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Complement in Kidney Disease: Core Curriculum 2015

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The complement cascade is part of the innate immune system and provides an important line of defense against invasive pathogens. However, the complement system also causes kidney injury in a variety of different diseases, and clinical evaluation of the complement system is an important part of the diagnostic workup of patients with glomerulonephritis. Complement activation is particularly important in the pathogenesis of atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy. Complement inhibition is effective for treatment of aHUS, and complement inhibitors will likely be tested in other kidney diseases in the future. While the role of the complement system in the pathogenesis of many kidney diseases is well established, however, there is not a simple algorithm for identifying which patients should be treated with complement inhibitors or for how long complement inhibition should be continued.

OVERVIEW OF THE COMPLEMENT SYSTEM

Complement proteins provide an important line of defense against bacteria, fungi, and viruses. The complement system also facilitates the efficient removal of damaged cells and immune complexes. Inactive complement proteins (zymogens) circulate in plasma, and are activated through three distinct pathways: the classical pathway, the alternative pathway, and the mannose binding lectin pathway (Figure 1). Once activated, the complement system generates several different activation fragments that have potent pro-inflammatory or cytolytic effects. Some of these fragments are soluble and can be measured in plasma (e.g. C3a and C5a), and some become covalently bound to target cells (e.g. C4d and C3d). A key feature of this system is that it is rapidly activated on pathogens and damaged cells, but it is not activated on host surfaces. The ability of the complement system to discriminate between different surfaces is accomplished by a finely tuned balance between activator proteins and regulatory proteins.

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Role of Complement in Fighting Infection

The importance of the complement system for protecting against pathogens is demonstrated by the susceptibility of patients with congenital complement deficiencies to opportunistic infections. Patients deficient in C3 are predisposed to bacterial infections. Levels of C3 can become depleted in patients deficient in the complement regulatory proteins factor I and factor H, and this acquired deficiency of C3 is also associated with recurrent pyogenic infections. Patients with deficiencies of classical pathway proteins (C1q, C1r, C1s, C2, and C4) are at increased risk of infections with encapsulated bacteria. Those with deficiencies of terminal complement proteins (C5, C6, C7, C8, or C9) and those with deficiencies of alternative pathway proteins (factor B, factor D, and properdin) are at increased risk of infections with Neisseria species. Similarly, patients treated with eculizumab are susceptible to Neisseria infections and should be immunized with the meningococcal vaccine prior to treatment.

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Role of Complement in Tissue Inflammation

Complement activation causes tissue injury in a wide range of autoimmune and inflammatory diseases. Complement activation can damage host tissues if the response to infection is sufficiently strong or widespread, when activated by autoantibodies and immune complexes, in patients deficient in complement regulatory proteins, and in patients with gain of function mutations. Systemic complement activation, as occurs during sepsis or dialysis with incompatible membranes, causes vascular leak and sequestration of leukocytes in the pulmonary circulation. At the local level, complement activation within the glomerular capillary walls causes cell activation, glomerular inflammation, and injury.

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Key Role of Complement Regulatory Proteins

As highlighted above, the complement system must adequately respond to pathogens, but activation must be controlled on host tissues. This balance is maintained by a group of complement regulatory proteins. Some of these proteins are expressed on cell membranes [such as membrane cofactor protein, decay accelerating factor, and CD59], whereas others (such as factor H and factor I) are soluble plasma proteins that are synthesized in the liver.

Several inflammatory diseases are directly caused by congenital or acquired deficiencies of regulatory proteins, permitting uncontrolled complement activation on host cells. Patients with paroxysmal nocturnal hemoglobinuria, for example, have a clonal defect that prevents the expression of two complement regulatory proteins (decay accelerating factor and CD59) on the surface of erythrocytes. As a result, complement activation causes lysis of erythrocytes and hemolytic anemia.

aHUS and C3 glomerulopathy are also strongly associated with defects in the proteins that regulate alternative pathway activation. These defects in complement regulation are generally systemic, and it is not clear why the kidney is vulnerable to complement-mediated injury in patients with these mutations. Factor H is a soluble protein, for example, and mutations should increase alternative pathway activation throughout the body. Injury of other organs does occur in patients with factor H mutations, but the kidney is the most frequent and the most severely affected organ.

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THE SPECTRUM OF COMPLEMENT-MEDIATED KIDNEY DISEASES

Complement proteins are seen in biopsies from patients with virtually all forms of glomerulonephritis, and each of the three activation pathways have been linked with various kidney diseases (Figure 2). Although complement activation occurs downstream of immune complex deposition or antibody-mediated injury in many of these diseases, complement activation is also observed in kidney diseases that are not antibody-mediated. The wide variety of kidney diseases associated with complement activation suggests there is something about the structure or function of the kidney that makes it particularly susceptible to complement-mediated injury.

aHUS (Atypical Hemolytic Uremic Syndrome)

HUS is a clinical syndrome of hemolysis, thrombocytopenia, and acute kidney injury. In approximately 90% of patients, HUS is triggered by enteric infections by Shiga-toxin producing bacteria. The remaining cases are referred to as “atypical HUS”. Over the past 15 years, work from laboratories around the world has shown that 40–60% of patients with aHUS have mutations in complement proteins, and approximately 10% of patients have

autoantibodies to factor H that impair its function. The 30–40% of patients with aHUS who do not have identified mutations in complement-related genes may have mutations in genes for other proteins, as yet unidentified, that also help control alternative pathway activation. It is also possible that even normal complement regulation can be overwhelmed during some illnesses. Thrombi form in the glomeruli and capillaries of patients with aHUS, similar to what occurs in Shiga toxin-associated HUS and thrombotic thrombocytopenic purpura (Figure 3), and studies have revealed multiple links between the complement system, the coagulation system, and platelet activation.

All of the complement defects associated with aHUS have similar functional effects and enhance alternative pathway activation (Table 1). The mutations in factor B and C3 are gain of function mutations. The mutations in the complement regulatory proteins, on the other hand, decrease the function of these proteins. Interestingly, most factor H mutations cluster in the region of the protein that mediates binding to endothelial cells. The mutant proteins can still control alternative pathway activation, but binding to endothelial cells and other surfaces is impaired. Autoantibodies to factor H bind this same region of the protein and likely have a similar functional effect. For patients with these defects, cells or tissues that require bound factor H for regulating the complement system are vulnerable to complement-mediated inflammation.

