

ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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Atypical Hemolytic Uremic Syndrome: The Role of Complement Pathway Gene Mutation Analysis



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H&O What causes atypical hemolytic uremic syndrome (aHUS)?

IW We think that most people with aHUS have problems with regulation of complement. As a result of excess complement, endothelial and organ damage occur. We know that mutations in the genes of complement regulatory proteins are associated with aHUS. In addition, factors other than underlying mutations may play a role in increasing activation and the expression of the clinical syndrome.

H&O How is the complement system activated and regulated?

IW The complement system is a part of the innate immune system that is necessary for fighting infections and aberrant immunologic stimuli. Complement has 2 main functions. One function is opsonization: coating pathogens—such as bacteria, viruses, and dead cells—with C3b for macrophage clearance. The other function is cell lysis; that is, punching a hole in the membrane of a cell or pathogen.

People who have a mutation in a complement regulatory protein do not have adequate capacity to limit complement activation. Some of the triggers for upregulation of complement activity are bacterial and viral infections, antigen-antibody complexes, autoimmunity, vaccination, pregnancy, and surgery. There may be a threshold for complement activation above which people who have some abnormality in their complement regulatory proteins become symptomatic.

H&O Which mutations in complement alternative pathway genes are linked to aHUS?

IW Multiple genetic mutations have been linked to aHUS, especially those involved in the complement alternative pathway. These include mutations in complement factor H, complement factor I, membrane cofactor protein, complement factor B, and C3 nephritic factor. Mutations may cause the protein to be normal but low in quantity, or normal in quantity but abnormal in function; the degree of the abnormality may depend on whether the patient is heterozygous or homozygous.

In addition, other factors such as thrombomodulin have been described that work through other enzymes. Thrombomodulin is involved in complement regulation by activating thrombin activatable fibrinolytic inhibitor (TAFI). TAFI enzymatically degrades the C5 cleavage products C5a and C5b, thereby limiting the generation of the terminal complement complex (C5b-9) as well as the anti-inflammatory and thrombotic effects of C5a. These mutations are rare and we may not be able to identify these specific abnormalities, which is a major limitation of looking at mutations. In addition, new mutations in other genes are being identified.

H&O How common are genetic mutations in people with aHUS?

IW We see mutations in approximately half of patients, and about one-third of patients have more than 1 mutation. However, about 25% to 30% of patients will not

have an identifiable mutation. There have also been some recent descriptions of mutations in proteins related to the clotting system that may play a role in aHUS, such as mutations in plasminogen.

H&O How has analysis of these mutations evolved over time?

IW It has taken a long time—approximately 30 years—to identify the most important 6 or 7 mutations. Now that we are able to do genome sequencing, we are likely to pick up many more interesting mutations.

H&O Does having information about the gene mutations affect diagnosis or treatment of aHUS?

IW If patients have the clinical syndrome, it does not matter whether they have a mutation or not because the diagnosis of aHUS is a clinical one. Having information about the presence of a mutation is sort of the “icing on the cake”; it can confirm your suspicion and might make you feel more confident in your treatment approach. The presence of a mutation clearly would encourage patients to continue treatment, particularly if they have had organ damage. However, waiting for the results of a mutation analysis before deciding on treatment will be detrimental to the patient, as it can take months to get a result.

At this time, it is not recommended to routinely perform mutation analysis on individuals with aHUS. Having this information does not predict the clinical activity of the disease or the response to treatment. Even if we wanted to do more testing, mutation analysis takes an extraordinary amount of time—it takes approximately 3 months to get the test results—and is quite expensive. Genome sequencing technology will make this process much faster and more cost-effective, but as of today, it is not readily available.

H&O Could you talk about your recent case report in *Blood Transfusion*?

IW This case study was on an interesting patient whom we treated before the approval of eculizumab (Soliris, Alexion). A previously healthy 20-year-old woman presented to the hospital with an acute diarrhea syndrome. No Shiga toxin was identified with timely testing, and she was diagnosed with aHUS. Within 4 days of presenting to the hospital, she had renal failure, thrombocytopenia, and microangiopathic hemolytic anemia.

