Utilization of complement testing in clinical medicine

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The complement system consists of a number of high molecular weight proteins that exist in plasma as inactive protein precursors. The complement system can be activated by a number of pathways including the classical and the alternative pathways. The classic pathway is initiated as a result of binding of the first complement component (C1) to immune complexes, while the alternative pathway is initiated when modified C3 binds to unprotected targets such as bacterial cell wall (**Figure 1**). Activation of the complement system through both pathways is tightly controlled by a variety of soluble and cell-surface bound proteins (such as C1-esterase inhibitor [C1-I], C4 binding protein [C4bp], factors I and H) (**Figure 1**). In addition to playing an important role in the body defense against infectious agents, the complement system plays an important role in the pathogenesis of a variety of autoimmune-inflammatory conditions. Assessment of serum complement levels is therefore employed in the investigation, not only of complement deficiency states, but also of a variety of pathological states; particularly connective tissue diseases, renal and vasculitic conditions (**Table 1**). In the present brief communication, we will discuss how assessment of serum complement levels (mainly C3 and C4) can be used in the diagnosis, prognosis and monitoring of these important conditions.

Assessment of serum complement levels is used in the investigation of connective tissue diseases; particularly systemic lupus erythematosus (SLE).[1] In this disease, immune complexes (such as anti-dsDNA antibodies, cryoglobulins) are deposited in various organs leading to chronic complement activation resulting in reduction of both C3 and C4 levels. However, reduction of C4 level, with apparently normal C3, can sometimes be observed. The latter may be explained by mild complement activation which, because of the narrow and the wide normal ranges of C4 and C3; respectively, results in reduced C4 with apparently normal C3 level. Increased production of C4 binding protein has also been proposed as another explanation for the normal level of C3.

Reduction of complement levels (C3 and C4), therefore, can act as a pointer towards the diagnosis of SLE. However, since complement tests would be requested along with other more specific SLE tests (such as anti-dsDNA antibodies, antinuclear antibodies [ANA], extractable nuclear antibodies [ENA]), complement testing plays a more important role in the prognosis and monitoring of the disease. Thus, low complement levels, with high affinity anti-dsDNA antibodies, tend to predict a bad prognosis with renal and central nervous system involvement, while decreased complement levels, with anti-C1q antibodies, tend to predict more specifically renal disease with proliferative glomerulonephritis. In patients with established SLE, normalization of the complement levels is associated with improvement of the disease, while decreases of complement levels tend to predict a flare of the disease. However, it must be remembered that low serum complement levels can occur independently of the diseases activity. Thus, low serum C4 level can be due to congenital deficiency of C4 protein which is very frequent in SLE patients (occurring in approximately 40% of patients). Moreover, complement activation with reduction in C4 level can occur in a small percentage of SLE patients with acquired C1-I deficiency. Furthermore, reduction of C3, with normal C4 level, can also occur in SLE patients with C3-nephritic

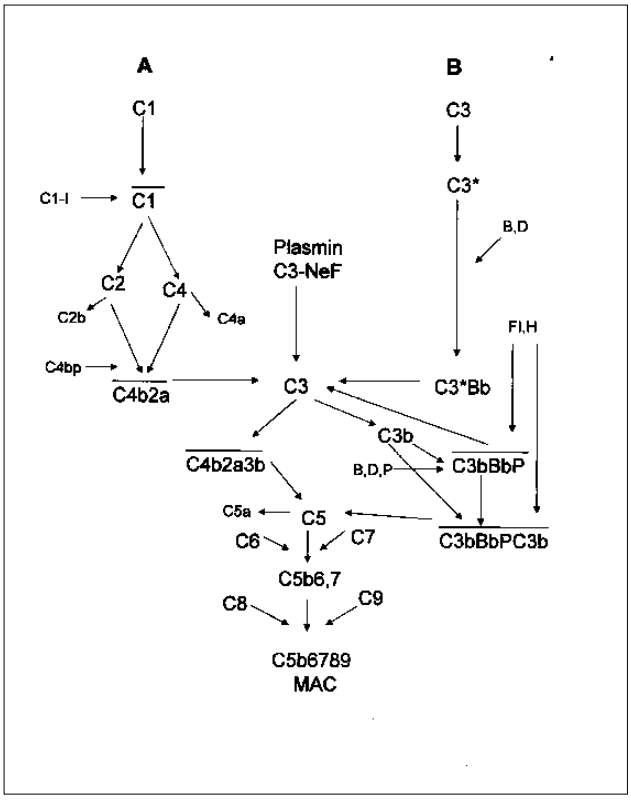

Figure 1 - Activation of the complement system by the classical (A) and the alternative (B) pathways.