Although patients with congenital complement mutations usually present in childhood, some patients present as adults. Furthermore, the mutations have incomplete penetrance. Most of the mutations are heterozygous, but 3% of patients carry compound mutations in more than one complement related gene. Patients carrying these mutations have impaired ability to control alternative pathway activation, and complement regulation is overwhelmed by events that promote intravascular complement activation, including infections, pregnancy, and medications.

Plasma exchange is beneficial in some patients with aHUS. This treatment removes autoantibodies or dysfunctional complement proteins, and replacement of patient plasma with fresh frozen plasma can restore deficient proteins (factor H and factor I). Thus, it addresses many of the possible underlying defects. Unfortunately, many patients with aHUS do not respond to plasma, and in some patients the administration of plasma simply provides additional substrate for complement activation. Case reports describe patients who did not respond to plasma exchange but quickly responded to eculizumab. Eculizumab was approved by the US Food and Drug Administration (FDA) for treatment of aHUS, primarily based on two trials showing its efficacy. One of the trials included patients considered to have stable disease controlled by regular plasma exchange, and the kidney function in this group of patients improved after starting treatment with eculizumab. Because eculizumab blocks the complement cascade at the level of C5, activation through C3 is not directly blocked by the drug, and it is not known to what degree C3 activation fragments contribute to tissue injury in this disease.

It can be challenging to distinguish aHUS from thrombotic thrombocytopenic purpura (TTP). Although there is probably some overlap in the underlying mechanisms of tissue injury in aHUS and TTP, aHUS is regarded as a disease of uncontrolled complement

activation and TTP as a disease of abnormal ADAMTS13 activity. ADAMTS13 is a metalloprotease that cleaves von Willebrand factor multimers, and up to 90% of patients with TTP have deficient ADAMTS13 activity (<10%). Clinical findings suggestive of deficient ADAMTS13 activity and TTP are a platelet count <30,000/μL and a serum creatinine level less than 1.7 mg/dL. Distinguishing aHUS and TTP is clinically important because eculizumab may be more effective than plasma exchange in patients with aHUS, whereas plasma exchange is the treatment of choice for TTP. However, there are case reports of patients with TTP who have been successfully treated with eculizumab, and it is possible that a single approach to these diseases will be possible in the future.

Shiga-Toxin aHUS—There is evidence of complement activation in patients with HUS triggered by Shiga toxin. A few patients with particularly severe courses have been identified as having mutations in genes for complement regulatory proteins. Several case reports have described patients who were successfully treated with eculizumab. Eculizumab was also used in an open label, multi-center trial of patients in France and Germany during a severe outbreak of HUS in 2011caused by Escherichia coli O104:H4. Outcomes of patients treated with plasma exchange and eculizumab were not better than those treated with plasma exchange alone, although the treatments were not randomized and some clinical parameters were worse in the group that received eculizumab. Thus, the role of eculizumab in the treatment of non-aHUS forms of thrombotic microangiopathy is unclear at this point.

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C3 Glomerulopathy

C3 glomerulopathy is a recently described disease diagnosed by the detection of glomerular C3 deposits in the absence of concomitant immunoglobulin (Figure 4). Alternative pathway mutations have been identified in many patients with C3 glomerulopathy, indicating that this is a disease of dysregulated alternative pathway activation. Dense deposit disease (DDD; formerly referred to as membranoproliferative glomerulonephritis type 2) is considered a subset of C3 glomerulopathy, but it is distinguished by the presence of electron dense deposits within the glomerular basement membrane by electron microscopy, and it may have a worse prognosis than C3 glomerulopathy in the absence of dense deposits.

Similar to aHUS, C3 glomerulopathy (and DDD) is associated with mutations in the genes for C3, factor H, factor B, and for complement factor H related proteins 1, 2 and 5 (CFHR1, CFHR2, and CFHR5). More than 70% of patients with DDD and more than 40% of patients with other forms of C3 glomerulopathy have a circulating auto-antibody, referred to as C3 nephritic factor (C3Nef) that stabilizes the alternative pathway C3-convertase and protects it from inactivation by factor H. It is still not known whether C3Nef is a pathogenic factor, but its functional effect is similar to the described mutations in factor H, C3, and factor B that are associated with the disease. Investigators have also discovered autoantibodies to factor H, C3, and factor B in some patients.

Several therapies have theoretical ability to block the complement defects associated with C3 glomerulopathy. Rituximab could potentially ameliorate disease in those with autoantibodies, and some case reports describe successful use of rituximab in patients with C3Nef or autoantibodies to complement proteins. Standard immunosuppressive drugs do not directly reduce complement activation. There are anecdotal reports of some patients improving with standard immunosuppressive drugs, but a benefit was not detected in other reports. Plasma exchange could theoretically benefit those patients with autoantibodies or mutations in complement proteins. There are case reports of this therapy helping some patients, but the evidence to support this treatment is also quite limited. Eculizumab has had mixed results in C3 glomerulopathy. Treatment with the drug was associated with clinical improvement in at least four reported patients, but has not been of benefit in all cases. In a recent series of 6 patients with C3 glomerulopathy (three of whom had DDD) who were treated with eculizumab, three of the patients seemed to respond to therapy with the drug. Thus, although a great deal has been learned about the pathogenesis of C3 glomerulopathy, at this time there are no validated therapeutic strategies.

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C3 Glomerulopathy Versus aHUS: Flip Sides of the Same Coin?

C3 glomerulopathy and aHUS are both associated with defective control of the alternative pathway. In particular, many patients with these diseases have defective function of the factor H protein. What, then, determines whether a patient develops one of these diseases or the other? Factor H has several functional domains, including a complement regulatory domain at the amino terminus of the protein and a binding region at the carboxyl terminus of the protein (Figure 5). It controls alternative pathway activation in the fluid phase (i.e. in plasma) and on tissue surfaces (i.e. on glomerular basement membrane and endothelial cells). The factor H mutations associated with aHUS cluster in the carboxyl-terminal region of the protein that is responsible for binding tissue surfaces. Factor H protein with these mutations can regulate the alternative pathway in the circulation but has an impaired ability to regulate activation on surfaces. Similarly, autoantibodies to the carboxy-terminus of factor H impair alternative pathway regulation on tissue surfaces.

