, while 5% remain positive at a titre of 1:160[4]. This finding emphasises the importance of performing this test only in individuals who are suspected to have an ANA-associated illness. Once a diagnosis has been established, repeat testing for ANA in positive individuals is generally not useful unless clinical features change. This is because there is no evidence to indicate that alteration in ANA titre (or immunofluorescence pattern) provides useful information to guide the management of any illness[4-6]. In summary therefore, there is no clear indication to repeat a positive ANA unless the result is not compatible with the clinical presentation, in which case the test should not have been requested in the first instance.

Because ANA-associated illnesses are often dynamic, it is sometimes reasonable to retest individuals with such a diagnosis where ANA testing has been negative. In general, the presence of ANA generally precedes diagnosis in relevant rheumatological conditions[7]. However, in systemic lupus erythematosis (SLE) for example, ANA may occasionally be undetectable at the onset of symptoms ("ANA negative SLE"). While many factors may account for this finding, it has been estimated that up to 18% of such ANA negative SLE patients will "seroconvert" over the course of their illness[6,8], providing a rationale for re-testing in highly selected cases.

Anti-double stranded DNA antibody

The only disorder for which the detection of anti-double stranded (ds)DNA antibodies is diagnostically useful is SLE. The level or titre of these antibodies is dynamic and can provide useful information in monitoring the activity of SLE. A rise in anti-dsDNA antibodies often precedes SLE exacerbation by a few weeks[9]. High titres have been identified in lupus nephritis and their levels tend to rise and fall in direct proportion to disease activity[10]. However, the relationship between titre of anti-dsDNA antibodies and disease activity in SLE is not absolute. Thus, an increment in the titre of anti-DNA antibody does not predict with certainty that a subsequent SLE flare will occur[11]. The converse is also true in that SLE patients may have a disease flare without a prior increase in anti-dsDNA antibody levels[11]. Two recently conducted prospective case series have highlighted the limitations of anti-dsDNA antibody testing in mirroring disease status[12,13]. Consequently, alterations in the level of these antibodies should never be used in isolation to guide disease management. Against this background, the optimum frequency of retesting of anti-dsDNA antibodies is unknown[11]. However, since the half-life of most immunoglobulin G (IgG) sub-classes is approximately 3 wk, the minimum period required to detect significant alterations of antibody titres should be at least an interval of 4 or more weeks[5,6,14,15].

Antibody against extractable nuclear antigens

Antibodies against extractable nuclear antigens (ENA) (*e.g.*, SSA/Ro, SSB/La, Sm and/or U1-RNP) are commonly used to support the diagnosis of several conditions, notably SLE, Sjogren's syndrome and mixed connective tissue disease. The presence of these antibodies tends to be stable over time so that if the test is positive, repeat testing is generally not helpful[16,17]. Furthermore, alterations in titre of these antibodies do not predict the need for changes in disease management[18]. Similarly, anti-centromere and anti-scl70 antibodies tend to remain stable over time and do not correlate with disease activity[19]. Occasionally however, it is recognised that autoantibodies within the above groups may either emerge or recede over time in patients with rheumatological conditions[8], providing a rationale for selected retesting.

Rheumatoid factor

Rheumatoid factor (RF) is a generic term for antibodies that react with the Fc portion of the IgG molecules. The presence of RF in both children and adults with rheumatoid arthritis (RA) correlates with a more aggressive and erosive disease course[20]. Recent studies have indicated that RF tends to be stable over time other than a trend to reduction in levels[21-23]. Consequently, there seems little point in repeating this test over time.

Anti-cyclic citrullinated peptide antibodies

Anti-cyclic citrullinated peptide (CCP) antibodies are directed against citrulline residues, formed as a result of the post-translational modification of arginine. The presence of anti-CCP antibodies is a highly specific diagnostic marker of RA, correlating like RF with more aggressive disease. Current (2010) American College of Rheumatology guidelines include the use of anti-CCP antibodies in the classification of rheumatoid arthritis. In further similarity to RF, recent data indicate that anti-CCP antibodies remain stable over a period of up to 5 years. Consequently, re-testing over this period is not supported

by evidence[21-23]. Furthermore, repeated measurement of anti-CCP-antibodies does not help to evaluate disease activity[24].

