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ORIGINAL ARTICLE

# Diagnostic Utility of Complement Serology for Atypical Hemolytic Uremic Syndrome



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## Abstract

**Objective:** To investigate the clinical utility of a 9-analyte complement serology panel (COMS) covering complement function (CH50 and AH50), components (C3, C4), factor B (CFB), factor H, and activation markers (C4d, Bb, and soluble membrane attack complex) for the diagnosis of atypical hemolytic uremic syndrome (aHUS).

**Methods:** Physician orders for COMS from January 19, 2015, through November 4, 2016, were reviewed. Demographic characteristics, patient diagnosis, and laboratory parameters were recorded.

**Results:** There were 177 COMS orders for 147 patients. The median patient age was 44.9 years (range, 0.9-88.0 years). Common reasons for ordering COMS included monitoring and diagnosis of C3 glomerulopathy and renal dysfunction and differentiation of aHUS from other thrombotic microangiopathies (TMAs). Forty-four patients had COMS ordered for TMAs: 8 had aHUS and all had 1 or more abnormalities within the alternative pathway of complement. Although the sensitivity of this finding for the diagnosis of aHUS is 100%, the specificity is only 28%, with a positive likelihood ratio of 1.39. Patients with aHUS had lower CH50, C3, and CFB than did those with secondary non-aHUS TMA (all $P<.01$). A combined CFB of 20.9 mg/dL or less and CH50 of 56% or less led to sensitivity of 75% with increased specificity of 88.9% and a diagnostic odds ratio of 24.

**Conclusion:** A COMS abnormality should not be interpreted in isolation. In conjunction with clinical presentation, a decrease in both CFB and CH50 may be an important clue to support the diagnosis of aHUS.

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The complement (C) system, a key component of innate immunity and a regulator of tissue homeostasis, can be activated via 3 different pathways: classical, alternative, and lectin (mannose binding).[1] Although the recognition molecules that trigger activation of each pathway differ, all converge to a complement component 3 (C3)—mediated amplification loop by pathway-specific C3 convertases. The classical pathway is activated by an antigen-antibody immune complex or C-reactive protein, whereas the lectin pathway is activated directly by mannose-containing bacterial surfaces. The alternative pathway is constantly active at low levels, in a surveillance role, and can be initiated by spontaneous hydrolysis of C3 when a potential threat is detected. The activation of C3 by the C3 convertases generates C3b, which will attach to foreign surfaces, and leads to the lytic pathway by forming the C5 convertase. This initiates a process to form the membrane attack complex (MAC) and promote cell lysis. In addition to formation of the MAC, the anaphylotoxins C3a and C5a, which have potent inflammatory effects and promote chemotaxis, are generated after C3 and C5 cleavage, respectively.[2] Complement activation is controlled by a set of membrane-bound and fluid-phase regulators to prevent overactivation. Any imbalance between the acting and regulatory mechanisms caused by genetic variants or acquired autoantibodies to the complement components may trigger disease processes, frequently fueled by inflammatory and thrombotic routes.[3]

The dysregulation of the alternative pathway of complement plays a role in the pathogenesis of atypical hemolytic uremic syndrome (aHUS), a thrombotic microangiopathy

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(TMA) characterized by normal ADAMTS13 activity (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, which cleaves von Willebrand factor), more severe renal failure compared with thrombotic thrombocytopenic purpura (TTP), and variable treatment response to plasma exchange.[4,5] The pathophysiologic mechanism of aHUS involves increased continuous spontaneous hydrolysis of C3 to C3b, leading to tissue deposition of C3b, MAC formation, and subsequent tissue injury. The underlying susceptibility factors include germline mutations in complement proteins or their regulators or acquired autoantibodies that fail to protect the endothelium from complement activation and result in TMA and renal failure.[6-8] Similar to TTP, without early recognition and treatment, aHUS causes significant morbidity and mortality. The integral role of the complement system in the pathogenesis of aHUS is further supported by the robust hematologic and renal responses witnessed with eculizumab, a humanized monoclonal antibody that serves as a complement inhibitor by binding to C5.[9,10]

There are currently no clear diagnostic criteria or a gold standard laboratory assay for the diagnosis of aHUS. Atypical HUS is a diagnosis of exclusion in patients known to have a TMA phenotype but also found to have ADAMTS13 activity greater than 10% and no Shiga toxin–producing infection or other clear cause of TMA.[4,11,12] Given that specific therapy exists for the treatment of aHUS, better diagnostic tools for early recognition are necessary to improve patient outcomes.

The use of quantitative serologic complement assays in aHUS has previously been described[13]; however, a uniform panel of complement analytes offering consistent diagnostic results has not been reported. Complement serology can be used in conjunction with complement genetic testing; however, genetic testing may not be available in a timely manner, delaying time to a final diagnosis and initiation of treatment. In addition, results may demonstrate genetic variants of uncertain significance (VUS), which provide clinicians with unclear information. Absence of complement genetic variants does not exclude aHUS because approximately 30% to 48% of patients with aHUS have no identifiable complement genetic variants.[14]

On January 19, 2015, a complement serology panel (COMS) became available at Mayo Clinic to assess dysregulation of the complement alternative pathway. We aimed to assess our institutional practices in the ordering of the COMS and in determining its clinical utility for diagnosis of aHUS.

## METHODS

Patients who had COMS testing from January 19, 2015, through November 4, 2016, were identified by retrieving data through the laboratory information system. The study protocol was approved by Mayo Clinic's Institutional Review Board. Retrospective medical record review was performed by a hematologist-oncologist in training (M.S.). Diagnoses were established based on all information available in the electronic medical records. Demographic characteristics, patient diagnosis, and laboratory parameters, including serologic and genetic complement analyses, complete blood cell counts, and renal function, were recorded. Patient final diagnosis was recognized based on a combination of clinical and laboratory data. In this study, complement serology was not used to determine patient final diagnosis because it was thought that this would skew the analysis assessing the clinical utility of complement serology. Thrombotic microangiopathy was defined as evidence of microangiopathic hemolytic anemia (nonimmune hemolytic anemia and schistocytes in a blood smear) and thrombocytopenia, or kidney biopsy results demonstrating TMA. The care process model by Go et al.[12] was used to characterize patients into secondary TMA or TMA likely due to complement abnormalities (aHUS).

As noted previously herein, the diagnosis of aHUS is one of exclusion. Both TTP and HUS should be excluded before a patient can be diagnosed as having aHUS. However, after exclusion of patients with HUS and TTP, differentiation of patients with aHUS (TMA mediated by dysregulation of the alternative pathway of complement) from patients without aHUS remains challenging. In our practice, if a person presents with TMA with a known secondary or precipitating cause the recommendation is to manage and treat those


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