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Complement Biomarkers of Hemolytic Uremic Syndrome—If Not One Thing, Maybe Another

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Great progress has been made in our understanding of atypical hemolytic uremic syndrome (aHUS) during the past 15 years. Genetic and serologic studies have revealed that the disease is strongly associated with molecular defects causing overactivity of the complement cascade.[1] Several different animal models have confirmed that these genetic defects in complement regulation are causative of disease and that complement activation is a critical driver of tissue injury.[2] Based on these findings, patients with aHUS have been treated with the complement inhibitory drug eculizumab. Eculizumab is a monoclonal antibody to C5 that blocks the generation of C5a and C5b-9, complement activation fragments with proinflammatory and cytotoxic effects. Eculizumab has significantly improved outcomes for patients with aHUS and is now the standard of care for the disease in many countries.[3]

As exciting as these advances have been, the diagnosis and treatment of aHUS remain challenging. Atypical HUS is just 1 of several causes of thrombotic microangiopathy (TMA), a syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia, and damage to the kidneys and other organs. Patients with TMA do not always present with all of these laboratory findings, and many other diseases can cause anemia, thrombocytopenia, and organ failure. Thus, although the diagnosis of aHUS can be straightforward in some cases, such as in those with familial or recurrent disease, it can be quite difficult in patients who do not have the usual hallmarks of disease or who have other comorbidities.

Distinguishing among the various causes of TMA can also be a challenge. Thrombotic thrombocytopenic purpura and shiga toxin—producing *Escherichia coli* HUS can usually be diagnosed by measurement of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) or by detection of shiga toxin in stool, respectively. Pregnancy, drugs, infections, hypertension, and various other illnesses, however, are also associated with TMA. It is currently not clear whether these various conditions are simply triggers of aHUS in susceptible patients or whether they represent distinct pathophysiologic processes. Genetic analysis of complement genes can be useful. Many patients with aHUS do not carry known disease-causing mutations, however, and patients who do not have identifiable complement mutations still often respond to treatment with eculizumab.[3]

Perhaps the most useful indicator that a patient has complement-mediated disease is whether he or she responds to therapeutic complement inhibition. In this regard there are barriers to the use of eculizumab when the diagnosis is uncertain. The drug is expensive and it increases patients' risk of infection, particularly with meningococcus. It is not yet clear whether eculizumab is effective in the other forms of "secondary" HUS, although reports suggest that it is beneficial in postpartum disease[4] and hematopoietic stem cell transplant recipients with HUS.[5] In other forms of secondary HUS, cost and infectious risks of eculizumab weigh against empirical use of the drug. Atypical HUS can very quickly

lead to irreversible kidney damage, and, accordingly, clinicians need to quickly recognize that a patient has a TMA and make a decision whether to treat. Thus, better methods for accurately identifying patients with complement-mediated aHUS are greatly needed.

Complement activation can consume circulating C3 and C4, and it generates various activation fragments. Complement measurements such as these hold great appeal as potential biomarkers for identifying patients with complement-mediated HUS and for monitoring the effects of treatment on complement activation. Measurement of these proteins in plasma samples can provide evidence of complement activation in tissues. Indeed, a role for complement in HUS was originally suspected because some patients have very low C3 values.[6] The alternative pathway of complement is activated in aHUS, and levels of Ba (specific to the alternative pathway) and sC5b-9 (a marker of terminal complement activation) are elevated in some patients.[7,8] Effective complement blockade should prevent the consumption of intact complement proteins (increasing the plasma levels of C3, for example), while also preventing generation of complement cleavage fragments such as C5b-9 (lowering the plasma levels of sC5b-9).

Unfortunately, levels of complement proteins and fragments may be artifactually altered simply by vagaries in sample handling. Furthermore, the standard complement assays are normal in many patients with aHUS.[8] We do not yet have, therefore, a reliable serologic method of diagnosing the disease. To help address this unmet need, in the current issue of *Mayo Clinic Proceedings*, Sridharan et al[9] examine the utility of a panel of 9 complement-related tests for the diagnosis of aHUS: CH50, AH50, C3, C4, factor B, factor H, C4d, Bb, and sC5b-9. In their retrospective review, this panel of tests was performed in 44 patients with a diagnosis of TMA, 8 of whom had a clinical diagnosis of aHUS. Interestingly, in 11 of the patients the diagnosis of TMA was made only by kidney biopsy, and the standard hematologic parameters had not revealed the diagnosis ("renal-only TMA"). Three of these were transplant patients. The results in the complement panel were analyzed individually and in combinations to compare with the findings in patients with the different forms of TMA.

Atypical HUS is believed to be primarily driven by uncontrolled alternative pathway activity, and one would expect abnormalities in laboratory test results related to the alternative pathway (eg, low AH50, low factor H, and elevated sC5b-9 levels) but normal indices related to the classical complement pathway (eg, a normal C4 level). In aggregate, the patients with aHUS did have lower average CH50, AH50, C3, and factor B levels and higher sC5b-9 levels. Individually, all of the patients with aHUS had at least 1 alternative pathway abnormality. Yet only 1 of the 8 patients with aHUS had the expected abnormalities in all of the alternative pathway—related laboratory results, and there was overlap between the patient groups for each of the analytes. Consequently, the authors found that having a single alternative pathway abnormality was only 28% specific for aHUS in patients with TMA. The authors did find that a factor B level of 20.9 mg/dL or less was reasonably sensitive (75%) and specific (80.6%) for aHUS, and when combined with a CH50 level of 56% or less, the specificity for aHUS increased to 88.9%. Thus, although no single complement test seems sufficient for distinguishing aHUS from other forms of TMA, evaluating patients with a comprehensive panel of complement tests can identify aHUS with reasonable accuracy.

The patients with aHUS in this study already had a clinical diagnosis of the disease, and this study does not provide us with a method for making the diagnosis of aHUS in challenging cases. The results do, however, provide further evidence that the complement cascade is activated in aHUS. It would be particularly useful to identify serologic markers of disease for patients with renal-limited TMA, as the diagnosis was made only after patients underwent kidney biopsy. The results of the present study suggest that complement tests are closer to normal in this group of patients than in the other groups. This does not mean that the complement system is not activated in the other forms of TMA, such as renal-limited TMA, but the

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magnitude of activation seems to be greatest in patients with a clinical diagnosis of aHUS. Unfortunately, the number of patients in the study is too small to draw conclusions about the frequency and pattern of complement perturbations in specific subgroups of patients with secondary or renal-limited disease. This study is also too small to determine whether complement tests can predict which patients (with any form of TMA) would benefit from complement inhibition. Nevertheless, given the rarity of aHUS, and TMA in general, this study expands our understanding of the clinical characteristics of aHUS and brings us a little closer to the ultimate goal of securing a quick and accurate diagnosis.

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