Anti-phospholipid antibodies

Anti-phospholipid antibodies are a heterogenous group of antibodies with reactivity against cell surface phospholipids and/or associated cofactor proteins. These antibodies may be found in patients with the anti-phospholipid syndrome (APS). Commonly, the diagnosis of APS is based upon the revised Sapporo classification criteria (also called the Sydney criteria)[25,26] and requires one clinical (thrombotic or pregnancy-associated morbidity) and one laboratory criterion. The laboratory requirements for diagnosis of APS include either the presence of (1) lupus anticoagulant; (2) IgG and/or immunoglobulin M (IgM) anti-cardiolipin antibodies; or (3) IgG and/or IgM antibodies against β2 glycoprotein I. However, false positive antibodies may be found in several circumstances, including infection and even in normal individuals. Consequently, in patients with initial positive results, the test must be repeated and found positive on a second occasion, after a minimum interval of 12 wk. Experience indicates that in the majority of cases, repeat testing does not confirm the presence of these antibodies, highlighting the importance of such confirmatory testing[27].

In light of these considerations, it is recommended that the minimum re-testing interval for all three tests is set at 12 wk. Although evidence is limited, it appears that anti-phospholipid antibodies are generally stable for over 2 years from diagnosis and are not influenced by commonly used therapies such as warfarin, aspirin and/or hydroxychloroquine[28]. Poor assay standardisation makes comparison of serial results from different assays challenging and the relationship of titre to disease activity in SLE is also contentious[28]. Consequently, the role of repeated testing beyond the diagnostic phase would appear to be limited.

Anti-neutrophil cytoplasmic antibody

Anti-neutrophil cytoplasmic antibody (ANCA) detection is a valuable tool for diagnosing small-vessel vasculitis, including granulomatosis with polyangiitis (GPA, Wegener's granulomatosis), microscopic polyangiitis, eosinophilic GPA (Churg-Strauss syndrome) and primary pauci-immune crescentic glomerulonephritis. International guidelines for ANCA testing recommend screening for ANCA by indirect immunofluorescence microscopy, followed by enzyme-linked immunosorbent assay (ELISA) testing of positive samples[29,30]. Positive immunofluorescence staining may be cytoplasmic (c-ANCA) or peri-nuclear (p-ANCA) in distribution. Commonly, but not always, c-ANCA positivity is associated with ELISA reactivity against proteinase 3 (PR3) while p-ANCA correlates with binding of myeloperoxidase (MPO). Initially, it was reported that ANCA quantification could reliably predict relapse of necrotisingvasculitides and thereby guide treatment decisions[31,32]. More recently however, evidence from several sources has indicated that this application of ANCA testing is not sufficiently robust to be clinically useful in the majority of cases[33-35]. This viewpoint has been confirmed in a recent meta-analysis[36]. For individual patients in whom an association between PR3-ANCA levels and disease activity has been established, serial ANCA testing may have some predictive value for subsequent relapse[37]. It is well recognised that PR3-ANCA positive patients are more likely to relapse than those with MPO-ANCA, indicating the need for closer monitoring of the former group[38]. Furthermore, there is evidence that in PR3-ANCA positive patients, it is safer to progress from cyclophosphamide-based remission induction therapy to azathioprine maintenance therapy when ANCA have reverted to negativity[39]. However, once clinical remission has been achieved, renal transplantation is equally successful, regardless of ANCA levels[40]. Taken together, this indicates that serial ANCA measurement may be useful in some selected cases but that a straightforward role linking test results to disease activity is lacking.

Complement C3 and C4

In SLE, serum C3 and C4 levels commonly fall close to the time of a flare and return to higher concentrations after a minimum of several weeks of appropriate treatment. However, these findings are not uniform. Some patients have disease flares without a reduction in C3 or C4 levels. In keeping with this, a recent 6-year prospective study demonstrated that C3, C4 and anti-dsDNA antibody measurements had a satisfactory negative predictive value for lupus nephritis but that positive predictive value was inadequate[12]. Furthermore, a second prospective study has highlighted the inadequacy of C3, C4 and anti-dsDNA antibody testing in monitoring disease activity in lupus[13]. There are a number of reasons why reductions in C3 and C4 in particular may not necessarily indicate disease flare. For example, low levels of C4 are commonly seen in inactive SLE due to inherited partial C4 deficiency, which is common in SLE[41]. Furthermore, C4 may be reduced even during the inactive stage of SLE. Nonetheless, serial C3 and C4 measurements may be useful in disease monitoring in individual patients. Furthermore, it has been suggested that C3 and C4 levels should be monitored monthly during pregnancy in patients with lupus[42].

