Role of immunology laboratory in diagnosing renal diseases

Sabiha Anis*


Immune mediated injuries comprise a major bulk of renal disorders that mostly manifest as acute or chronic glomerulonephritides. Besides initial investigations for renal disorders, diagnosis of immune mediated renal diseases requires a battery of immunological tests and renal histopathology. The laboratory tests include anti-nuclear antibodies (ANA) followed by anti-double stranded deoxyribonucleic acid antibodies (anti-dsDNA) and/or anti-extractable nuclear antigens (anti-ENA), complement levels (C3, C4, factor H), rheumatoid factor (RF), C reactive proteins (CRP), anti-streptococcal antibodies including anti streptolysin-O titer (ASOT) and anti-deoxyribonuclease B (anti-DNAse B), anti-neutrophil-cytoplasmic antibodies (ANCA), anti-glomerular-basement membrane antibodies (anti-GBM), cryoglobulins detection, anti-phospholipid antibodies, nephritific factor, anti-phospholipase A2 receptor antibodies (anti-PLA2R), anti- thrombospondin type-1 domain-containing 7A (THSD7A) etc. The correct interpretation of these tests by an immunologist in collaboration with histopathologists and nephrologists is the key to an accurate diagnosis and successful management of patients with renal diseases.

A brief overview of the tests from the immunologist’s perspectives for the diagnosis and monitoring of renal disorders is given in this review.

Key Words: Anti-neutrophil cytoplasmic antibodies; Anti-nuclear antibodies; anti-phospholipase A2 receptor antibodies; Anti-streptococcal antibodies; Complement proteins; C-reactive protein; Glomerulonephritis; Immunological investigations of renal diseases; Rheumatoid factor

INTRODUCTION

Customarily renal injury or failure are broadly classified as pre-renal (azotemia), post-renal (obstructive) and interstitial renal (vascular, parenchymal or tubular) diseases. Depending on the clinical course and development of renal dysfunction, renal disorders are also categorized as acute and chronic diseases [1,2].

Baseline investigations in any kind of renal disorder include complete blood picture, detailed urine report with or without culture and sensitivity, serum creatinine, glomerular filtration rate (GFR) and creatinine clearance, 24 h urinary protein excretion, fraction excretion of sodium and albumin, cystatin C and radiological investigations such as ultrasound, computed tomography scanning etc. [3,4].

Most of the renal diseases result due to immune mediated injury mainly manifesting as glomerulonephritides (GN) [4]. GN can be primary or it may be a part of other autoimmune disorders such as systemic lupus erythematosus (SLE) with multiple organ involvement or vasculitis [5]. Immunopathogenesis of GN involves activation of complement cascades and coagulation pathways, recruitment of inflammatory cells and release of proinflammatory cytokines. Other important mechanisms include failure of apoptosis and intraglomerular hemodynamic changes. Progression to fibrosis and scarring depends upon the rate of antigen clearance or their persistence [1,5].

The immunological investigations for workup of patients presenting acutely or with chronic GN include anti-nuclear antibodies (ANA) followed by anti-double stranded deoxyribonucleic acid antibodies (anti-dsDNA) and/or anti-extractable nuclear antigens antibodies (anti-ENA), complement levels (mostly C3 and C4), rheumatoid factor (RF), C reactive proteins (CRP), anti-streptococcal antibodies such as anti-streptolysin O titer (ASOT) and anti-deoxyribonuclease B (anti-DNAse B), anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement membrane antibodies (anti-GBM), cryoglobulins detection and characterization, anti-phospholipid antibodies (APLA), nephritific factor and recently added anti-phospholipase A2 receptor antibodies (anti-PLA2R). Some of these tests are done routinely while others are done depending upon the presenting clinical features or histopathological findings and other laboratory parameters [5-10].

The value of these tests (immunological laboratory parameters) will be discussed in detail with their clinical association. The overview of the tests to evaluate immune dysregulation with renal manifestations is given in Table 1. Table 2 summarizes the clinical and laboratory features in various types of GN.

Table 1: Overview of laboratory tests done in patients with renal diseases.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA¹, anti-dsDNA², anti-ENA³</td>
<td>Autoimmune/connective tissue disorders such as lupus nephritis, systemic sclerosis, Sjögren’s syndrome, inflammatory dermatomyositis</td>
</tr>
<tr>
<td>Anti-neutrophil cytoplasmic antibodies (ANCA)</td>
<td>Rapidly proliferative GN; Pauci immune glomerulonephritis, pulmonary-renal syndrome</td>
</tr>
<tr>
<td>Anti-glomerular basement membrane antibodies (anti-GBM)</td>
<td>Rapidly proliferative GN; Anti-GBM disease, pulmonary-renal syndrome</td>
</tr>
</tbody>
</table>