Table 1 - Conditions associated with serum complement reduction.

Low C4 and C3	Low C4 normal C3*	Low C3 normal C4
Systemic lupus erythematosus	C4 deficiency	Gram negative sepsis
Cryoglubulinemia†	Hereditary angioedema	Post infectious GN
Hypocomplementemic urticarial vasculitis	Acquired angioedema	C3-nephritic factor‡
Membranoproliferative glomerulonephritis-1§	(Systemic lupus erythematosus,	SBE
Protein loss	malignancies)	Genetic deficiency (C3, I,H)**
Severe liver disease		
Severe hemolysis		
Embolism		
Sepsis		

*Low C4, with apparently normal C3, can also occur with SLE and cryoglobulinemia.
†Idiopathic or secondary to chronic infections (such as HCV, HBV, EBV, CMV, HIV, SBE, shunt nephritis, malaria, schistosomiasis), Connective tissue diseases (such as SLE, SS, RA with vasculitis), inflammatory conditions (such as IBD, Sarcoidosis, PBC, CF) and hematological malignancies (MM, WMG, B-CLL, NHL). ‡Disease associated with C3-NeF; partial lipodystrophy, MPGN-2 and SLE. §Idiopathic or secondary to cryoglobulinemia. **Diseases associated with Factors I/H deficiencies; MPGN-2, TTP, HUS.
C - complement, SLE - Systemic lupus erythematosus, HCV, HBV - hepatitis C/B virus, EBV - Epstein-Barr virus, CMV - cytomegaly virus, HIV - human immunodeficiency virus, SBE - subacute bacterial endocarditis, MM - multiple myeloma, SS - Sjögren's syndrome, RA - rheumatoid arthritis, IBD - inflammatory bowel diseases, PBC - primary biliary cirrhosis, CF - cystic fibrosis,
WMG - Waldenstrom macroglobulinemia, B-CLL - B-chronic lymphocytic leukemia, NHL - non-Hodgkin's lymphoma.

factor of the alternative pathway and, rarely, with deficiency of C3 protein. However, in these latter situations, other additional clinical manifestations (such as angioedema, recurrent infection, renal diseases) would be apparent. Although treatment of patients with SLE, based on decreased complement levels, has in the past resulted in reduction of relapses and the severity of the disease, treatment of patients based on the reduction of complement levels per se is not generally recommended. However, reduction in complement levels, with rising anti-dsDNA antibodies, should lead to close monitoring of patients; particularly for renal function. Complement activation, with reduction of C4 ± C3 levels, can also occur in patients with other CTD including Sjögren's syndrome (SS) and rheumatoid arthritis (RA) with vasculitis.[2,3] In both conditions, complement activation correlate with the presence of cryoglobulins. In SS, low complement levels occurs in approximately 24% of patients and correlate with the severity of the disease and extraglandular manifestations; including glomerulonephritis (membranoproliferative glomerulonephritis [MPGN-1]), vasculitis and lymphoma. Complement activation also occur in other CTD, however, activation does not normally lead to reduction of serum complement levels. Assessment of complement levels is also used in the investigation of renal diseases.[3,4] Thus, reduction of C4 ± C3 is seen in patients with idiopathic membranoproliferative glomerulonephritis type-1 (MPGN-1), or MPGN-1 secondary to cryoglobulinemia (**Table 1**). In contrast, reduction of C3 alone occurs in post infectious GN and MPGN-type 2 (dense body disease). In post-infectious GN, streptococcal antigens are deposited in the glomeruli leading to activation of the complement system, via the alternative pathway, with consequent reduction of C3 level. Normalization of the complement level normally occurs within a period of 2 months, although, occasionally, this may take longer. In such occasions, gradual increase in the complement level, rather than normalization, would be observed. However, persistent reduction of complement C3 level beyond 2 months should lead to testing for the C3-nephritic factor (C-3NeF); an IgG autoantibody that is associated with SLE, MPGN-type-2 and partial lipodystrophy. The nephritic factor binds to, and stabilizes, the C3-convertase of the alternative pathway (by inhibiting the binding of factors I and H) leading to prolonged activation of the complement system. Deposition of C3 on the glomeruli, and the mesangium, is associated with the development of MPGN-2. Low C3, with the nephritic factor, can also occur in patients with out renal involvement. However, such patients are at increased risk of developing MPGN type-2 and, therefore, close monitoring of these patients would be advisable. Finally, MPGN-2, with low C3, can also occur as a result of congenital deficiencies of C3, or the alternative pathway-regulatory proteins (factors I and H). Deficiency of the latter factors leads to prolongation of the C3-convertase with resultant reduction of C3 levels. In addition to developing MPGN-2, patients with Factor I and H deficiency may also develop hemolytica uratemic syndrome and thrombotic thrombocytopenic purpura (HUS/TPP) and be at increased risk of infections. Assessment of complement levels is also