In C3 glomerulopathy, the defects (mutations and autoantibodies) tend to decrease all alternative pathway regulation by factor H. This presumably affects both fluid phase and surface regulation. Animal models support the concept that C3 glomerulopathy is a consequence of overactive fluid phase complement activation, whereas aHUS is caused by activation specifically on the capillary walls. An elegant series of experiments by Pickering's group showed that an absolute deficiency of factor H in mice causes fluid phase complement activation and kidney disease similar to C3 glomerulopathy, whereas the absence of the binding region of factor H predisposes mice to thrombotic microangiopathy. Excessive fluid phase complement activation could even prevent thrombotic microangiopathy by reducing the amount of C3 available for surface activation.

In reality, this distinction between alternative pathway regulation in the fluid phase and on glomerular surfaces is probably not absolute. There are patients with complement mutations or autoantibodies who have developed membranoproliferative glomerulonephritis (MPGN) and thrombotic microangiopathy at different times, indicating a shared pathophysiology. The analysis of glomeruli from patients with DDD also demonstrates that complement activation occurs within the glomeruli of some patients. Patients with C3 glomerulopathy also develop macular drusen that are similar to those seen in macular degeneration. This has been attributed to similarities in the fenestrated capillaries of these two tissues, and may also

suggest alternative pathway activation on tissue surfaces. Finally, mutations in the complement factor H related proteins have been associated with C3 glomerulopathy, and this appears to be due to the ability of the mutant complement factor H related proteins to displace factor H from surfaces.

Immune-Complex Glomerular Diseases

Immune complexes deposit within the glomeruli in a number of different kidney and systemic autoimmune diseases. Immune complexes directly activate the classical pathway of complement, and the alternative pathway amplifies this process.

Membranoproliferative Glomerulonephritis—MPGN is a histologic pattern of injury caused by a number of different pathologic processes. Immunofluorescence microscopy and electron microscopy of MPGN kidneys reveals different patterns, and subcategories of the disease have been developed. In MPGN type 1, immune-complexes in the mesangium and subendothelial space activate the classical pathway of complement. Clinical evidence of complement activation includes deposits of complement proteins in the glomeruli and consumption of plasma C3 and C4. Some patients have profound complement abnormalities, although levels are normal in approximately 30% of patients. About 40% of patients have circulating C3nef. Heterozygous mutations in factor H or factor I have been identified in some affected patients, suggesting that dysregulated alternative pathway activation is also a risk factor for this disease.

MPGN, as a disease entity, has been modified several times. Ultrastructural examination led to the development of the MPGN type 2 and MPGN type 3 sub-classifications. The electron dense deposits that are pathognomonic for MPGN type 2 can also occur with histologic patterns of injury other than MPGN, so the name MPGN type 2 was subsequently supplanted by the term “dense deposit disease”. As discussed above, patients with DDD also frequently have defects in their control of alternative pathway activation and fulfill the diagnostic criteria for C3 glomerulopathy. This evolution of disease classifications – MPGN type 2 to DDD to C3 glomerulopathy – represents the transition from a classification based on light microscopy to one based on the underlying pathophysiologic process. As our understanding of the molecular causes of this disease improves, the nomenclature and classification will likely change further.

Cryoglobulinemia—Cryoglobulinemic kidney disease is usually associated with a MPGN pattern of injury. Immune-complexes are seen within capillary loops and in the subendothelial space, and C1q, C3, and C4 are usually detected. The involvement of classical pathway proteins (C1q and C4) is not surprising since this is an immune-complex disease. Cryoglobulinemic kidney disease is associated with a drop in C4 greater than what is typically seen in other forms of immune-complex glomerulonephritis.

Lupus nephritis—The complement system has a paradoxical role in the development of lupus nephritis. Complement deficiencies, particularly of components of the classical pathway, are strong risk factors for the development of lupus due to defective clearance of nuclear antigens released by injured and apoptotic cells. On the other hand, complement

activation mediates glomerular injury in lupus nephritis. C3 and C4 levels are depressed in greater than 90% of patients with diffuse proliferative lupus nephritis, and a fall in these proteins often reflects an increase in disease activity. In patients with congenital deficiency of C4, the levels of this protein do not rise during remission, and serial measurements of C4 must be performed to determine whether the level changes with disease activity or whether it is always low or absent in an individual patient.

Membranous nephropathy—Membranous nephropathy is caused by immune-complexes in the subepithelial space of the glomerular capillary wall. Extensive work in animal models demonstrates that complement activation is central to the pathogenesis of the disease. An exciting breakthrough was the identification of M-type phospholipase A2 receptor (PLA2R) as the target antigen in idiopathic membranous nephropathy. Most of the IgG in the glomeruli and most of the anti-PLA2R antibody in the serum of patients with idiopathic membranous nephropathy is of the IgG4 subclass, and IgG4 is a poor activator of the classical pathway of complement. Nevertheless, C3 deposits are seen in 60–80% of the biopsies of patients with membranous nephropathy. The mechanisms by which the IgG4 deposits engage the complement system are not yet understood, although it is possible that the class of antibody changes during the course of the disease. Mannose binding lectin is also often detected in the glomeruli of patients with membranous nephropathy, suggesting that complement activation proceeds through the mannose binding lectin pathway. In secondary membranous nephropathy, the glomerular immune deposits usually contain IgG1 and IgG3, which are effective activators of the classical pathway.

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Other Antibody-Mediated Kidney Diseases

Antibody-Mediated Rejection—Acute antibody-mediated rejection of kidney allografts carries a poor prognosis and is resistant to most standard immunosuppressive therapies. Detection of C4d deposition in the peri-tubular capillaries is one of the diagnostic criteria for

the condition, and probably reflects classical pathway activation by donor specific antibodies against HLA. It is not clear why C3 fragments and immunoglobulin are less reliable markers of antibody-mediated rejection than C4d, but these other proteins are frequently absent in C4d positive biopsies. The association of positive C4d staining with the titer of donor specific antibodies and graft failure suggests a pathogenic role of complement. Treatment of patients with acute antibody-mediated rejection often includes therapies aimed at reducing the levels of donor specific antibodies, such as plasmapheresis, IV Ig, and rituximab. Eculizumab has been used to prevent antibody-mediated rejection in sensitized patients and as salvage treatment for patients with refractory disease. A randomized controlled open-label trial of eculizumab in sensitized allograft recipients is currently underway.