C1q antibodies

Anti-C1q antibodies bind to C1q, which is the first component of the classical complement pathway. These antibodies have been found in patients with SLE in addition to several other inflammatory rheumatological conditions, notably hypocomplementaemic urticarial vasculitis. In SLE, these antibodies are found more commonly in patients with lupus nephritis. Furthermore, antibody titre tends to be higher in association with active disease. Although disease flare may be seen in their absence, evidence is emerging to suggest that these antibodies provide one of the strongest biomarkers of active disease and serial measurement warrants further investigation[12,43,44].

Anti-glomerular basement membrane antibody

Glomerular basement membrane (GBM) antibody disease is associated with auto-antibodies that bind the non-collagenous 1 domain of the α3/α5 chains of type Ⅳ collagen, found in the GBM and alveoli. Patients with anti-GBM antibodies commonly present with pulmonary renal syndrome and require intensive immunosuppression using cyclophosphamide, corticosteroids and plasmapheresis. In this context, frequent repeated antibody measurement can be used to guide therapy[45]. Typically, plasmapheresis is performed every 1-2 d for at least 14 d or until anti-GBM antibodies are no longer detectable[46]. In general, anti-GBM disease does not relapse and consequently does not require long-term immunosuppression. However, a proportion of patients also have ANCA and behave clinically more like patients with ANCA-associated vasculitis (*e.g.*, with a propensity to relapse). In patients with anti-GBM disease who require renal transplantation, it is recommended that anti-GBM antibodies are undetectable for at least 6 mo previously in order to minimise the risk of disease recurrence[46].

NEUROLOGY

Disorders of the neuromuscular junction

Myasthenia gravis (MG) is characterised by an immune-mediated failure of neuromuscular transmission. In part, the disease is mediated by auto-antibodies that bind to the postsynaptic nicotinic acetylcholine receptor (AChR) and muscle-specific receptor tyrosine kinase (MuSK) receptor complex. However, no studies have confirmed the usefulness of serial auto-antibody measurement in MG[47].

Antibodies directed against the AChR are detected in approximately 80% of patients with MG and thus contribute importantly to diagnosis of this disease. Although individual patients may vary, neither the presence nor the level of AChR antibodies on serial testing correlates well in general with disease severity[48,49] or response to immunosuppressive therapy[50]. Nonetheless, about 15% of patients who are initially seronegative may become seropositive within 12 mo, warranting re-testing in this population[51]. Furthermore, such seroconversion can be delayed for several years in some cases[51].

About 70% of the AChR seronegative population has antibodies directed against MuSK. In this scenario, a correlation between antibody titre and disease severity has been observed[52]. Patients with MuSK-specific antibodies tend to respond less well to acetylcholinesterase inhibition and often require immunosuppressive therapy. Consequently, it is possible that repeated measurement of these antibodies may be useful to aid disease monitoring, in conjunction with other parameters.

Anti-striated muscle antibodies may also be found in patients with myasthenia gravis and these correlate with increased risk of co-existing thymoma. The primary target of these antibodies is Titin. Emergence of these antibodies in a patient with myasthenia have also been linked to thymoma[53].

Autoimmune "channelopathies"

Antibodies to the water channel, aquaporin-4, are a sensitive and highly specific serological marker for neuromyelitis optica. Furthermore, titres of aquaporin-4 antibodies correlate at the population level with disease activity[54]. In the individual patient however, changes in antibody levels are not sufficiently predictive of disease relapse and the need to modify therapeutic approach[55].

Antibodies that bind to neuronal voltage-gated potassium channels may be found in neuromyotonia, limbic encephalitis and Morvan's syndrome. These conditions commonly respond to immunosuppressive therapy although they may occasionally signal the presence of underlying malignancy. A correlation has been observed in several cases between reduction in antibody titre and therapeutic response[56], providing a rationale for repeated measurement of antibodies in these patients.

Antibodies that bind to neuronal voltage-gated calcium channels are associated with both paraneoplastic and idiopathic forms of the Lambert-Eaton myasthenic syndrome. They antibodies are not diagnostic however in that they also occur in other neurological and malignant disorders. Antibody titre does not correlate well with clinical and electrophysiological parameters of disease. Serial antibody titres may reflect clinical status, at least in some patients, although clinical remission may be accompanied by persistence of high titre antibody levels[57,58].

Finally, a series of complex encephalitic conditions may be considered in this category of which the prototype is associated with antibodies to an ion channel, namely the N-methyl-D aspartate receptor. Other targets include the glycine, $GABA_B$ and AMPA receptors. Once again, some cases may be tumour-associated (*e.g.*, ovarian teratoma). Autoantibody concentrations are generally higher in serum than in CSF in these conditions. However, intrathecal synthesis of antibody also occurs, and the role of serological monitoring of these patients is presently unclear[59].