Department of Immunology and Molecular Biology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan

*Correspondence: Sabiha Anis, Department of Immunology and Molecular Biology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan, Tel: +922132725543; E-mail: Sabiha_anis@hotmail.com

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Table 2: Laboratory Findings in Immune Mediated Renal Diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Renal involvement</th>
<th>Autoantibodies</th>
<th>Complement proteins</th>
<th>Other proteins</th>
<th>Other supportive laboratory features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>APSGN¹</td>
<td>5-52% of RPGN²</td>
<td>Anti-streptococcal antibodies (ASOT)¹⁴</td>
<td>C₃ mostly low, sometime C₃ and C₄ both low. Normal C₃ and C₄ do not rule out APSGN¹⁷</td>
<td>CRP⁵</td>
<td>Mild anemia, Hematuria with or without significant proteinuria</td>
<td>[7,11]</td>
</tr>
<tr>
<td>Anti-GBM²</td>
<td>10-20% of RPGN²</td>
<td>Anti-GBM²</td>
<td>Normal C₃ and C₄</td>
<td>CRP⁵</td>
<td>Hematuria ± proteinuria, Renal biopsy: linear deposition of IgG and C₃ on GBM⁶</td>
<td>[12]</td>
</tr>
<tr>
<td>ANCA¹⁸</td>
<td>74% of RPGN², GPA⁰, 70-80%, MPA¹⁰, 90-100%, eGPA¹¹:45%</td>
<td>C- and P-ANCA², anti-PR², anti-MPO¹³</td>
<td>Normal to high C₃ and C₄</td>
<td>CRP⁵</td>
<td>Paucimmune, necrotizing and or crescentic GN⁰, RPGN², usually preceded by upper respiratory infections, nasal polyps and nasal septum involvement in GPA² and eGPA¹¹, I/o asthma in eGPA¹¹</td>
<td>[13-15]</td>
</tr>
<tr>
<td>Henoch Schonlein Purpura</td>
<td>20-50%</td>
<td>Negative for autoantibodies, ASOT³</td>
<td>C₃ may be low</td>
<td>High IgA levels</td>
<td></td>
<td>[11,16]</td>
</tr>
<tr>
<td>Lupus Nephritis</td>
<td>40-75% of SLE¹⁴</td>
<td>Positive ANA¹⁵, anti-ds DNA¹⁶, anti-ENA¹⁷, anti-Smith (anti-Sm¹⁸, -SSA¹⁹, -SSB²⁰, -ribosomal P protein, -PCNA²¹, etc.)</td>
<td>Low C₃ and C₄</td>
<td>Normal or low CRP⁵</td>
<td></td>
<td>[17,18]</td>
</tr>
<tr>
<td>Renal APS²⁰</td>
<td>2.7% of APS²² 30% of SLE¹⁴ with APLA²³, 78% of catastrophic APS²²</td>
<td>Persistence of APLA²⁵</td>
<td>C₃ and C₄ may be low</td>
<td>Other coagulation proteins such as protein C and protein S may be low</td>
<td>Hypertension, Hematuria, proteinuria, renal dysfunction; Renal biopsy: TMA²⁵, thrombosis, infarction</td>
<td>[19-21]</td>
</tr>
<tr>
<td>Renal involvement in other connective tissue disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[17,22,23]</td>
</tr>
</tbody>
</table>

ANA, anti-dsDNA and anti-ENA

ANA are the hallmark of most of the connective tissue disorders [25]. These are detected by various methods such as indirect immunofluorescent (IFA) assay using Hep-2 cell lines or rat tissue (liver, kidney, stomach) as substrate, enzyme linked immunosorbent assay (ELISA) and immunodiffusion techniques, etc. The widely accepted assay for ANA detection is IFA using Hep-2 cell lines. The sensitivity of the assay is higher with this method especially for diagnosing SLE but at the expense of specificity [26].

Renal involvement occurs in various connective tissue disorders including SLE, systemic sclerosis (SSc), Sjögren’s syndrome (SS), inflammatory myositis [27,28] and others [17]. The histopathological evidence and detection of ANA followed by anti-dsDNA and anti-ENA antibodies has become the mainstay to make a final diagnosis of GN [5,29,30].

ANA can be positive in various other clinical conditions besides autoimmune disorders. They are also present in 5-10% of healthy individuals in low titers [31]. Therefore ANA results should be interpreted carefully in association with clinical features, other laboratory results and histopathological findings in GN patients.

Anti-dsDNA antibodies are detected most preferably by IFA using *Cribidia luciliae* as substrate. Other methods for anti-dsDNA detection including ELISA and agglutination assays are either less specific or less sensitive [32].