Antiphospholipid Antibody Syndrome—Antiphospholipid antibody syndrome can cause thrombotic microangiopathy or kidney failure due to thrombosis in renal capillaries, arterioles, and arteries. Animal studies indicate that complement activation by the antibodies is an important mechanism of disease pathogenesis. Serum C3 and C4 are low in some patients with primary antiphospholipid antibody syndrome, and levels of C3a and C4a (generated during activation) are frequently elevated. Cases of C3 glomerulopathy in patients with antiphospholipid antibody syndrome have also been reported, suggesting that complement activation within glomeruli by antiphospholipid antibodies triggers C3 glomerulopathy in susceptible patients. “Catastrophic” antiphospholipid antibody syndrome is a rare but serious form of antiphospholipid antibody syndrome involving three or more organ systems, and kidney involvement is reported in 78% of these patients. Eculizumab has been successfully used as salvage therapy in patients with catastrophic antiphospholipid antibody syndrome. It has also been used prophylactically in patients with catastrophic antiphospholipid antibody syndrome undergoing kidney transplantation and in patients with antiphospholipid antibody syndrome who have developed recurrent thrombotic microangiopathy post-transplant. A randomized trial of eculizumab is currently underway for patients with catastrophic antiphospholipid antibody syndrome undergoing kidney transplantation. Complement inhibition may also be beneficial in patients with catastrophic antiphospholipid antibody syndrome who have failed conventional therapy.

Antineutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis—Studies in animal models of ANCA-associated vasculitis have shown that complement activation contributes to the pathogenesis of this disease. Activation is through the alternative complement pathway, and agents that block C5 cleavage or C5a signaling are protective in these models. In humans, active disease is associated with elevations in plasma levels of C3a, Bb, C5a, and sC5b-9, but not with elevations in plasma C4d, consistent with alternative pathway activation. Although ANCA-associated glomerulonephritis is often called “pauci-immune”, most biopsies contain some immunoglobulin and/or complement proteins. One report identified deposits of alternative pathway proteins (factor B and properdin), but C4d was absent. Complement activation in humans with the disease therefore mirrors that described in the animal models. There are not, at this time, any reports of patients with ANCA-associated vasculitis who have been treated with eculizumab.

IgA Nephropathy—IgA nephropathy is associated with aberrant glycosylation of IgA1 molecules, and the development of autoantibodies specific for the altered IgA1. IgA1-containing immune complexes deposit within the mesangium, and likely initiate glomerular injury. Although plasma C3 levels are usually normal, plasma C3a is elevated in some patients and glomerular C3 deposits are detected in approximately 85% of biopsies. IgA activates the complement system through either the alternative or mannose binding lectin pathway. Glomerular mannose binding lectin is seen in a subset of patients with IgA nephropathy, and mannose binding lectin deposition correlates with greater disease severity and a worse prognosis. C4d deposition, which may be the result of mannose binding lectin pathway activation, has also been linked with worse outcomes. A genome wide association study in patients with IgA nephropathy found that an allele with deletion of CFHR1 and CFHR3 was protective. Recent work indicates that the complement factor H related proteins can compete with factor H, positively activating the alternative pathway. Thus, the deficiency of the complement factor H related proteins may make factor H more effective and reduce complement activation within affected glomeruli, explaining the association of this allele with protection from disease.

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Complement and Tubular Injury

Tubulointerstitial Injury in Chronic Proteinuria Diseases—Complement regulatory proteins are not expressed on the apical (urinary) surface of tubular epithelial cells. Ordinarily the complement proteins are restricted from passing through the glomerular filtration barrier and accessing this surface, so complement regulation is unnecessary. In proteinuric diseases, however, complement proteins enter the urinary space and the absence of regulatory proteins on the apical surface of tubular cells permits complement activation. Complement activation fragments are detectable in the urine of patients with many forms of nephrotic syndrome. In general, a higher degree of proteinuria is associated with a worse renal prognosis, and complement activation on the renal tubules may be a mechanism linking the glomerular process with progressive tubulointerstitial injury.

Acute Kidney Injury—The alternative pathway is activated in the tubulointerstitium of rodents with ischemic acute kidney injury, and complement activation in the tubulointerstitium directly contributes to kidney injury. C3d is also seen in kidney biopsies with histologic evidence of acute tubular injury. Cross-talk between the complement system and the adaptive immune system may affect the immune response to foreign antigens. In the transplant setting, this may link kidney injury and complement activation at the time of transplantation with increased risk of rejection and worse long-term outcomes. Therapeutic complement inhibitors could, in that context, reduce delayed graft function and also improve long-term graft survival. APT070 is an experimental complement inhibitor that binds to cell membranes, and a Phase II clinical trial of this agent is currently underway in the United Kingdom to test whether it protects kidney allografts from delayed graft function.

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CLINICAL COMPLEMENT TESTS

The most common complement labs used by nephrologists are measurement of complement protein levels (C3 and C4), immunostaining of biopsies for complement proteins (C3 and C4 fragments), and measurement of the hemolytic potential in a patient's serum (CH50 and AH50). These tests have long been used to focus the differential diagnosis in patients with glomerular disease and to monitor disease activity. More recently, screening for genetic mutations has been used for diagnostic and prognostic evaluation of aHUS and C3 glomerulopathy.

Genetic testing of disease-associated complement genes is not widely available, but several laboratories can help get this testing done. A list of complement laboratories in Europe is available from the European Complement Network website (www.ecomplement.org). Genetic testing is under development at several labs in North America, including the Molecular Otolaryngology and Renal Research Laboratories at the University of Iowa (www.healthcare.uiowa.edu/labs/morl/) and the Molecular Genetics Laboratory at The Hospital for Sick Children (SickKids) at the University of Toronto (www.sickkids.ca/molecular). The Complement Laboratory at the National Jewish Medical and Research Center (www.nationaljewish.org/professionals/clinical-services/diagnostics/adx/about-us/

lab-expertise/complement) can quantitatively measure a broad range of complement proteins and activation fragments, and also performs functional tests of the complement system.

