Immunological diagnosis of vasculitis

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ABSTRACT

Inflammation of blood vessels, or vasculitis, is caused by a heterogenous group of autoimmune conditions with wide spectrum of systemic, and often overlapping, clinical manifestations. Some of these conditions present acutely and result in major organ's damage and, therefore, require prompt diagnosis and treatment in order to avoid the high morbidity and mortality that otherwise occur. The clinical immunology laboratory plays a vital role in the diagnosis of vasculitis. Moreover, due to the availability of simple tests, with quick turn around time, immunological findings can provide an early picture of the type of vasculitis involved thereby allowing initiation of prompt treatment in life threatening situations. In the present review, we will outline the various tests available in the immunology laboratory for the investigation of vasculitides, discuss the assays used to carry out these tests and, finally, comment on the significance of the results produced in relation to the diagnosis, or exclusion, of vasculitis. We hope that such information would prove of great importance to physicians and immunologists alike and lead to more efficient diagnosis of these important and, often, life threatening conditions.

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Vasculitis refers to inflammation of blood vessels and is caused by a heterogeneous group of autoimmune conditions occurring either as primary (idiopathic), or secondary to a variety of systemic inflammatory, malignant and infectious diseases (**Table 1**).[1-4] Some of these vasculitic conditions present acutely and cause major organ damage and, therefore, require prompt diagnosis and intensive treatment in order to avoid the high morbidity and mortality that would, otherwise, ensue. Due to the diverse and complex clinical picture of vasculitis which overlaps with a variety of other conditions, recognition of vasculitis can be difficult. However, the greatest difficulty is encountered when trying to diagnose individual vasculitic conditions, particularly when a small vessel vasculitis is suspected.[1,4] Diagnosis of specific vasculitides, therefore, requires a high clinical suspicious of vasculitis, supported by extensive laboratory investigations (**Table 2**). The clinical immunology laboratory plays a vital role in this diagnostic process. Some of the immunological findings are almost diagnostic; being highly specific and sensitive for a specific vasculitis.[5-7] Moreover, due to the availability of simple tests, with quick turn around time, immunological findings can provide an early indication of the type of vasculitis involved and thus allow appropriate treatment to be commenced in life threatening situations.[8,9] For proper and efficient use of the immunology laboratory in the investigation of vasculitides, full awareness of the vasculitic test repertoire, and the significance of the results produced, is needed. In contrast, lack of knowledge of the tests available and/or the significance of the results produced can lead to delay in, or even the wrong, diagnosis of a particular vasculitis.[10,12] In the present review, we will outline the various tests available in the immunology laboratory for the investigation of vasculitides, discuss the assays used to carry out these tests, and then comment on how the results produced

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Table 1 - Primary and secondary vasculitides.

Large vessel vasculitis
- Giant cell (temporal) arteritis
- Takayasu's arteritis

Medium vessel vasculitis
- Polyarteritis nodosa
- Kawasaki disease

Small vessel vasculitis

Anti neutrophil cytoplasmic antibodies-associated vasculitis
- Wegner granulomatosis
- Microscopic polyangiitis
- Churg-Strauss syndrome

Immune complex vasculitis

Primary (Idiopathic) vasculitis
- Henoch-Schonlein purpura
- Anti-glomerular basement membrane disease (Goodpasture syndrome)
- Hypocomplementemic urticarial vasculitis
- Behcet's disease
- Essential cryoglobulinemia

Secondary vasculitis
- Serum sickness
- Lupus vasculitis
- Rheumatoid vasculitis
- Sjogren's syndrome vasculitis
- Drug induced vasculitis*
- Infections induced vasculitis
- Cystic fibrosis
- Sarcoidosis
- Lymphoproliferative neoplasm induced vasculitis
- Inflammatory bowel disease

Table modified from reference-1

**Drugs:* penicillins, aminopenicillins, sulfonamides, allopurinol, thiazides, pyrazolones, retinoid, quinolones, hydantins, propylthiouracil, minocycline, azithromycin, penicillamine, sulfapyridine, streptomycin, hydralazine, streptokinase, cytokines, monoclonal antibodies.

Mimics of vasculitis: for example; endocarditis, cholesterol embolization, embolic disease, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, antiphospholipid syndrome, disseminated intravascular coagulation, systemic infections, arterial myxoma, connective tissue disease.

Table 2 - Immunological, and non-immunological, tests used in the investigation of vasculitides.