Anti-ENA antibodies can be detected by double immunodiffusion, counter current immunoelectrophoresis, ELISA and immunoblot assays. Detection of these autoantibodies are helpful in distinguishing various connective tissue disorders associated with renal manifestations such as anti-Scl-70 for diagnosing SSc, anti-Jo1 and anti-Pm/Scl for inflammatory myositis [33,34], anti-Sm for SLE and anti-SSA and anti-SSB for SLE based on clinical features. In rare cases of ANA negative SLE, identification of anti-ribosomal P protein (anti-P antibodies) and rarely anti-SSA antibodies become very helpful in reaching a correct diagnosis. However, with ANA detection by IFA using Hep-2 cells, a negative ANA with a high titre for anti-SSB is now a rarity. The characterization of ANA for their specificity is more important in GN patients with significant proteinuria [26].

Anti-phospholipid antibodies (APLA)

APLA are associated with antiphospholipid syndrome (APS). The syndrome is characterized by the manifestations of recurrent arterial and venous thrombosis in the presence of APLA. The most important antibodies, included in the classification criteria of APS are anti-beta-2 GP1 antibodies (anti-β2GP1), anti-cardiolipin antibodies (aCL) and lupus anticoagulant (LA). Complete work-up of APS require detection of LA and screening and isotypes (IgG, IgM and IgA) detection of anti-β2GP1 & aCL. These antibodies should be present persistently when tested 12 weeks apart with fresh samples [19,35].

APLAs can be primary or it can be secondary when found with other autoimmune disorders. Renal APS include renal vessel thrombosis, renal
Anti-complement antibodies containing 7A (anti-THSD7A) and other anti-phospholipase A2 receptor antibodies (anti-PLA2R) are of IgG4 subclass and correlate well with remission and response to therapy [48,50,51]. Anti-PLA2R antibodies have a high specificity (nearly 100%) and sensitivity around 70% for pMN. These autoantibodies are directed against M-type PLA2R, expressed normally on podocytes [49]. Anti-PLA2R are of IgG4 subclass and correlate well with remission and response to therapy [48,50,51]. Anti-THSD7A is also directed to the antigens present on podocytes named THSD7A. These antibodies can be detected in 2-5% of pMN, negative for anti-PLA2R. Anti-NEP and anti-BSA antibodies can be detected in children with pMN, however their clinical utility is not well established yet [48].

Complement levels
Complements are plasma proteins that provide protection against infections and autoimmune disease. Complement activation is involved in most of the renal injury with or without low complement (C3 and C4) levels in blood [52]. Usually classical or alternate pathways of complement activation are described in the pathogenesis of GN, C3 is found low in both pathways of complement activation while C4 is low in classical pathway complement activation [52,53]. The complement (C3 and C4) estimation assays are very useful not only in the diagnosis but monitoring of disease activity in various GN including LN, post-infectious GN, membranoproliferative GN (MPGN) and dense deposit disease (DDD). C3 is also found low sometimes in Henoch Schönlein purpura (HSP) [16,54]. Estimation of factor H, an alternate pathway complement regulatory protein, is done for the diagnosis of atypical hemolytic uremic syndrome (aHUS), MPGN type II and DDD. The complement protein assays are usually measured either by immunonephelometry or ELISA [53,55].

Anti-complement antibodies
These autoantibodies against various complement proteins or activated enzymes have been found in MPGN and DDD. These include C3 nephritic factor (C3NeF), C4 nephritic factor (C4NeF), anti-factor H (anti-fH), anti-factor B (anti-fB) and anti-C1q antibodies [56]. C3 NeF is an autoantibody of IgG or IgM isotype that prevents physiological decay of alternate pathway C3 convertase (C3Bb). These are of two types, properdin dependent (stabilizes C5 convertase as well) and properdin independent [56,57]. C4 NeF stabilizes classical pathway C3 convertase [58]. Anti-factor B antibodies have been detected in few patients presenting with DDD. Autoantibodies against the complement regulator, factor H are detected in some patients with aHUS [56,59]. Various assays are in use to detect these antibodies. Detection of anti-fH and anti-fB can be done by ELISA, but assays for nephritic factors are not very sensitive neither specific. This is because of the variability in antigenic epitopes recognized by these autoantibodies [56,58].

Cryoglobulins
Cryoglobulins are paraproteins comprising of abnormal immunoglobulins that precipitate at temperatures below 37˚C. They are strongly associated with hepatitis C virus (HCV) infection [60] but may be found in other infections, various autoimmune disorders or may be idiopathic [24,61]. Cryoglobulin detection requires stringent temperature control. The cryocrit or cryoprecipitate concentration does not correlate with the severity of vasculitis. However it is used for disease monitoring. Positive samples are characterized by immunofixation. Type II mixed cryoglobulins are associated with severe disease [61].