The rapid and accurate assessment of the complement system is particularly important for patients with aHUS and C3 glomerulopathy. Biomarkers of complement activation would help with the diagnosis of these diseases and be useful for monitoring disease activity. Given the limitations of the available tests, however, there is no strict diagnostic algorithm for these diseases. Nevertheless, the available tests provide valuable clinical information (Tables 2 and 3).

Immunofluorescence Microscopy

Most native kidney biopsies are immunostained for C3 and C4 fragments, and kidney allograft biopsies are now routinely immunostained for C4d. Although one generally refers to “C3” deposits, in truth the immunostaining is performed to detect C3 fragments that are covalently bound to kidney surfaces during complement activation (C3b, iC3b, C3d; see Figure 1). C3 is the central component to complement activation by all pathways, so the presence of C3 fragments reflects complement activation by any of the three activation pathways.

Immune Complex Glomerulonephritis—Glomerular immune complexes activate the classical pathway of complement, and classical pathway proteins (C1q, C4, and C3) are present in the deposits. C3 fragments are detected in nearly 100% of kidney biopsies from patients with membranoproliferative glomerulonephritis (MPGN) type 1, lupus nephritis, and post-infectious glomerulonephritis.

C3 Glomerulopathy—C3 glomerulopathy is a recently described disease that is diagnosed by detection of prominent glomerular C3 in the relative absence of immunoglobulin, C1q, or C4d. This immunofluorescence pattern indicates alternative pathway activation, and the absence of immunoglobulin distinguishes C3 glomerulopathy from immune complex diseases such as MPGN type 1 and lupus nephritis. The histologic pattern of injury varies and can resemble other forms of proliferative or crescentic glomerulonephritis. The diagnosis of this disease therefore depends upon the pattern of immune deposits seen on immunofluorescence microscopy.

Antibody-Mediated Rejection of the Kidney Allograft—In patients with antibody-mediated allograft rejection, C4d is seen in the peritubular capillaries. This finding is now incorporated into the diagnosis of acute and chronic antibody-mediated kidney allograft rejection. C4d is deposited due to classical pathway activation on endothelial cells by donor specific antibodies to human leukocyte antigens (HLA) class I and class II antigens. C4d, which becomes covalently bound to target endothelial cells, persists longer than the donor specific antibodies that triggered complement activation.

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Complement Levels

Measurement of C3 and C4 can narrow the differential diagnosis in patients with the nephritic syndrome (Box 1), and serial measurement of these proteins may detect disease remissions or flares. The concentration of complement proteins in plasma is influenced by the rate of production in the liver and consumption of the proteins throughout the body. Complement activation within the kidney is probably only responsible for a small component of the overall consumption of complement proteins. Consequently, intra-renal complement activation can cause tissue injury without causing a decrease in the plasma level of complement proteins. In membranous nephropathy, for example, complement fragments are seen in the majority of biopsies and complement activation probably causes podocyte injury, yet the levels of circulating C3 and C4 are usually normal. The level of C3 is thus a poor indicator of whether the complement system is "on" or "off", although in some diseases (such as lupus) the magnitude of the decrease in these proteins does reflect the overall disease activity.

Box 1

Serum C3 Levels in Patients with Various Kidney Diseases

Low Serum Complement Level	Normal Serum Complement Level
Systemic diseases	Systemic diseases
• SLE	• Polyarteritis nodosa
– Class III (75%)	• Hypersensitivity vasculitis
– Class IV (90%)	• Granulomatosis with polyangiitis
– Class V (60%)	• Microscopic polyangiitis
• Subacute bacterial endocarditis (90%)	• Henoch-Schönlein purpura
• "Shunt" nephritis	• Goodpasture syndrome
• Cryoglobulinemia (90%)	• Visceral abscess
• aHUS (50%)	
Primary kidney diseases	Primary kidney diseases
• Poststreptococcal glomerulonephritis (90%)	• IgA nephropathy
• MPGN type I (70%)	• Antiglomerular basement membrane disease
• C3 glomerulonephritis (75%)	• Renal limited ANCA-associated vasculitis
• Dense deposit disease (80%)	
• MPGN type III (80%)	

Note: The percentage of patients with low C3 levels is shown in parentheses

Abbreviations: SLE, systemic lupus erythematosus; MPGN, Membranoproliferative glomerulonephritis; ANCA, anti-neutrophil cytoplasmic antibody; aHUS, atypical hemolytic uremic syndrome; Ig, immunoglobulin.

In glomerular diseases that are associated with decreased C3 levels, the sensitivity of this test varies from 50–90% (Box 1). Immune-complex-mediated diseases, such as lupus nephritis, tend to decrease the levels of both C3 and C4, consistent with complement activation through the classical pathway (Figure 1). Alternative pathway driven diseases such as aHUS and C3 glomerulopathy, on the other hand, are sometimes associated with low levels of C3 and normal C4 levels. Low complement levels can be caused by decreased production of the proteins by the liver or consumption of the proteins in tissues other than the kidneys, so low C3 or C4 levels are not specific markers of glomerulonephritis. Other diseases can also cause C3 consumption, including sepsis, atheroembolic disease, pancreatitis, and HIV infection. Production of complement proteins may be decreased in patients with malnutrition or liver disease, and production can increase during pregnancy or the acute phase response. A pregnant patient with active lupus nephritis might therefore have normal C3 and C4 levels in spite of high consumption of these proteins.

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Hemolytic Assays: CH50 and AH50

The CH50 assay measures the overall hemolytic capacity of a patient’s plasma, and is used as a screening test for complement deficiencies. In this test, antibody coated sheep erythrocytes are incubated with patient serum, and the degree of lysis reflects classical pathway activity of the serum. Consequently, deficiency or consumption of classical pathway components, C3, or terminal pathway components causes a decrease in the CH50. In the AH50 assay, the lysis of rabbit or guinea pig erythrocytes is measured in a reaction that only permits activation via the alternative pathway. A patient with low C3 should have low hemolytic activity by both the CH50 and AH50 assays since C3 is central to complement activation through either pathway. Enzyme-linked immunosorbent assays

(ELISAs) have also been developed to measure the activation potential of the classical, alternative, or mannose binding lectin pathway, and are used in a growing number of clinical laboratories.