Disorders associated with antibodies to glycolipid and glycoprotein-related saccharides

Several neurological disorders have been associated with this class of autoantibody (Table 1). Pre-eminent among these are anti-ganglioside antibodies. Molecular mimicry between lipooligosaccharides on the surface of infectious agents (most commonly *Campylobacter jejuni*) and ganglioside antigens on neural cells may induce cross-reactive antibody responses, targeted against ganglioside complexes. Alternatively, these antibodies may manifest as non-malignant IgM monoclonal gammopathies. Complement fixation by these antibodies appears to be an important contributor to disease pathogenesis[60].

Although assays are not standardised, these antibodies have important diagnostic utility. Furthermore, several of these antibodies appear to have pathogenic relevance in these neurological conditions. In some cases, an association has been observed between antibody titre and disease activity[61] or response to immunosuppressive

Table 1 A summary of clinically relevant antibodies targeted against glycolipid and glycoprotein-related saccharides

Neuropathy syndrome	Antibody target	Antibody isotype
Chronic Sensory-Motor demyelinating	Myelin-associated glycoprotein Sulfoglucuronylparagloboside	IgM (monoclonal)
Chronic ataxic neuropathy	GD1b, GQ1b	IgM (monoclonal)
Multifocal Motor neuropathy	GM1, GM2, GD1b	IgM (polyclonal or monoclonal)
Sensory neuropathy	Sulfatide	IgM (monoclonal or polyclonal)
Acute motor axonal neuropathy	GM1, GD1a, GalNAc GD1a, GM1b	IgG
Acute inflammatory demyelinating polyneuropathy	Variable	Variable
Miller-Fisher syndrome	GQ1b ("anti-GQ1b antibody syndrome"), GT1a	IgG
Bickerstaff's brainstem encephalitis		
Acute ophthalmoparesis		
Ataxic Guillain-Barré syndrome		
Pharyngeal-cervical-brachial weakness	GT1a (GQ1b)	IgG
Amyotrophic lateral sclerosis	GM1	IgG

Modified from http://www.aetna.com/cpb/medical/data/300_399/0340.html (Accessed December 8, 2012). IgM: Immunoglobulin M; IgG: Immunoglobulin G.

therapy[62]. Once again however, the temporal relationship between antibody levels and disease status is not straightforward. To further complicate matters, IgG antibodies directed against some of these targets can be found in pooled immunoglobulin, which is commonly used to treat these disorders.

Antibodies associated with paraneoplastic disorders

Several antibodies have been described in paraneoplastic disorders, including anti-Hu, Ri, Yo, Tr, CV2/CRMP5, amphiphysin, recoverin and Ma1/Ma2[63]. When used diagnostically, detection of these antibodies may be predictive of the presence of underlying malignancy. Some, but not all anecdotal reports have suggested that antibody levels may increase prior to emergence of clinical cancer relapse[64]. Once again however, the role of repeated measurement of these antibodies in disease monitoring is not established.

Monitoring of paraproteins is discussed elsewhere in this review.

ENDOCRINOLOGY

Type 1 diabetes

Antibodies against islet cells and associated autoantigens (glutamate decarboxylase, islet antigen-2 and insulin) are commonly detectable in individuals with type 1 diabetes and in latent forms of this disease[65]. Multiplicity of antibody positivity is associated with greater risk of disease development. However, in the absence of proven interventions to decease disease risk, such use of these tests would appear unwarranted outside of research protocols. These antibodies may also be detected in a sub-group of patients with type 2 diabetes who have a greater risk of progression to require insulin. Once again however, there is no firm evidence that this information either leads to improved outcome or altered management. The presence of islet-reactive autoantibodies can be useful in categorising patients with features of both type 1 and type 2 diabetes, or in predicting risk of future diabetes in women with gestational diabetes. In none of these situations however is there evidence to support repeat antibody measurement.

Steroid cell autoantibodies

Adrenal antibodies are found in individuals with established or evolving primary adrenal failure[66], either alone or as part of one of the autoimmune polyglandular syndromes. In about 15% of cases, antibodies cross-react with other steroid hormone producing cells (*e.g.*, Leydig cells, theca cells, syncytiotrophoblast), indicating a high risk of progression to primary gonadal failure, particularly in women with high titre antibodies. The principal autoantigen recognized by adrenal antibodies is 21-hydroxylase whereas the latter "steroid cell antibodies" commonly react with 17 hydroxylase. While these antibodies have no pathogenetic role, their detection is useful both for diagnostic purposes and also in the identification of subjects at high-risk for future development of clinical adrenal failure.