Rheumatoid factors (RFs)
RFs are autoantibodies against IgG molecule. The target antigenic epitope is in the Fc region of the molecule at the interface of Cγ2 and Cγ3. Under physiological conditions and in certain infections, polyclonal IgM-RFs are produced by CD5+B-cells that help in the removal of immune complexes and maintenance of immune homeostasis [62,63]. Pathological RFs are produced in rheumatoid arthritis, mixed cryoglobulinemia, chronic autoimmune diseases and certain infections. These pathological RFs are usually monoclonal and may be of other isotypes besides IgM (IgG, IgA or IgD) [63]. RF is a very useful test in the diagnostic workup and monitoring of GN. These auto antibodies have been reported to be involved in the pathogenesis of various GN including LN, IgA nephropathy, membranous GN [64], ANCA associated GN [65,66] and cryoglobulinemic GN with or without HCV [61,67]. Various assays are available to detect RF including latex agglutination assay, ELISA, immunonephelometry and multiplexed immunoassays [63].

C-reactive proteins (CRP)
CRP is an acute phase protein that increases several folds non-specifically in response to infections and inflammation and hence is a very useful surrogate marker in these conditions. In blood, it starts increasing in six to 12 h and peak at 19 h and is not influenced by other blood proteins or cellular constituents. Moreover, its concentration decreases more rapidly than erythrocyte sedimentation rate at the decline of inflammation or infection. Therefore, it is a much more reliable inflammatory marker for disease monitoring [68,69]. CRP is included in the diagnostic testing repertoire of GN and monitoring the response to treatment. In SLE patients, CRP helps to differentiate between active LN disease and infections [69-72].

Immunoglobulins, immunofixation electrophoresis (IFE) and free light chain assay
There is a polyclonal increase in serum immunoglobulins in chronic infections and autoimmune diseases [73]. This may also be reflected in immune mediated renal disorders [22]. However in GN patients with glomerular damage, serum IgG is often found low due to their urinary excretion [74]. In HSP, serum IgA is usually found high, though not used as a surrogate diagnostic marker [11,16]. The utility of serum immunoglobulins mainly lies in multiple myeloma (MM) along with serum and urine electrophoresis and immunofixation and free light chain assay [75]. In MM and monoclonal gammopathy of renal significance (MGRS), electrophoresis determines the presence of monoclonal proteins in the
form of M spike which is then characterized by immunofixation [76]. Recently serum free light chain assay has become an integral part of the diagnostic work-up and for monitoring and determining prognosis of patients with monoclonal gammopathy. Its role becomes more important in non-secretory multiple myeloma and light chain disease. It is more sensitive than urine protein electrophoresis and IFE (for Bence Jones proteins) [77,78].

**Serum IgG subclasses**

There are four IgG subclasses including IgG1-IgG4. The utility of serum IgG subclass in renal diseases lies in the determination of IgG4 when there is a suspicion of IgG4 related renal disease (IgG4-RD). This is a recently recognized entity characterized by storiform fibrosis and dense lymphoplasmacytic infiltrates, mostly as tubulointerstitial nephritis. As IgG4 is increased in many other disorders including autoimmune diseases, vasculitis and malignancies, therefore it is important to use this test judiciously only when there is a clinical suspicion of IgG4-RD. moreover this test is interpreted along with the histopathological findings of the organ involved [79,80].

**Viral markers**

Certain viruses are implicated in renal diseases as they cause immune dysregulation, resulting in immune complex formation and deposition with subsequent complement activation [81].

Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are associated with various forms of GN including cryoglobulinemic GN, MPGN and polyarteritis nodosa causing pauci immune GN. Therefore, anti-HCV and HBV surface antigen (HBs Ag) detection tests are done routinely in these patients [82].

Human immunodeficiency virus (HIV) is also associated with renal pathology, either directly or indirectly due to co-infections or as side effects of anti-retroviral therapy. HIV associated nephropathy (HIVAN) is mostly found in African-American population. KIDDO (Kidney disease: improving global outcomes) has given pathological classification and guidelines for the management of these patients [83]. It is to be noted that in the setting of certain risk factors and characteristics histopathological features, anti-HIV testing should be done for better patient management.

Less commonly Epstein Barr virus (EBV) can also cause acute or chronic kidney disease. Therefore in case of infectious mononucleosis and renal manifestation, consider EBV infection also and keep it in your investigating repertoire [45,81].

**CONCLUSION**

Immunology laboratory has a very important role in the diagnosis and monitoring of disease in patients with renal disorders especially with GN. Immunopathologist have a very big responsibility in proper interpretation of the results in correlation with clinical features that affects patient management. It is important that immunologists, histopathologists and nephrologists or treating physicians should collaborate with each other for there is a suspicion of IgG4 related renal disease (IgG4-RD). This is a sensitive than urine protein electrophoresis and IFE (for Bence Jones proteins) [77,78].

**REFERENCES**

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