Genetic Analysis

A number of genetic defects in complement regulation have been identified in patients with kidney disease. The most striking example is aHUS, in which disease-associated mutations have been identified in the genes for factor H, factor I, C3, factor B, membrane cofactor protein, thrombomodulin, and genes for the complement factor H related proteins (Table 1). Mutations in complement regulatory genes have also been identified in patients with C3 glomerulopathy, other forms of thrombotic microangiopathy, and MPGN type 1. The functional consequence of these different mutations is, in most cases, over-activity of the alternative pathway.

Because these kidney diseases are associated with so many different mutations, identifying the specific mutation in an individual patient is complicated. The genetic studies can be very helpful, however. Patients with aHUS do not always present with all of the typical clinical findings, and the identification of an associated complement mutation provides support for this diagnosis. The underlying complement defect also influences the prognosis, although genetic analysis takes too long to guide therapy during acute flares. For patients with aHUS who develop end stage kidney disease, detection of an underlying mutation is also important for transplant planning. Membrane cofactor protein is a transmembrane protein, and a kidney allograft from a healthy donor corrects the defect. On the other hand, kidney donation from a relative is contraindicated if the relative carries the same complement mutation as the patient, even if the potential donor is disease free. It is worth noting, however, that the recurrence of aHUS after transplantation in patients with membrane cofactor protein mutations is higher than 10% in some series. This may be due to co-existing mutations in other complement-related genes in some patients. Because factor H, factor I, C3, and factor B are primarily synthesized in the liver, transplant recipients with mutations in the genes for these proteins have limited ability to control complement activation within the allograft and are at high risk of disease recurrence during the peri-transplant period.

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THERAPEUTIC COMPLEMENT BLOCKADE

Complement Inhibitory Drugs

Eculizumab has been approved by the FDA for the treatment of aHUS, and has been tested in several other kidney diseases. It is a humanized murine monoclonal antibody to complement C5 that prevents the formation of C5a and C5b-9, but it does not prevent the generation of C3a and C3b. Leaving the early complement system intact may reduce the risk of infection, but C3a and C3b may also contribute to the pathogenesis of some inflammatory diseases. Because eculizumab prevents formation of the membrane attack complex, the CH50 in treated patients should be close to 0. This functional readout aids in monitoring the dosing of the drug. C5 blockade does not directly affect the levels of circulating C3 and C4, nor does it block C3 deposition within glomeruli. The primary risk of complement inhibition is that of infection, and all patients who receive eculizumab should be either immunized for meningococcus or prophylactically treated for this infection with antibiotics.

Eculizumab is an expensive drug, limiting its use for diseases in which its benefit is uncertain or that have other effective treatments. Even in patients with aHUS, the optimal duration of treatment is unknown. Patients with complement mutations are at lifelong risk of recurrence, and it is not clear whether patients should be treated indefinitely or only during periods of active disease. Disease flares are often triggered by illness (particularly diarrheal illnesses), pregnancy, and certain drugs. In some cases disease recurrence has been attributed to very minor stressors, such as vaccination. A dilemma, then, is that flares may be triggered by minor events and may rapidly lead to irreversible kidney injury, yet prevention of these flares could require life-long therapy.

Other complement inhibitory drugs are in development, some of which block specific activation pathways or activation fragments. One difficulty in developing new drugs for the treatment of aHUS and C3 glomerulopathy is the large number of underlying genetic mutations and autoantibodies associated with these diseases. Depending upon their

mechanisms of action, many of the new drugs will likely not work for patients with particular underlying complement defects. For example, gain of function mutations in C3 that resist inactivation by endogenous complement regulatory proteins might also be resistant to inactivation by some anti-complement drugs. Although complete genetic and molecular testing of each patient may take weeks or months to complete, functional assays of complement inhibition may provide a rapid means of testing complement inhibition by a specific drug in an individual patient.

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FUTURE DIRECTIONS

The complement system plays a central role in the pathogenesis of a wide range of kidney diseases. Mutations and auto-antibodies that impair control of the alternative pathway are associated with the development of aHUS, C3 glomerulopathy, and several other kidney diseases. Eculizumab has been approved for the treatment of aHUS, and a variety of new anti-complement drugs are in development. Additional therapeutic options may lead to greater complexity regarding which drugs to use, but improved biomarkers of complement activation may improve our ability to identify appropriate patients for treatment. Nevertheless, it is clear that the complement system is an important mediator of kidney injury and the role of anti-complement therapies in nephrology will expand in the coming years.

Acknowledgments

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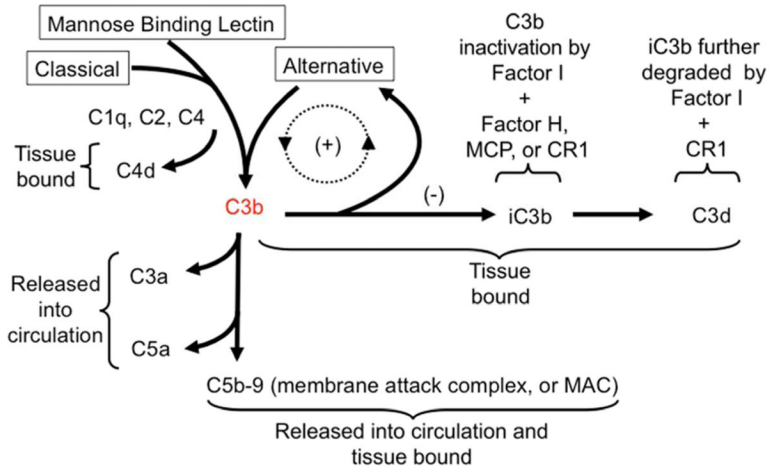

Figure 1. Overview of the complement system

The complement system is activated through three distinct pathways: the classical pathway, the mannose binding lectin pathway, and the alternative pathway. Activation through the classical and mannose binding lectin pathways causes cleavage of the protein C4, and fixation of C4d (a fragment of C4) to nearby tissues. Activation through all pathways leads to cleavage of C3. The cleavage of C3 generates a soluble fragment (C3a) and a tissue bound fragment (C3b). Further proteolysis of C3b generates iC3b and finally C3d. Full activation of the complement system also generates C5a and C5b-9, important mediators of tissue inflammation and injury.