There is no evidence to support retesting for these antibodies, either in the event of positive or negative results. In the context of polyglandular autoimmunity, periodic reassessment for the presence of several autoantibodies would appear to be justifiable. In this context, it has been suggested that in patients with primary adrenal failure, autoantibody testing is repeated every 2-3 years[67]. The rationale underlying this proposed strategy is the detection of serological markers of other clinical manifestations of one of the associated autoimmune polyglandular syndromes, leading in turn to appropriate functional testing and diagnostic reclassification.

Thyroid autoantibodies

Antibodies with reactivity to thyroid peroxidase are commonly used markers of autoimmune thyroid disease, including Hashimoto's disease, Graves disease, autoimmune post-partum thyroiditis and sub-clinical autoimmune thyroid disease. However, antibody levels do not correlate with disease severity. Consequently, repeated

Table 2 Common clinical associations of paraproteins[70]

Disorder	Diagnostic features
Monoclonal gammopathy of undetermined significance	All three criteria must be met: Serum monoclonal protein < 30 g/L Clonal bone marrow plasma cells < 10% Absence of end-organ damage ("CRAB"), *e.g.*, hypercalcemia, renal insufficiency, anaemia and bone lesions due to the plasma cell disorder
Smouldering myeloma	Both criteria must be met: Serum monoclonal protein (IgG or IgA) > 30 g/L and/or clonal bone marrow plasma cells > 10% Absence of "CRAB", as defined above
Multiple myeloma	All three criteria must be met: Clonal bone marrow plasma cells > 10% Presence of serum and/or urinary monoclonal protein (except in patients with true non-secretory multiple myeloma) Evidence of "CRAB", as defined above
Waldenström's macroglobulinaemia	Both criteria must be met: IgM monoclonal gammopathy and 10% bone marrow infiltration (usually intertrabecular) by lymphoplasmacytic cells (morphology/immunophenotype)[1]
IgM monoclonal gammopathy of undetermined significance	All three criteria must be met: Serum IgM monoclonal protein < 30 g/L Bone marrow lymphoplasmacytic infiltration < 10% No evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy or hepatosplenomegaly
Smoldering Waldenström's macroglobulinemia	Both criteria must be met: Serum IgM monoclonal protein > 30 g/L and/or bone marrow lymphoplasmacytic infiltration > 10% No evidence of end-organ damage such as anemia, constitutional symptoms, hyperviscosity, lymphadenopathy or hepatosplenomegaly due to a lymphoplasma cell proliferative disorder

[1]Note that other clonal B-cell disorders may be associated with paraproteins and may require careful clinical, morphological and immunophenotypic assessment for diagnosis (*e.g.*, chronic lymphocytic leukaemia, mantle cell lymphoma, *etc.*). IgM: Immunoglobulin M; IgG: Immunoglobulin G; CRAB: Hypercalcemia, renal insufficiency, anaemia and bone lesions.

measurement of these antibodies in general has no clinical value[68].

HAEMATO-ONCOLOGY

Monitoring of serum and urinary paraproteins

A paraprotein or M (monoclonal) protein is a clonal intact immunoglobulin or light chain that is produced in excess. Paraproteins can be detected in multiple myeloma, Waldenström's macroglobulinaemia, other clonal B-cell/plasma cell disorders or, more commonly, as a manifestation of monoclonal gammopathy of undetermined significance (MGUS). When intact, paraproteins are best detected in the serum. By contrast, light chain paraproteins (also known as Bence Jones proteins) are often easier to detect in urine, owing to their smaller size. Paraproteins are generally detected and/or quantified using one of four assay systems. In serum, this is achieved by agarose gel electrophoresis or capillary zone electrophoresis. By contrast. light chain paraproteins are traditionally detected by urine electrophoresis but may also be detected and quantified using the recently developed free light chain assay. This is a sensitive, latex-enhanced immunonephelometric test that quantitates free κ and λ light chains, generally in serum samples[69]. Finally, in some carefully selected cases, paraprotein quantification is best achieved by measurement of the relevant immunoglobulin isotype, although this approach requires caution.

The role of paraprotein measurement in the diagnosis of haematological malignancy/MGUS is beyond the scope of this review and the reader is referred to a number of recent articles for a more comprehensive discussion of this area[70,71]. The common causes of paraproteins are listed in Table 2, together with relevant diagnostic features. In the discussion that follows, the role of these assays in disease monitoring is considered.