Tests	Purpose
Immunological tests	
ANCA	ANCA-associated vasculitis (WG, CSS, MPA, RPGN)
Anti-GBM antibodies	Anti-GBM disease
Cryoglobulins	Cryoglobulinemia (essential/ secondary)
RF	Cryoglobulinemia
ANA/dsDNA	Exclude CTD (for example; SLE)
Complement (CH50, C3, C4)	Cryoglobulinemia, HUVS, SLE
IgE	Raised in CSS
Skin/renal biopsies immunofluorescence	Immune complex vasculitis (cryoglobulinemia, HSP, HUVS) Pauci-immune vasculitis (WG, MPA, CSS, RPGN)
CRP/ESR	Raised in vasculitis
ACA	Exclude APC
Serum immunoglobulins and electrophoresis	Cryoglobulinemia, HSP, malignancies
Other laboratory tests	
FBC and differential	Normocytic anemia, thrombocytosis, leukocytosis, lymphopenia eosinophilia (CSS >10%)
Coagulation screen	Exclude thrombosis
Blood cultures	Exclude infection
Viral serology/genetic (HCV, HBV, EBV, CMV, HIV)	Cryoglobulinemia, PAN
Cr/E/liver enzymes/urine analysis	Pointer to type, extent, and severity of vasculitis
Fecal blood	Gut associated vasculitis
Histopathology	For example; leukocytoclastic/ granulomatous vasculitis crescentic glomerulonephritis

ANCA - anti neutrophil cytoplasmic antibodies, WG - Wegener's granulomatosis, CSS - Churg Strauss syndrome, MPA - microscopic polyangiitis, RPGN - rapidly progressive glomerular nephritis, GBM - glomerular basement membrane, RF - rheumatoid factor, CTD - connective tissue disease, SLE - systemic lupus erythematosus, IgE - immunoglobulin E, HUVS - hypocomplementemic urticarial vasculitic syndrome, C - complement, CRP - C-reactive protein, ESR - erythrocyte sedimentation rate, ACA - anti-cardiolipin antibodies, APC - anti-phospholipid syndrome, HSP - Henoch Schonlein syndrome, PAN - polyarteritis nodosa, FBC - full blood count, ANA - antinuclear antibodies, dsDNA - double stranded deoxyribonucleic acid, HCV - hepatitis C virus, HBV - hepatitis B virus, EBV - Epstein-Barr virus, CMV - cytomegalovirus virus, HIV - human immunodeficiency virus, Cr - creatinine, E - electrolytes.

Table 3 - Antineutrophil cytoplasmic antibodies and associated conditions.

C-ANCA	C-ANCA + PR-3	P-ANCA	P-ANCA + MPO
WG	WG	MPA	MPA
CSS	CSS	RPGN	RPGN
MPA	RPGN	CSS	CSS
RPGN	MPA	RA	RA
Amoebiasis	Amoebiasis	SLE	SLE
TB		Anti-GBM disease	Anti-GBM disease
Others*		PSC	
		IBD	
		LPD	
		TB	
		Sarcoidosis	
		HSP	
		Cryoglobulinemia	

C/P-ANCA - cytoplasmic/perinuclear antineutrophil cytoplasmic antibodies, PR3 - proteinase 3, MPO - myeloperoxidase, WG - Wegener's granulomatosis, CSS - Churg Strauss syndrome, MPA - microscopic polyangiitis, RPGN - rapidly progressive glomerular nephritis, TB - tuberculosis. RA - rheumatoid arthritis GBM - glomerular basement membrane, SLE - systemic lupus erythematosus, HSP - Henoch Schonlein purpura, PSC - primary sclerosing cholangitis, IBD - Inflammatory bowel disease, LPD - lymphoproliferative disease.
*Positive C-ANCA, without PR3, are also reported in patients with human immunodeficiency virus, endocarditis, pneumonia, cystic fibrosis, malignancy, polymyalgia rheumatica, hypereosinophilic syndrome, tuberculosis.

can be used in the diagnosis and monitoring, or exclusion, of a specific vasculitis.

