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). 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.**

<table>
<thead>
<tr>
<th>Low C4 and C3</th>
<th>Low C4 normal C3†</th>
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<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>C4 deficiency</td>
<td>Gram negative sepsis</td>
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<tr>
<td>Cryoglobulinemia†</td>
<td>Hereditary angioedema</td>
<td>Post infectious GN</td>
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<tr>
<td>Hypocomplementemic urticarial vasculitis</td>
<td>Acquired angioedema</td>
<td>C3-nephritic factor†</td>
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<tr>
<td>Membranoproliferative glomerulonephritis-1</td>
<td>(Systemic lupus erythematosus, malignancies)</td>
<td>SBE</td>
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<tr>
<td>Protein loss</td>
<td></td>
<td>Genetic deficiency (C3, I,H)†</td>
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<tr>
<td>Severe liver disease</td>
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<td>Severe hemolysis</td>
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<td>Embolism</td>
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<td>Sepsis</td>
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</table>

†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. ‡Disease associated with Factors I/H deficiencies; MPGN-2, TTP, HUS.


Complement testing

Low C4 and C3

- Systemic lupus erythematosus
- Cryoglobulinemia
- Hypocomplementemic urticarial vasculitis
- Membranoproliferative glomerulonephritis-1
- Protein loss
- Severe liver disease
- Severe hemolysis
- Embolism
- Sepsis

Low C4 normal C3†

- C4 deficiency
- Hereditary angioedema
- Acquired angioedema
- (Systemic lupus erythematosus, malignancies)

Low C3 normal C4

- Gram negative sepsis
- Post infectious GN
- C3-nephritic factor†
- SBE
- Genetic deficiency (C3, I,H)†

The low C4 and C3 levels can be associated with various conditions, including glomerulonephritis, vasculitis, and infections. In cases of idiopathic or secondary to chronic infections, assessing complement levels is important. The presence of cryoglobulins, which can be associated with cryoglobulinemia, can also lead to complement activation. In such situations, close monitoring of patients is necessary to prevent complications such as renal failure.

**Table 1**

- **Diseases associated with C3-NeF:** partial lipodystrophy, MPGN-2 and SLE.
- **Diseases associated with Factors I/H deficiencies:** MPGN-2, TTP, HUS.

**Note:**

- 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 Factors I/H deficiencies; MPGN-2, TTP, HUS.

employed in the investigation of vasculitic conditions including cryoglobulinemia and hypocomplementemic urticarial vasculitis (HUVS). Thus, in patients with clinical suspicion 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 (hemolytic 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 altered 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). 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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