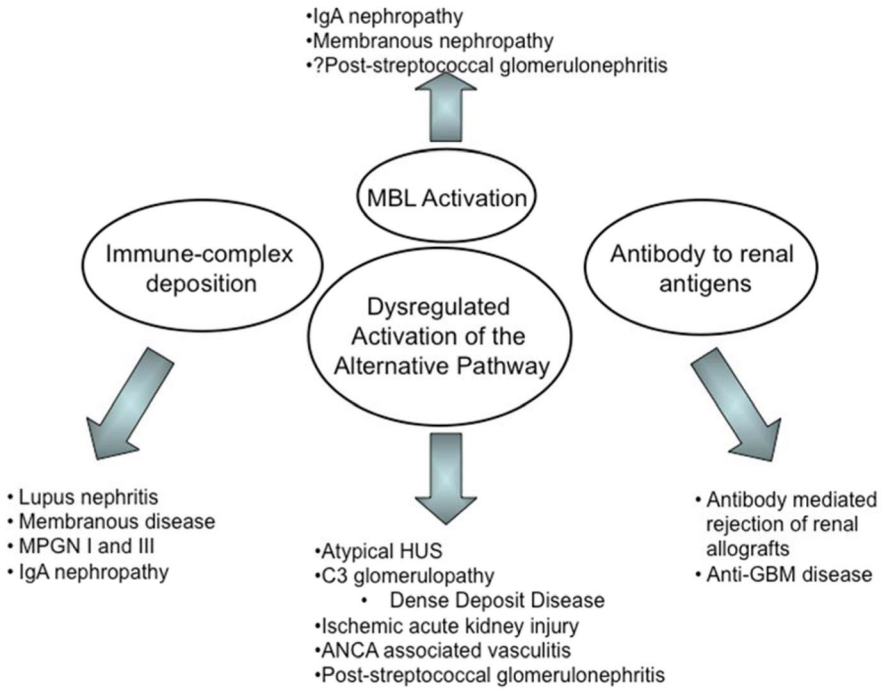

Figure 2. Mechanisms of complement activation in kidney disease

Immune complex deposition within glomeruli activates the classical pathway of complement in some forms of glomerulonephritis, and antibodies specific to renal antigens also activate the classical pathway. Uncontrolled activation of the alternative pathway of complement is associated with aHUS and C3 glomerulopathy, and the alternative pathway has also been implicated in ANCA-associated vasculitis and post-streptococcal glomerulonephritis. Mannose binding lectins have been detected in the kidneys of some patients with IgA nephropathy, membranous nephropathy, and post-streptococcal glomerulonephritis. Abbreviations: HUS, hemolytic uremic syndrome; ANCA, anti-neutrophil cytoplasmic antibody; MBL, mannose binding lectin; IgA, immunoglobulin A; MPGN, membranoproliferative glomerulonephritis; GBM, glomerular basement membrane

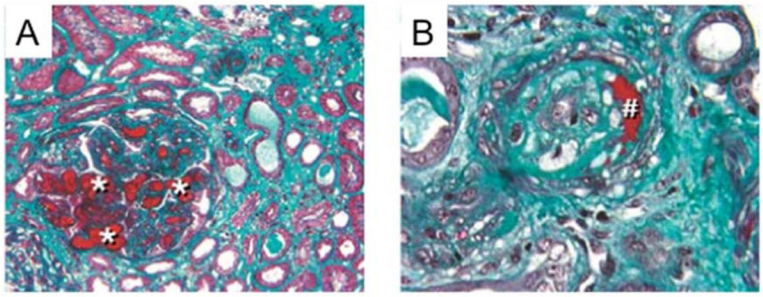

Figure 3. Histology of atypical hemolytic uremic syndrome

A kidney biopsy from a patient with atypical HUS and dysregulated alternative pathway activity demonstrates typical findings of thrombotic microangiopathy. (A) Thrombi are seen within the glomerular capillaries (labeled with asterisks). (B) A thrombus is also seen in an arteriole (labeled with a crosshatch). Tissue was stained with Masson's trichrome. Reproduced by permission from Macmillan Publishers Ltd: Fakhouri F and Fremeaux-Bacchi V. Does hemolytic uremic syndrome differ from thrombotic thrombocytopenic purpura? *Nat Clin Pract Nephrol* 2007;3:679–687.

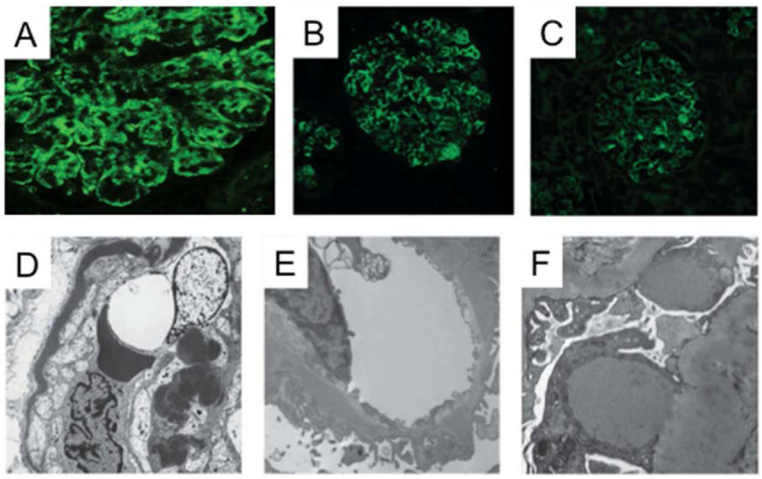

Figure 4. Biopsy characteristics of C3 glomerulopathy

The diagnosis of C3 glomerulopathy is based upon the detection of C3 in the relative absence of immunoglobulin or classical pathway proteins. (A) C3 fragments are seen in the mesangium and capillary loops of a patient with C3 glomerulopathy. C3 (B) and IgG (C) from the same patient with C3 glomerulopathy, demonstrating that some immunoglobulin deposition is occasionally seen. (D) Electron microscopy from a patient with dense deposit disease demonstrates dense intramembranous deposits. (E) Electron dense deposits are seen in the glomerular basement membrane of patients with C3 glomerulopathy, although they are not as dense or well defined as those in patients with dense deposit disease. (F) Large, humped subepithelial deposits are sometimes seen in patients with C3 glomerulopathy, resembling those seen in patients with post-infectious glomerulonephritis. Reproduced with permission from Macmillan Publishers Ltd: Pickering MC, D'Agati VD, Nester CM et al. C3 glomerulopathy: consensus report. *Kidney Int.* 2013;84(6):1079–1089 and with permission from BMJ Publishing Group Ltd: Servais A, Frémeaux-Bacchi V, Lequintrec M, et al. Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. *J Med Genet.* 2007;44(3): 193–199.