Immunological tests. ***Anti-neutrophil cytoplasmic antibodies (ANCA).*** Anti-neutrophil cytoplasmic antibodies are autoantibodies directed against various components of neutrophil cytoplasmic granule constituents including proteinase-3 (PR3), myeloperoxidase (MPO), elastase, cathepsin G, lactoferrin, bacteriocidal/permeability increasing protein (BPI), and glucuronidase.[13,14] Testing for ANCA is used in the investigation of ANCA-associated vasculitides (**Table 1**).[7,15,16] Testing for ANCA is performed by an indirect immunofluorescence (IIF)-assay, employing ethanol fixed human neutrophils; as substrate, and by enzyme-linked immunosorbent assay (ELISA). Three types of staining patterns are detected by the IIF-assay which includes the granular cytoplasmic staining (termed C-ANCA), the perinuclear staining (P-ANCA) and a third staining pattern termed atypical or X-ANCA.[7,16-18] The latter pattern can be either a diffuse, or fine granular cytoplasmic staining; without the nuclear interlobular accentuation, or fine perinuclear staining patterns without the nuclear extension.[16,19] Anti-neutrophil cytoplasmic antibody staining is produced by autoantibodies binding to neutrophil cytoplasmic enzyme PR3, while P-ANCA staining is produced by antibodies binding to a number of cationic enzymes and proteins (including MPO, elastase, cathepsin G, lactoferrin, BPI, glucuronidase and, occasionally, PR3) that become localized to the nuclear membrane during ethanol fixation. The X-ANCA is produced by autoantibodies binding to neutrophil cytoplasmic components other than PR3 (such as BPI, elastase) and autoantibodies binding to nuclear membrane proteins.[16-19] Out of all the individual ANCA autoantibodies, only PR3 and MPO antibodies have varying specificities for vasculitis, whereas other autoantibodies are associated with a variety of other non-vasculitic conditions (**Table 3**) and, therefore, in clinical practice only the PR3 and MPO autoantibodies are measured.[20-25] It is recommended that screening for ANCA is performed by an IIF-assay, and all positive samples are further typed for PR3 and MPO antibodies using ELISA assays.[26,27] Positive C-ANCA/PR3 are strongly associated with Wegener's granulomatosis (WG); with a specificity ranging from 95-100%.[16,28-32] However, the sensitivity varies considerably from one study to another (range 22-100%) and depends largely on the nature of the disease and the method used. Thus, in the systemic form of WG, the sensitivity can be as high as 100%, while in the limited (such as subglottic stenosis, upper respiratory tract) and the inactive forms, the sensitivities are below 50% and can be as low as 22%.[16,33-35] C-anti-neutrophil cytoplasmic antibodies/PR3 are also reported in 30% of patients with Churg Strauss syndrome (CSS), 5-10% with microscopic polyangiitis (MPA) and small percentage of patients with rapidly progressive glomerular nephritis (RPGN). In addition, these antibodies have also been reported in some patients with amebiasis, Tuberculosis and propylthiouracil induced vasculitis.[13,16] However, in a recent large study of vasculitides, C-ANCA/PR3 were largely found associated with WG; with <1% of patients with CSS, and no patients with MPA, being positive.[28] C-anti-neutrophil cytoplasmic antibodies alone is reported to be less specific for WG as it has been associated with a variety of other conditions beside vasculitis (**Table 3**).[16,25,36] However, it is unclear whether the C-ANCAs observed in these previous studies were the traditional granular C-ANCA, or the atypical (flat) C-ANCA (X-ANCA) that is associated with antibodies to other than PR3 (such as elastase, BPI).

Positive P-ANCA/MPO are strongly associated with MPA; with a sensitivity of 47-75% and specificity

of 85-99%.[16,28,33] These antibodies are also found in 40-60% of patients with RPGN, 30% with CSS, 10-40% with anti-GBM disease, 5-25% with WG.[16,37] In addition, these antibodies have also been reported in a wide variety of conditions beside vasculitides (**Table 3**).[16,25] However, in a recent large study of vasculitides, P-ANCA/MPO were found largely associated with MPA; with <7% of patients with CSS, and even small percentages of other conditions (such as systemic lupus erythematosus [SLE], rhuematoid arthritis), being positive.[28] P-ANCA alone is less specific for MPA as it is associated with a variety of other conditions (**Table 3**).[25,38]

Perinuclear staining-ANCA, or C-ANCA, with specificity for both MPO and PR3 have been detected in patients with WG, MPA, and propylthiouracil induced vasculitis and some unspecified conditions. In patients with WG, or MPA, switching to a single antibody type can occur with time.[39]

The difference in the sensitivity, and specificity, of ANCA for the diagnosis of WG and MPA is due to a number of variables that are associated with the assays used to measure ANCA (which include the method used for neutrophil/neutrophil-proteins preparation and fixation, dilution of samples [ranges from 1:8-1:40; with the majority of laboratories using 1:20]), dilution of conjugates, intensity of light in the microscopy room and the experience of the person reading the IIF-results, in addition to the types of patients investigated (limited vs systemic; active vs inactive disease).[16,22,30,35,39] In contrast to the IIF-assay, ELISA results are regarded as more specific, but less sensitive, for the diagnosis of WG and MPA. Reduced sensitivity has been largely attributed to loss of conformational epitopes of MPO- and PR3- proteins during coating, and fixation, to ELISA plates. Capture-ELISA, which tends to preserve these conformational epitopes, is associated with enhanced sensitivity and can be used in place of traditional ELISAs.[40-42]