Changes in paraprotein levels are the principal indicators used to evaluate therapeutic response in patients with multiple myeloma, except in those patients with oligo- or non-secretory disease. Owing to the limitations of the assays involved, measurable disease has been defined as a serum paraprotein of $\geqslant$ 10 g/L or a urine paraprotein of $\geqslant$ 200 mg/d[72]. In serum, reductions in paraprotein levels of at least 25% and 50% respectively are considered minimal and partial responses. By analogy, a reduction in urinary paraprotein (per day) of at least 50% and 90% respectively are classified as minimal and partial responses[72]. A "very good partial response" is defined as the situation where a serum/urine paraprotein is detectable by immunofixation but not on electrophoresis or where a 90% or greater reduction in serum paraprotein has occurred, together with a urine paraprotein level of < 100 mg/d. A complete response requires the demonstration that a previously identified paraprotein is no longer present when sought using immunofixation electrophoresis and that bone marrow plasma cells are < 5% with resolution of any plasmacytomas. A "stringent complete remission" also requires that the serum free light chains (see below) have normalised and clonal bone marrow cells are absent.

If the paraprotein level is 15 g/L or lower and the patient is asymptomatic with no other laboratory or clinical abnormalities, MGUS is probable. In patients with

low-risk MGUS, paraproteins can be rechecked in 6 mo, and then once every two years or only at the time of symptoms for evidence of progression. All other subsets of patients need to be rechecked in 6 mo, and then yearly thereafter[73]. Patients with smouldering multiple myeloma (Table 2) need more frequent follow up than those with MGUS; at least every 3-4 mo and especially with IgA paraproteins[73].

Patients who are treated with plasmapheresis may require frequent paraprotein measurements in addition to serum or plasma viscosity to guide this therapy.

Monitoring of serum free light chains

The serum free light chain assay quantifies circulating free κ and λ immunoglobulin light chains. The assay is useful in three circumstances. First, measurement of free light chains is useful as a component of screening for paraprotein-related illness. Second, baseline free light chain levels provide valuable prognostic information in virtually all paraprotein-related conditions, from MGUS through to myeloma. Third, and of greatest relevance to this review, repeated measurement of free light chains can be useful in monitoring of these disorders under the following selected circumstances.

In the absence of a serum or urine paraprotein that is "measurable" according to the definitions described above, myeloma can be considered as "oligosecretory". In this setting, the International Myeloma Working Group has recommended that the serum free light chain assay can be used to monitor disease, provided that the paraprotein (*e.g.*, involved free light chain) is ≥ 100 mg/L and that the free light chain κ/λ ratio is abnormal. Using this threshold for "measurable disease, a 50% decrease in paraprotein level indicates partial response[72]. A recent consensus statement has recommended the additional use of this assay for monitoring of other oligosecretory plasma cell disorders, including amyloidosis AL and the majority of patients with "non-secretory" myeloma[74]. The free light chain ratio is not a useful monitoring tool in this setting owing to treatment-related suppression of the disease-unrelated light chain. It is also important to emphasise that the free light chain assay is not recommended as a replacement for 24-h urine protein electrophoresis for monitoring of myeloma patients with measurable urinary paraproteins.

There is no clear evidence to guide appropriate retesting intervals. However, rapid changes are possible in particular since the half-life of serum free light chains (2 to 4 h) is much shorter than intact immunoglobulins (8-21 d). In myeloma kidney, recovery of renal function in patients requires an early substantial reduction in serum free light chain concentrations, warranting frequent retesting[75], perhaps at monthly or quarterly intervals.

Caution is warranted in the interpretation of repeated measurement of free light chains. There can be significant lot-to-lot variation between batches of polyclonal free light chain antiserum. Indeed, the κ/λ ratio can even "double" artifactually in a patient with a stable disease[76]. Thus, as in the case for 24-h urine protein measurements, a change of up to 50% in the involved free light chain concentration may not necessarily be significant. Furthermore, antigen excess errors can cause highly spurious trends in repeat measurements, which sometimes can lead to the erroneous conclusion that disease progression is occurring[77].

β2-microglobulin

β2-microglobulin concentration at presentation is the best prognostic indicator for multiple myeloma[78]. Half-life is only 1-3 h providing a rationale for frequent repeated measurement in this disease. However, falls in β2-microglobulin parallel those observed for paraproteins and it has been argued that little additional information is gained from measurement of both of these disease markers[79].