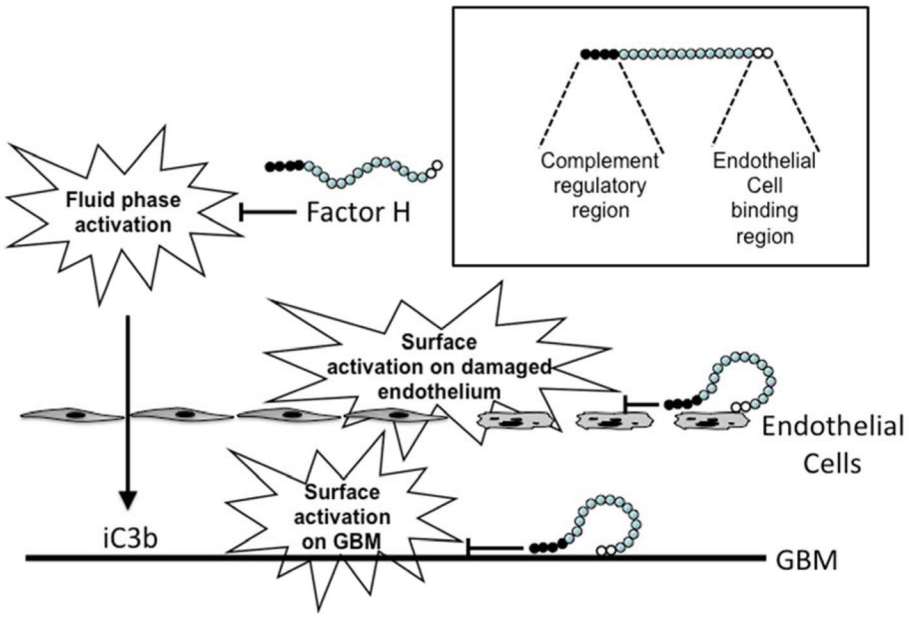

Figure 5. Complement regulation in the glomerulus by factor H

Factor H inhibits alternative pathway activation in the fluid phase and on the surface of cells and the basement membrane. It is a string-like protein; the complement regulatory region is at the amino terminus, and a region that mediates binding to surfaces is at the carboxy terminus. Factor H defects in patients with aHUS are predominantly in the binding region of the protein, suggesting that the disease is caused by impaired complement regulation by factor H on endothelial cells and/or the glomerular basement membrane (GBM). Some patients with C3 glomerulopathy have an absolute deficiency in complement regulation by factor H. Disease in these patients may be caused by uncontrolled fluid phase activation of the alternative pathway or insufficient regulation on the GBM.

Table 1

Kidney diseases associated with mutations or variations in the genes for complement regulatory proteins, categorized by syndrome.

Disease	Associated gene mutations or variations
Thrombotic microangiopathy	
Atypical hemolytic uremic syndrome	Factor H, Factor I, C3, Factor B, Membrane cofactor protein, CFHR1, CFHR3, thrombomodulin
Shiga toxin associated hemolytic uremic syndrome	Membrane cofactor protein
Thrombotic thrombocytopenic purpura	Factor H
HELLP syndrome	Factor H, Factor I, Membrane cofactor protein
De novo thrombotic microangiopathy after renal transplantation	Factor H, Factor I
Pre-eclampsia	Factor I, Membrane cofactor protein
Hematologic stem cell transplant related	CFHR1, CFHR3
C3 glomerulopathy	
C3 glomerulopathy	Factor H, C3, Factor B, Membrane cofactor protein, CFHR1, CFHR2, CFHR5
Dense deposit disease	Factor H, C3, CFHR5
Immune-complex glomerular disease	
MPGN type I	Factor H, Factor I
IgA nephropathy	CFHR1, CFHR3

CFHR, complement factor h related protein; HELLP, a syndrome of hemolysis, elevated liver enzymes, low platelets; MPGN, membranoproliferative glomerulonephritis; Ig, immunoglobulin.

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Table 2

Complement testing in patients with C3 glomerulopathy

Test	Interpretation	Limitations
C3 and C4 levels	C3 frequently depressed and supports diagnosis; normal C4 suggests an alternative pathway process	Non-specific
Soluble C5b-9	May be indicator of active disease; may identify patients who will benefit from C5 blockade	Test not widely available
C3 nephritic factor	Associated with C3 glomerulopathy; may identify patients who will benefit from B cell targeted therapies	Levels do not correlate with disease activity; also seen in MPGN type 1
Factor H protein levels	May identify underlying mechanism of alternative pathway activity; may identify patients who will benefit from plasma infusion/exchange	
Autoantibodies to factor H and factor B	May identify underlying mechanism of alternative pathway activity; may identify patients who will benefit from B cell targeted therapies	Test not widely available
Genetic mutation screening: • Factor H • CFHR1, 2, and 5 • Factor I • C3 • Factor B	May identify underlying mechanism of alternative pathway activity	Not widely available; clinical implications unknown

Abbreviations: MPGN, membranoproliferative glomerulonephritis.

Table 3

Complement testing in patients with aHUS

Test	Interpretation	Limitations
C3 and C4	Low C3 supports that disease involves the complement system; normal C4 suggests an alternative pathway process	An insensitive indicator of complement activation in aHUS
Soluble C5b-9	A sensitive indicator of complement activation and may also reflect active disease	Test not widely available
Levels of factor H, factor I, MCP	May identify underlying mechanism of alternative pathway activity	Levels may be normal in patients with dysfunction protein
Autoantibodies to factor H	May identify underlying mechanism of alternative pathway activity	Test not widely available
Genetic mutation screening: • Factor H • MCP • Factor I • C3 • Factor B • Thrombomodulin	May identify underlying mechanism of alternative pathway activity	Tests not widely available; tests take too long to help with acute care

Abbreviations: aHUS, atypical hemolytic uremic syndrome; MCP, membrane cofactor protein.