Finally, rapid spot test ELISA have sensitivities and specificities equal to that associated with traditional ELISAs, and thus can be used in emergency situations for the detection of MPO and PR3 antibodies.[8] Regarding ANCA-testing, there has been a debate on which patients to test for ANCA. Some have recommended that testing should only be carried out for patients with chronic destructive airway diseases, pulmonary nodules, subglottic stenosis, retro-orbital mass, pulmonary renal syndrome, RPGN, mononeuritis multiplex; while others have recommended that ANCA-testing should be carried out for patients with all types of glomerulonephritis, cutaneous vasculitis with systemic features, long standing sinusitis and otitis, peripheral neuropathy and undiagnosed inflammatory diseases.[19,38,41,42] However, since vasculitides can manifest in many ways, often indistinguishable from other systemic inflammatory and connective tissue diseases, and since untreated vasculitis can be associated with high morbidity and mortality, testing for ANCA may need to be left to the judgment of individual clinicians. It has been estimated that the financial saving gained from early detection of a few patients with ANCA-associated vasculitis would offset the financial expenditure that would be saved from restricting ANCA testing.

Anti-glomerular basement membrane (GBM). Anti-glomerular basement membrane antibodies are autoantibodies directed against the α-3 chain of type 4 collagen that is found in the glomerular- and alveolar-basement membranes, as well as in other special membranes including that of the retina capillary, cochlea and the choroid plexus in the brain.[5,6,43] High titres anti-GBM antibodies are associated with anti-GBM disease (Goodpasture's disease). Screening for anti-GBM antibodies can be performed by an IIF-assay using rodent, or human, renal tissues as substrates. Linear staining of IgG, and complement, is detected along the GMB.[6,43] However, similar staining can be produced in many other conditions with raised serum immunoglobulins (such as SLE, diabetes mellitus, severe nephrotic syndrome, Alport's syndrome) thereby reducing the specificity of the assay for anti-GBM disease.[6] This assay has, therefore, been largely replaced by a more sensitive, and specific, ELISA assay (**Table 4**). Enzyme-linked immunosorbent assay detects high titre anti-GBM antibodies with a sensitivity and specificity of more than 98% and 99%, respectively.[6,43] Since many diseases can produce linear immunoglobulin (Ig) G and complement staining, positive direct immunofluorescence staining of renal biopsy should always be confirmed by an ELISA assay. Enzyme-linked immunosorbent assays are also used to monitor response of the disease to therapy.[44]

Cryoglobulins. Cryoglobulins are immuno-globulins that precipitate at low temperature and re-dissolve when heated to 37°C. There are 3 types of cryoglobulins. Type-1 cryoglobulins consist of a monoclonal protein (usually IgMk) and are associated with hematological malignancies. Type-2 consists of a monoclonal protein (IgMk), with rheumatoid factor activity, and polyclonal IgG antibodies. The third type consists of polyclonal IgM, with rheumatoid factor activity, and polyclonal IgG antibodies. Types 2 and 3, also known as mixed cryoglobulins, are associated with hematological malignancies, chronic inflammation and infections.[45-47] The physiochemical properties of cryoglobulins determine the temperature at which

Table 4 - Sensitivities and specificity's of serological tests used in the investigation of vasculitides.

Types of vasculitis	IIF		IIF + ELISA		IIF	ELISA
	C-ANCA	P-ANCA	C-ANCA + PR3	P-ANCA + MPO	RENAL	GBMA
WG						
Sen	63-100%		22-100%			
Spec	95-99%		99-100%			
MPA						
Sen		50-75%		47-75%		
Spec		76-94%		85-99%		
GBMD						
Sen					75%	98%
Spec					Low	99%

IIF - indirect immunofluorescence, C/P-ANCA - cytoplasmic/perinuclear-anti neutrophil cytoplasmic antibodies, PR3 - proteinase 3, WG - Wegner granulomatosis, MPA - microscopic polyangiitis, GBMA/D - anti-glomerular basement membrane antibodies/disease, Sen - sensitivity, Spec - specificity.