ALLERGY

Specific IgE in food allergy

In general, the magnitude of specific IgE that is reactive against a food allergen correlates with the likelihood but not severity of true IgE-mediated allergy. In keeping with this, values of specific IgE have been defined that have a positive predictive value of 95% for childhood allergy to egg, milk, peanuts, tree nuts or fish[80]. With lower levels and depending upon the history, diagnosis of food allergy may also require additional testing such as skin prick tests and supervised open food challenge.

In the setting of previously diagnosed food allergy, serial measurement of specific IgE may also be useful in monitoring of allergic status. This is particularly the case in children with allergy to cows milk and egg. Over 90% of children with cows milk allergy will become tolerant by 6 years of age[81] while 66% of children with egg allergy acquire tolerance after 5 years of follow-up[82]. Progressive development of tolerance can be predicted by lowered levels of specific IgE, combined with declining reactivity in skin prick testing and may be confirmed by judicious use of open food challenge[83].

Total IgE

In some circumstances, serial measurement of total IgE may be if use in disease monitoring. For example, in patients with allergic bronchopulmonary aspergillosis, total serum IgE provides a measure of disease status and response to corticosteroids[84]. However, IgE measurement is not useful in monitoring the depletion of this isotype by anti-IgE (omalizumab) therapy since commonly used assays do not distinguish between free and omalizumab-complexed forms of this antibody[85].

GASTROENTEROLOGY AND HEPATOLOGY

Coeliac serology

Coeliac screening generally involves the measurement of

IgA anti-tissue transglutaminase (tTG) antibodies, often followed by confirmatory testing for IgA anti-endomysial antibodies. Serological testing for these antibodies is highly useful in identifying individuals who may have coeliac disease (and who generally require duodenal biopsy while on a gluten-containing diet to confirm the diagnosis). Antibody testing has been advocated to aid disease monitoring in two circumstances. First, periodic retesting may be performed in an effort to assess compliance with a gluten-free diet in patients with an established diagnosis of coeliac disease. Second, repeated serological screening is commonly undertaken to detect the development of coeliac disease, particularly individuals at higher risk such as patients with type 1 diabetes. Importantly however, sequential serological testing in coeliac disease is compromised by the fact that assays are not standardised[86].

In the sections that follow, these potential areas of disease monitoring are considered in turn.

Monitoring compliance with a gluten-free diet: Both anti-tTG IgA and anti-endomysial IgA antibodies can normalise on a gluten-free diet and can increase in titre following re-exposure to gluten. However, the value of repeated measurement of these antibodies in the monitoring of patients with coeliac disease remains complex and controversial. Anti-tTG IgA antibody levels were reported to be scarcely reliable in some[87-89], but not all[90] studies in detecting compliance with a gluten-free diet, when compared with duodenal biopsy. Despite following such a diet for 2 years with normalisation of anti-tTG IgA antibody levels, villous atrophy had not improved in 25% of asymptomatic adults[91]. Furthermore, in patients with associated autoimmune disorders such as type 1 diabetes, anti-tTG IgA antibody levels may remain elevated despite dietary compliance and thus may not be useful for this purpose in such patients[92]. In keeping with this, Armstrong and colleagues have recently concluded that repeat testing of anti-tTG IgA in patients diagnosed with coeliac disease and managed with a gluten-free diet is not indicated to confirm response to treatment. They acknowledge that studies have shown that quantitative results of anti-tTG IgA assays do commonly change in response to the initiation of a gluten-free diet. However, they point out that there is no evidence to suggest that regular, continued monitoring of anti-tTG titres improves outcomes[93].

The Ontario Health Technology Advisory Committee have also commented on this issue. They concluded that: "Repeat serologic testing for patients diagnosed with celiac disease is reasonable for those patients who remain symptomatic despite strict adherence to a gluten-free diet. In this case, serologic testing for celiac disease should not be repeated more that once a year for each patient"[94]. Taken together, these considerations argue that repeated testing of anti-tTG IgA antibodies in patients with an established diagnosis of coeliac disease is of highly limited value.

Monitoring high-risk populations for development of coeliac disease: High-risk populations such as individuals with type 1 diabetes are often monitored periodically using anti-tTG IgA and/or anti-endomysial IgA assays for the development of coeliac disease. To facilitate this process, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have recently recommended initial testing for coeliac disease-associated human leukocyte antigen (HLA) alleles (HLA-DQ2/ DQ8) in order to ascertain which of these children warrant such monitoring. However, no firm evidence exists as to how frequently coeliac serology should be re-tested in individuals who have these disease-associated alleles. In the face of this lack of evidence, it was the opinion of the ESPGHAN working group that testing should be undertaken every 2 to 3 years[95]. To complicate matters further, recent data suggests that positivity for one or both coeliac disease-associated antibodies can spontaneously revert in some individuals, particularly children, who maintain a gluten-containing diet[96,97].