The only treatment available at that time was supportive treatment with plasma exchange. She required 2 volume exchanges and was very symptomatic, with reactions to the plasma. She was on high-dose steroids and was not getting better, so she was transferred to our facility (USC-Los Angeles

County Medical Center) approximately 6 weeks after initial presentation. I already had some experience with eculizumab from treating patients with paroxysmal nocturnal hemoglobinuria and wanted to use it in this patient, which we were able to do under a compassionate use protocol.

Although the patient was slow to respond at the beginning of treatment, within 5 weeks she was dramatically and completely better. Her only residual abnormality was hypertension. The issue was how long to continue treatment with eculizumab in aHUS at that point. We decided to have a mutation analysis done as an additional guide to help us decide whether it was a congenital issue. What we found, after 3 months of waiting, was that our patient was heterozygous for the complement factor H–related 1 through 3 (*CFHR1-CFHR3*) genes, which act as accessory genes to complement factor H. In addition, 3 months later a previously undescribed mutation was identified in the C3 binding area of complement factor H, making our patient a compound heterozygote. Of course, the more mutations patients have, the more likely they are to become symptomatic in the presence of stressors such as infection or pregnancy.

The patient did well on treatment, but she decided on her own to discontinue therapy after approximately 9 months. This would not have been my decision; she had been very ill and nearly died, and we were especially concerned about recurrence given the findings on the mutation analysis. One month after discontinuing treatment she became pregnant. She did very well until 34 weeks of gestation, when she developed preeclampsia. Her baby was delivered right away and both mother and child seem to be doing well, although the patient still has hypertension.

There is a study by a French group that was published in *Blood* in 2008 that looked at patients with hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) and preeclampsia. What the researchers found is that approximately one-third of the patients had underlying complement mutations and regulatory defects.

One of the concerns I have with this patient is what might happen with a second pregnancy, because the highest incidence of preeclampsia and HELLP syndrome in the French cohort occurred with the second pregnancy. The fact that she remains hypertensive suggests to me that she has ongoing endothelial damage.

H&O What are some of the most important studies related to gene mutations in aHUS that have come out in recent years?

IW One of these, led by Richard Smith, was recently published in the *Journal of the American Society of Nephrology*. The researchers conducted a comprehensive genomic screen of the complement and coagulation pathways in 36 patients with aHUS. They found 19 genes implicated in

the pathogenesis of aHUS, including several genes in the coagulation pathway. Notably, *PLG* carried 3 plasminogen deficiency mutations.

I think that researchers will continue to find links between aHUS and the coagulation pathway, as well as abnormalities in other pathways. In a recent publication, researchers identified a group of infants younger than 9 months who presented with aHUS but did not appear to respond to complement inhibition. These infants had a unique mutation in diacylglycerol kinase (DGK) epsilon. The DGK epsilon enzyme is found in platelets and podocytes and endothelial cells, all of which are involved in aHUS. How a mutation in DGK epsilon interacts with other complement disorders is not really clear and needs to be examined.

Another recent paper, by Jodele and colleagues in *Blood*, demonstrated CFHR1-3 heterozygous mutations in 6 children with aHUS that occurred after bone marrow transplant. Of significance was the fact that 3 out of the 6 were autotransplanted, so presumably they were not receiving cyclosporine or tacrolimus. It certainly would suggest that endothelial damage related to the preparatory medications in the setting of an underlying mutation may have induced the clinical syndrome.

Another important finding, which came from a new prospective trial of eculizumab in 41 patients with aHUS, was that approximately one-half of the patients did not have identifiable mutations.

H&O What have been the most important recent advances in aHUS?

IW The use of eculizumab has dramatically changed the way we treat the disease. Although plasma exchange may

be effective initially, long-term responses to plasma are quite poor, and patients wind up with renal failure or other organ complications of this disease. I suspect that eculizumab will change the natural history of aHUS, just as it has with paroxysmal nocturnal hemoglobinuria. It does not treat the mutations, but it clearly reduces the effect of the complement activation in these patients.

Suggested Readings

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