they precipitate, which in turns determines the clinical manifestations and the severity of the disease. Thus, cryoglobulins that precipitate at high temperatures are associated with severe disease, whereas cryoglobulins that precipitate at low temperatures may not be associated with any clinical manifestations.[46-48] Precipitation of cryoglobulins occur as a result of immunoglobulin interaction, occurring non-specifically (between the same class, as well as between different classes, of immunoglobulins) and specifically (through binding of rheumatoid factor (RF) IgM to the fragment crystallize portion of IgG molecules). Non-specific binding occur as a result of increased glycosylation of immunoglobulins. These altered chemical properties of immunoglobulins also cause the precipitates to bind to the blood vessel wall leading to activation of the complement system and initiation of inflammatory reaction leading to vessel wall damage.[46-53] Immune complexes are deposited in blood vessels of many organs including the skin and kidneys. For the investigation of cryoglobulins, it is vital that blood is drawn into pre-warmed syringes and maintained at 37^{O}C until separation of serum from clotted blood. For parallel measurement of cryogfribinogen, a separate blood sample should be taken into EDTA-tubes. Separated serum, and plasma, is then left at 4^{O}C for a period of seven days.[46,47] The tubes are then centrifuged and examined for presence of precipitates. In some laboratories, absence of visible precipitates is taken as absence of cryoglobulins, and results are reported as negative, while other laboratories proceed with quantification of cryoglobulins regardless of the presence or absence of precipitates.[46,47] When precipitates are observed, tubes are heated to 37°C to re-dissolve the precipitates and thus confirm presence of cryoglobulins. Precipitates that fail to dissolve would suggest presence of contaminating fibrinogen resulting from either inadequate time given for blood clotting, or blood obtained from patients who are on heparin therapy.[46] Precipitates are then washed several times to remove non-specific bound proteins and the cryoglobulins are then reported as cryocrits (percentage precipitates in relation to the starting volume of serum), or quantified nephelometrically and reported in mg/L; taking the normal values as 60-80 mg/L.[46,53] Washed precipitates are also investigated for the presence of paraproteins and rheumatoid factor activity.[46,53] False negative results are common and are due to failure to maintain blood at 37^{O}C and, consequently, cryoprecipitates are lost with clotted blood during serum separation. It is therefore vital that blood is always taken into pre-warmed syringes and maintained at 37°C until separation of serum.

Anti-C1q IgG antibodies. Anti-C1q IgG antibodies with specificity for the collagen like head of C1q are associated with hypocomplementemic urticarial vasculitic syndrome (HUVS).[54,55] Quantification of these antibodies is performed by ELISA assays. High titre antibodies are found in 100% of patients with HUVS and are reported to be diagnostic for the disease.[54,55] These antibodies have also been associated with a few other diseases including rheumatoid arthritis (particularly with associated vasculitis, Felty's syndrome) and SLE.[7,56,57]

Other tests used in the investigation of vasculitis. Evaluation of serum complement is essential in the investigation of vasculitis. The patterns of serum complement can suggest a diagnosis. Thus, in patients with clinical suspicious of vasculitis, low C4 ± C3 would suggest a diagnosis of cryoglobulinemia, or HUVS. Assessment of serum immunoglobulins (IgG,

IgM, IgA) by nephelometry, and electrophoreses, is also essential. IgA is raised in more than 50% of patients with Henoch Schonlein purpura (HSP) and IgA nephropathy (believed to be a limited form of HSP).[59] Presence of paraproteins could indicate presence of cryoglobulinemia and/or malignancies. Hypogammaglobulinemia, with normal serum albumin, may indicate presence of cryoglobulins which have been lost during improper blood handling. Anti-nuclear antibodies (ANA) are autoantibodies directed against the various components of the cell nucleus including dsDNA. Anti-nuclear antibodies are measured by an indirect immunofluorescence using Hep-2 cell as substrate, while anti-dsDNA antibodies are measured by an IIF-assay, using Crithidia Lucilae as substrate, and by ELISA.[45] Both ANA and anti-dsDNA antibodies should be measured when investigating vasculitides in order to exclude possible presence of connective tissue diseases (such as SLE) whose clinical presentation overlaps with the vasculitides. While anti-dsDNA antibodies (by IIF) are specific for SLE; ANA (and anti-dsDNA antibodies measured by ELISA) are not as they are found in many other conditions including hepatitis C virus-associated cryoglobulinaemia.[45] Rheumatoid factor is an autoantibody directed against the FC portion of IgG and it is measured by ELISA, or nephelometry. Although this is non-specific test, in patients with clinical suspicious of vasculitis, its presence, along with low C4 and a paraprotein, would suggest a diagnosis of cryoglobulinemia.

Finally, anticardiolipin antibodies (ACA) may need to be tested in certain situations (such as in patients with clinical suspicious of vasculitis and thrombocytopenia) in order to exclude antiphospholipid syndrome (APS). The syndrome is characterized by recurrent arterial and venous thrombosis, recurrent miscarriage and thrombocytopenia. The clinical manifestations include renal failure, hypertension, pulmonary embolism, myocardial infraction, Budd Cherry syndrome, skin ulcers, digital gangrene, livedo reticularis, intermittent claudication, migraine, chorea, amaurosis and stroke and thus can mimic vasculitis.[60] Anticardiolipin antibodies are measured by ELISA. Strong IgG antibodies are strongly indicative of APS, whereas the significance of low IgG, or IgM alone, is debatable and, therefore, need to be interpreted in relation to the clinical picture. Lupus anticoagulant test need to be requested as well, since the antibodies involved in each test can exist independently of the other.[45,60]