employed in the investigation of vasculitic conditions including cryoglobulinemia and hypocomplementemic urticarial vasculitis (HUVS).[3] Thus, in patients with clinical suspicious of vasculitis, low serum complement levels (C4 ± C3), with cryoglobulins and rheumatoid factor (RF), would strongly suggest a diagnosis of cryoglobulinemia, while reduction in serum C4 ± C3 levels, together with presence of anti-C1q autoantibodies, would suggest a diagnosis of HUVS; having first excluded SLE. However, it must be remembered that low complement levels can sometimes occur with a variety of other conditions that can mimic vasculitis. Thus, low C4 ± C3 can occur in many embolic conditions (including cholesterol embolism [occurring in 3/4 of cases] and endocarditis), while low C3 level is associated with thrombotic conditions (hemolytica uremic syndrome/ thrombotic thrombocytopenic purpura [HUS/TTP]). Low C4 can also occur in patients with recurrent fibril and nodular panniculitis (Weber-Christian syndrome). In the alter condition, IgG paraprotein is believed to bind the globular head of the C1q molecule resulting in C1-esterase activation and reduction of C4 level. Finally, assessment of complement level is used in the investigation of patients with angioedema (hereditary and acquired forms).[5] Angioedema (AE) is characterized by non-pitting edema affecting any part of the body including the extremities, face, tongue, throat and the abdomen. Angioedema of the abdomen can mimic acute abdomen and result in many unnecessary investigation before the diagnosis is made, while the larynx angioedema can result in respiratory failure and death. Symptoms can occur spontaneously, or brought about by stress or trauma. Hereditary angioedema (HAE) results from congenital deficiency (type-1; affecting 80% of patients), or abnormality (type-2) of C1-esterase inhibitor (C1-I). In the acquired form of AE (seen in patients with CTD [such as SLE] and malignancies), binding of an autoantibody to C1-I result in a deficiency (largely functional) of this inhibitor (acquired angioedema due to angiotensin converting enzyme inhibitors is not associated with complement activation). In both forms of AE, deficiency of the C1-I result in sustained activation of the C1-esterase leading to depletion of serum C4 component. The level of C3 component remains normal and this has been attributed to the large increase in C4 binding protein, which binds C4a and prevents the formation of C3-convertase. It is also possible that activation of the complement in the fluid phase fails to achieve the formation of C3-convertase. Reduced C1q level is said to distinguish the acquired AE from the hereditary form. However, testing for C1q is unreliable, and distinction can be made on clinical ground with the support of other laboratory tests (such as ANA, anti-dsDNA antibodies, paraproteins, immunophoresis, beta-2 microglobulin).

In conclusion, complement testing is used in the investigation of a wide variety of clinical conditions. For the proper utilization of complement results, good knowledge of these conditions is essential. In addition, complement results should be interpreted in relation to well validated normal ranges (produced within a given laboratory serving a particular region) as well as with other laboratory and clinical data. For this, to happen, easy access to such information by the Immunologist is imperative.

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