The National Institute for Clinical Excellence has also commented on the issue of repeated serological testing for coeliac disease. It has concluded that "there is a lack of evidence on the need for repeat testing. Studies are needed to determine whether serological tests should be repeated if the initial results are negative and there is no high clinical suspicion of coeliac disease, and if so, when and how often they should be repeated"[98]. It is clear that further research is required to clarify the optimum means to achieve such monitoring.

Autoimmune liver disease

Autoimmune hepatitis is characterized by hypergammaglobulinaemia accompanied by either ANA and anti-smooth muscle antibodies (type 1) or anti-liver kidney microsomal-1 antibodies (type 2). Anti-LC1 antibodies may also be detected, more commonly in European patients and in the absence of ANA or SMA[99]. However, autoantibody titres in adults correlate imprecisely with disease severity, clinical course, and treatment response[4,100]. By contrast, IgG levels and autoantibody titres have all been correlated with disease activity in children with this disease[101], providing a rationale for repeated measurement.

Anti-mitochondrial antibodies reactive against the 2-oxodehydrogenase complex - previously named anti-M2 mitochondrial antibodies - are excellent serological markers for the serological diagnosis of primary biliary cirrhosis. However, these antibodies do not correlate with any clinical, histological or laboratory parameters of disease. Antibody titres remain stable over time[102] and are not influenced by ursodeoxhcholate treatment. Furthermore, antibody titres decrease only transiently after liver transplantation[103,104].

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

CD4 counts in human immunodeficiency virus

Monitoring CD4 cell counts in asymptomatic human im-

munodeficiency virus-1 infected patients to decide when to start antiretroviral therapy is unanimously recommended and is cost-effective. However, no recommendations have yet been formulated regarding the optimal frequency of CD4 monitoring, which most United Kingdom practitioners perform every 3-4 mo[105]. Although formal guidelines do not exist, a recent study suggests that this frequency of testing may be excessive in those whose CD4 counts are well above the treatment threshold[106].

DERMATOLOGY

In autoimmune blistering skin disease, diagnosis relies upon histopathological analysis of a skin biopsy together with direct immunofluorescence microscopy to demonstrate tissue-bound autoantibodies and/or C3 in the patient's skin or mucous membranes. Nonetheless, skin antibody testing (commonly by indirect immunfluorescence) provides useful information in the monitoring of these conditions. Increasingly however, as target autoantigens are being identified, ELISAs are being produced which can be used to measure antibody levels over the course of the disease. Target antigens include desmoglein 3 (pemphigus vulgaris), desmoglein 1 (pemphigus foliaceus), envoplakin (paraneoplastic pemphigus), NC16A domain of BP180 (bullous pemphigoid/pemphigus gestationis/mucous membrane pemphigoid) and type Ⅶ collagen (epidermolysis bullosa acquisita). In general, a reduction in antibody level can be demonstrated with disease remission, although the correlation is by no means absolute[107-109].

IMMUNE MONITORING IN CLINICAL IMMUNOLOGY

Serum immunoglobulin measurements

Serum immunoglobulin concentrations (IgG, IgA and IgM) are markedly raised in many autoimmune diseases. However, the magnitude of increase of these proteins is not related to the severity of disease; repeated measurements are therefore not useful[110].

Trough IgG levels provide a useful marker of adequacy of immunoglobulin replacement therapy in many patients with hypogammaglobulinaemia. Repeated measurement at least quarterly is recommended by the United Kingdom primary immunodeficiency network. (http://www.ukpin.org.uk/home/standards-CVID.htm, accessed February 21st, 2013).

CONCLUSION

Immunology laboratories make an enormous contribution to the diagnosis of several disorders. However, the role of immunology testing in disease monitoring has been subject to considerably less scrutiny. In this review, evidence has been evaluated to assess the role of several commonly requested tests in disease monitoring. Some investigations, such as paraprotein quantification or measurement of anti-GBM antibody titres, provide critical information to inform therapeutic decision-making. However, in many other cases, repeated performance of immunology tests generates data that do not alter disease management or accurately reflect disease status. As ever, liaison between clinical and laboratory staff remains vital to achieve optimal evidence-based selection and interpretation of test results to guide disease management.

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