The clinical immunology laboratory plays a vital role in the diagnosis, and monitoring, of vasculitides. The tests available in the laboratory include ANCA, anti-GBM antibody test, cryoglobulins, anti-C1q-IgG, complement, Igs, RF, ANA, dsDNA, ACA, and histological examination of skin and renal biopsies.Anti-neutrophil cytoplasmic antibodies testing is used in the investigation and monitoring of ANCA-associated vasculitides (**Table 1**). Positive C-ANCA and PR3 are strongly suggestive of WG.[2,3,16,28-32] Confirmation of the disease is obtained, in most instances, from skin and renal biopsies showing necrotizing leukocytoclastic vasculitis, and necrotizing crescentic glomerular nephritis, respectively, with pauci immune deposition.[2,15] However, it should be remembered that 20% of patients with WG have associated IgA-nephropathy and, therefore, granular deposition of IgA, IgM and complement components in both renal and skin biopsies may be observed.[12,61] Such findings have in the past led to a wrong diagnosis of HSP.[12] It is, therefore, important that in patients with a strong suspicion of WG, presence of immune complex deposition in renal and/or skin biopsies should not be taken to exclude WG. In patients with allergy (asthma and allergic rhinitis), presence of C-ANCA with PR3 specificity, raised IgE and eosinophilia (>10%), would suggest a diagnosis of CSS, whereas in renal limited disease, the results would suggest a diagnosis of RPGN. Finally, C-ANCA and PR3 have been associated with a minority of patients with MPA.[16] In the absence of PR3, classical granular C-ANCA would still strongly suggest a diagnosis of WG.[62] However, since considerable experience in recognizing such a pattern is required, C-ANCA, with negative PR3, should be interpreted in relation to the clinical picture and other laboratory and radiological findings.

Presence of P-ANCA, with anti MPO specificity, would strongly suggest a diagnosis of MPA.[16,42,48] In renal limited disease, the results would suggest a diagnosis of RPGN, or anti-GBM disease and, therefore, anti-GBM antibodies should always be measured at the same time as ANCA.[16] In patients with allergy, P-ANCA/MPO would suggest a diagnosis of CSS.[15,16] Finally, P-ANCA and MPO have been reported in small number of patients with other diseases (**Table 3**). Results of other immunological tests (ANA, dsDNA, RF, complement) and skin and/or renal biopsies would serve to exclude, or include, these diseases. The titres of anti PR3- and MPO-antibodies correlate with disease activity in the majority of patients with ANCA-associated vasculitis and, therefore, monitoring of these antibodies is recommended. In the majority of patients with WG, anti-PR3 titre decreases with treatment and increases with disease relapse. However, in a small percentage of patients, the titre remains elevated despite clinical improvement.[44,62-64]

Anti-GBM disease, is a very rare (one per 2 million), but life threatening condition characterized by RPGN and pulmonary hemorrhage[5,6] and, therefore, like ANCA-associated vasculitis, requires prompt diagnosis and treatment. Presence of strong anti-GBM antibodies is diagnostic of the disease, and confirmatory results are obtained from renal biopsy showing linear deposition of IgG and complement components along the GMB.[19] However, since anti-GBM disease can be associated with membranous glomerulonephritis, the finding of granular deposition of other Igs should not be taken as an exclusion of anti-GBM disease.[65] Close examination of renal biopsy should reveal the linear deposition of IgG.

Low anti-GBM antibody titres are found in 10-30% of patients with anti-GBM disease in whom ANCA (largely P-ANCA/MPO) coexist. Such patients, in whom the disease behaves more like a vasculitis, tend to have a better prognosis.[16,43] Monitoring of anti-GBM antibodies is recommended. Intensive therapy with plasma exchange and chemotherapy leads to reduction in the anti-GBM antibody titre. Disappearance of the antibody can take between a few weeks to several years, with an average of 12-15 months period.[5,6,43] Kidney transplantation should be delayed until after the disappearance of the antibodies; ideally > 6, preferably 12, months after disappearance of the antibodies, since residual antibodies can result in the reoccurrence of the disease in the transplanted organ.[43] However, occurrence can still occur in patients with the inherited deficiency of α- 3, 4 and 5 collagens (Alport's syndrome) due to generation of antibodies against these types of collagens in transplanted normal kidneys. In such patients, direct immunofluorescence of renal biopsy would be positive, however, ELISA may show low or absent (where deficiency is mostly due to α- 5) anti-GBM antibodies.[66]

Henoch Schonlein purpura is largely a disease of childhood and present classically with skin purpura, migratory arthralgias and abdominal pain. However, the disease can also present with clinical signs and symptoms that are indistinguishable from other vasculitic conditions (**Table 5**).[59] Currently there are no specific serological tests for the investigation of HSP. Anti-C1q IgA antibodies have been associated with a proportion of patients, however, these antibodies are not routinely measured.[67] In the appropriate clinical setting elevated serum IgA, which occur in more than 50% of patients, would suggest a diagnosis of HSP.[44,59] However, diagnosis of HSP is based on the clinical picture, supported by the findings of renal and/or skin biopsy investigation. The findings of IgA ± IgG, IgM and complement in renal (in the mesangium and along the GMB), and skin (around blood vessels and along the epidermal-dermal junction) biopsies would confirm the diagnosis of HSP. Similar findings are also associated with IgA-nephropathy; believed to be a renal limited form of HSP.[19,42,57]

Cryoglobulinemia is classified amongst small vessel vasculitides and can be primary (essential), or secondary to a variety of inflammatory, malignant and infectious conditions.[45-57] Most of what was referred to in the past as mixed essential cryoglobulins are now known to be associated with HCV infection.[47] The syndrome is characterized by a protean range of clinical manifestations which are shared by many other forms of small vessel vasculitides and, therefore, the syndrome can be difficult to diagnose on clinical grounds (**Table 5**).[46,47,53] Cryoglobulin investigation should be performed in patients with clinical suspicious of vasculitis, with renal failure and in patients with cold intolerance.[2,3,7] In the laboratory, cryoglobulin-test may be added when other laboratory results indicate the possibility of cryoglobulinemia (such as IgM and complement deposition in skin and renal biopsies, low serum complement components [particularly C4] ± hypogammaglobulinemia, high RF, paraprotein). In patients with clinical suspicious of vasculitis, presence of cryoglobulins, low complement levels (particularly C4), RF and a paraprotein would strongly suggest a diagnosis of cryoglobulinemia. Confirmation of the disease is obtained from biopsies showing immune deposition (IgM [± IgG, IgA] and complement) in renal (in the mesangium, along the GBM and in blood vessels) and skin (around blood vessels).[49-54] However, it should be remembered that the amount of immune deposition in both skin and renal biopsies varies considerably.[49,53] In many samples, traces of IgM and/or complements may be all that is found and, therefore, such findings should not be taken as an exclusion of cryoglobulinemia and the investigation shifted towards the pauci-immune vasculitis. Equally, since cryoglobulins are not associated with cryoglobulinemic syndrome in the majority of patients, and since cryoglobulins can accompany many other diseases beside vasculitis; their presence per se should not be taken as evidence for cryoglobulinemia. The results need to be interpreted, not only in relation to the clinical picture, but also in relation to other immunological and non immunological findings. Finally, like all patients with paraproteins, patients with type-2 cryoglobulinemia should be monitored indefinitely, since many patients (3-38%) develop non-Hodgkin's lymphoma within a period of 10 years.[3]

In addition to cryoglobulinemia, low serum C4 ± C3 is also associated with HUVS, a disease that is characterized by chronic urticaria and SLE like picture,

Table 5 - Vasculitic conditions and their clinico-pathological correlates.

Types of vasculitis	Serology/serum proteins	Histology/immunohistology renal/skin	Clinico-pathological picture
WG	ANCA (69-100%) C-ANCA/PR3 (22-100%) P-ANCA/MPO (5-10%) ANA (low titre) raised-normal complement normal Ig RF (50%)	necrotizing crescentic GN necrotizing leukocytoclastic vasculitis ± granulomatous inflammation pauci Immune deposition *	Triad of respiratory tract, renal and systemic vasculitis respiratory (sinus pain, epistaxis, nasal ulceration, necrosis of nasal septum → perforation and saddle nose deformity, otitis media with facial paralysis, subglottic stenosis→ stridor, pulmonary infiltration/nodules, pulmonary hemorrhage, cough, dyspnea,), ocular (orbital pseudotumor/proptosis, episcleritis, uveitis, conjunctivitis, blindness), renal (RPGN, suppurative interstitial nephritis), cutaneous (purpura, ulcer, urticaria), neuropathy, abdominal, cardiac infarction.
MPA	P-ANCA (47-75%) P-ANCA/MPO (47-75%) C-ANCA/PR3 (0-25%)	as above, but no granulomas	Lower respiratory and renal involvement (nephritis, renal insufficiency, hypertension, pulmonary hemorrhage), nervous system (polyneuropathy, mononeuritis multiplex, seizures, monoplegias, optic neuropathy, sensorineural deafness, subarachnoid hemorrhage, pseudobulbar palsy), cutaneous (purpura, splinter hemorrhage, ulceration), others (episcleritis, coronary arteritis).
CSS	C-ANCA/PR3 (1-30%) P-ANCA/MPO (7-30%)	necrotizing vasculitis eosinophilic rich inflammatory infiltration ± granulomas in tissues and blood vessels pauci-immune deposition	Respiratory (allergic rhinitis, sinusitis, nasal obstruction, nasal polyps/pain, eosinophilic pneumonia, asthma, pulmonary infiltrates/nodules, alveolar hemorrhage, pleural effusion, hilar adenopathy), cardiac (eosinophilic endocarditis, pericarditis, coronary arteritis, congestive heart failure), nervous (mononeuritis multiplex, polyneuropathy, palsies of cranial nerves, psychosis, optic neuritis, cerebral hemorrhage-infraction, convulsion), gut (eosinophilic infiltrates, ischemia, blood diarrhea, cholecystitis, pancreatitis), cutaneous (petechia, ecchymoses, nodules).
HSP	elevated IgA (>50%) anti-C1q-IgA (40%)	leukocytoclastic vasculitis IgA/C deposition in mesangium/GBM and around blood vessels in kidneys/ skin	Purpura, arthralgia and colicky abdominal pain, cutaneous (purpura, urticaria), gut (colicky pain, hematemesis, blood per rectum, vomiting), others include nephritis, myocardial infarction, hepatosplenomegaly, testicular torsion/swelling/, nervous (headache, focal deficit, mononeuropathies, seizures), pulmonary hemorrhage, pleural effusion.
Cryo	RF (47%) ANA low C4/C3 (84%) low CH50 (56%) cryo type II/III IgM paraprotein (56%) hypo G (25%) hyper G (20%)	membranoproliferative GN leukocytoclastic vasculitis IgM/IgG/C (variable) in mesangium and GBM and blood vessels of kidneys and skin	Cutaneous (palpable purpura, urticaria, ulcers, nodules, Raynaud's syndrome), renal (nephritic/nephrotic syndrome, proteinuria, hematuria), peripheral neuropathy (mononeuritis multiplex, symmetrical polyneuropathy), abdominal pain, lymphadenopathy, sicca syndrome, liver abnormalities, other; cardiac (mitral valve insufficiency, myocardial infraction, pericarditis), pulmonary infiltration and hemorrhage, encephalopathy and cerebro-vascular accident.
Anti-GBMD	anti-GBM antibodies (98%) P-ANCA/MPO (10-20%)	crescentic GN linear IgG/C deposition in GBM (100%) ** and TBM	Renal-pulmonary involvement, renal (RPGN → ARF), ± pulmonary (bilateral alveolar infiltrates, hemoptysis, hemosiderosis, dry cough, mild dyspnea)
HUVS	anti-C1q IgG RF ANA (low titre) low C4, CH50. C1q paraprotein (few)	leukocytoclastic vasculitis IgG and C in blood vessels and along the basement membrane	Chronic urticaria (>24h), Raynaud's syndrome, arthralgias, angioedema, uveitis, conjunctivitis, episcleritis abdominal pain (vasculitis/angioedema), chronic obstructive pulmonary disease (reversible airway obstruction, emphysema, interstitial fibrosis, cough, dyspnea) pericarditis and mild renal disease.

* (20% may have IgA and complement deposition due to associated IgA-nephropathy). **(10% will have IgG, IgM, IgA and C deposition due to associated membranous GN). Cryo - cryoglobulinemia, GN - glomerular nephritis, C - complement, RPGN - rapidly progressive glomerular nephritis, ARF - acute renal failure, anti-GBMD - anti-glomerular basement membrane disease, TBM - tubular basement membrane. C/P-ANCA - cytoplasmic/perinuclear antineutrophil cytoplasmic antibodies, PR3 - proteinase 3, MPO - myeloperoxidase, WG - Wegener's granulomatosis, CSS - Churg Strauss syndrome, MPA - microscopic polyangiitis, GBM - glomerular basement membrane, HSP - Henoch Schonlein syndrome, HUVS - hypocomplementemic urticarial vasculitic syndrome, ANA - anti-nuclear antibodies, RF - rheumatoid factor

but with absent/mild renal disease and absent SLE serology.[54,55] Anti-C1q IgG antibodies are associated with 100% of patients with HUVS. Although these antibodies are also found in a few other conditions, under the right clinical setting, with exclusion of SLE, anti-C1q IgG antibodies would suggest a diagnosis of HUVS. Confirmation of the disease is obtained from skin biopsy showing leukocytoclastic vasculitis with IgG and complement deposition along blood vessels and at the dermo-epidermal junction.[19,56,57] In some patients with HUVS, a paraprotein may be found. Low complement with paraproteins (IgG) are also found in rare patients with hypocomplementemic panniculitis.[68] Finally, low complement levels can also occur with many other non-vasculitic conditions (such as connective tissue disease, thrombotic/embolic and infectious diseases) whose clinical manifestations can overlap with vasculitis.[58,69] However, these conditions would be excluded by the results of other laboratory tests that would be requested, along with the vasculitic tests (**Table 2**).

In conclusion, the clinical immunology laboratory plays a central role in the diagnosis and monitoring of vasculitic conditions. For efficient utilization of immunological results in the diagnosis of vasculitis, all immunological results need be interpreted in relation to each other, as well as to other laboratory and clinical findings. This requires easy access to all other laboratory and clinical data, as well as close liaisons between immunologists and physicians.

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