Diagnosis of atypical HUS using Genetic Testing

ABSTRACT

The patient is a 33 year old woman at 31 weeks gestation with twins who presented to the ER complaining of shortness of breath, headache, and blurry vision. The patient's preliminary complete blood count (CBC), RBC morphology, coagulation testing, and certain metabolic indicators were characteristic of a hemolytic process caused by microcirculatory lesions known as thrombotic microangiopathies (TMAs). The major pathologies of this hemolytic process are Thrombotic Thrombocytopenic Purpura (TTP), Hemolytic Uremic Syndrome (HUS), Disseminated Intravascular Coagulation (DIC), and Hemolysis, Elevated Liver Enzymes, Low Platelets (HELLP). Additional coagulation and biochemical testing indicated that the patient probably was experiencing HELLP syndrome, but atypical HUS (aHUS) could not be ruled out. Consequently an aHUS genetic susceptibility panel was also ordered on this patient. The results of the genetic testing revealed that the patient did indeed have aHUS. Atypical hemolytic uremic syndrome is a disease of complement dysregulation. In approximately 50% of patients, mutations have been described in the genes that encode complement regulator factors.

With an accurate diagnosis established, the patient was able to receive treatment utilizing an anti C5 monoclonal antibody aimed specifically at controlling the dysregulated complement protein C5.

CASE REPORT

A 33 year old woman at 31 weeks gestation with twins presented to the ER complaining of shortness of breath, headache, and blurry vision in her left eye. Her pregnancy to date was

without complications except for significant edema and very recently, a urinary tract infection being treated with amoxicillin. Her blood pressure (BP) was 158/98. Her inaugural hematology workup showed her to have a markedly increased white blood cell (WBC) count, while displaying neutrophilia, anemia, and thrombocytopenia (Figure 1, Table 1). A manual leukocyte differential performed at this time revealed the presence of shistocytes – although the numbers were modest (Figure 1, Table 1). The patient had normal protime (PT), activated partial thromboplastin (APTT) times, and fibrinogen levels, but her fibrin degradation products (FDP) were modestly increased. Significantly abnormal chemistry values included a moderately elevated blood urea nitrogen (BUN), elevated creatinine, and mildly to markedly elevated liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH))(Table 1). A urinalysis done at this time revealed the presence of blood, leukocytes, protein, bacteria, and yeast. A urine culture and sensitivity done at the time of admission could not be interpreted due to specimen contamination (data not shown).

LABORATORY FINDINGS

The patient's preliminary complete blood count (CBC), RBC morphology, coagulation testing, and certain metabolic indicators were characteristic of a hemolytic process caused by microcirculatory lesions known as thrombotic microangiopathies(TMAs).[1, 2] Laboratory testing proceeded so that the major pathologies of this hemolytic process could be ruled out. Hemolysis in TMAs is caused by damage to the endothelial lining of the smallest blood vessels, the damage activates the coagulation cascade, and the resulting fibrin strands fragment erythrocytes caught in the fibrin structure.[3] Several diseases underlie the development of TMA including

antiphospholipid antibody syndrome, disseminated intravascular coagulation (DIC), malignant hypertension, thrombotic thrombocytic purpura (TTP), hemolytic uremic syndrome (HUS), atypical HUS (aHUS), and a severe form of preclampsia known as hemolysis, elevated liver enzymes, and low platelets count (HELLP) syndrome.[3, 4, 5] The patient's normal International Normalized Ratio (INR) and APTT testing indicated that her TMA was not the result of antiphospholipid antibodies.[6] These same coagulation results, along with a normal fibrinogen level and only slightly elevated FDPs concomitant with a non-supportive clinical presentation, allowed us to rule out DIC for this patient as well.[6, 7] Malignant hypertension remained a possibility, particularly since the patient presented to our facility pregnant, with a headache, and with blurry vision.[8, 9] To investigate the possibility of this disorder, creatine kinase-muscle/brain (CK-MB) levels, Troponin I testing, and a careful eye examination were carried out. Both of the cardiac markers indicated that the patient had a low probability of cardiac damage, and she tested negative for burry vision, diplopia, scotoma, photophobia, coryza, and oculorrhea (data not shown). Considering the results of her cardiac markers and her visual test results, it was determined that pathologies other than malignant hypertension be considered as the likely cause of her TMA.[8, 9] To investigate the possibility of Thrombotic Thrombocytopenic Purpura (TTP), a blood sample was sent to a reference lab to be tested for *a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13* (ADAMTS13) activity.[5, 6, 10] The result of the ADAMTS13 activity assay test was charted 5 days after the patient was admitted. Reduced activity of ADAMTS13 (NR > 61%) indicated that the cause of our patient's hematology troubles could be complicated by Thrombotic Thrombocytopenic Purpura, although her ADAMTS13 activity was not as critically low as is usually seen in classical TTP.[5, 6, 10] Accordingly, the patient began plasma exchange therapy. At the same time that ADAMTS13

activity was investigated, testing for the presence of Shiga Toxin 1 and 2 was carried out in order to rule out Hemolytic Uremic Syndrome (HUS).[5, 6, 11] Negative test results for the presence of Shiga toxins supported a conclusion that this patient's hemolytic troubles were not due to HUS, but there remained the possibility that our patient's trouble was a variant of HUS – aHUS. This prospect was investigated with the assessment of complement proteins. The results of complement testing done on day 5 revealed normal results for both C3 and C4 which indicated that the underlying problem was not likely aHUS since C3 levels would be expected to be decreased in this pathology.[5] The patient's ongoing BUN and creatinine levels indicated that she was experiencing renal failure, so a renal biopsy was done on day 10, a few days after a caesarian section was performed and twins delivered. Changes consistent with toxemia of pregnancy with progression to frank thrombotic microangiopathy were noted on the biopsy specimen (Table 1). Given the history of recent pregnancy, it was felt that these tissue findings most likely represented HELLP syndrome, although it was noted that aHUS could not be ruled out. On day 10, the patient's B type natriuretic peptide (BNP) was interrogated to clarify whether or not her difficulty was indeed (HELLP) syndrome.[12] As can be seen in Table 1, our patient's BNP strongly supported our early suspicion that the patient was suffering from HELLP syndrome, again with TTP complicating her troubles.[12] However, to fully investigate the possibility of aHUS, an aHUS genetic susceptibility panel was also ordered. This multi-gene panel interrogates pathogenic variants in the genes that are associated with genetic aHUS (more discussion to follow).[13] When the results of the genetic susceptibility panel were received, they indicated that a diagnosis of aHUS was a strong candidate for this patient since one of her alleles contained a CFHR3-CFHR1 deletion (Table 2). At this point the patient's therapy was changed so that she began receiving Eculizumab, a monoclonal antibody that is efficacious in the

treatment of aHUS.[7, 14] The patient's problems rapidly resolved, confirming aHUS as the cause of her troubles.

DISCUSSION

The complement system of proteins is part of the innate immune system.[3, 4] Activation of the complement cascade of proteins occurs by one of three pathways; classical, lectin, and the alternative pathway. All three pathways produce an enzyme that is active midway in the complement cascade - complement component 3 (C3) convertase. C3 convertase activates a complement protein 5 (C5) that in turn may activate the terminal portion of the complement cascade.[3, 4, 5] Once it is fully activated, the complement cascade must be tightly regulated to avoid cell damage.[3, 9] Eculizumab is an anti C5 monoclonal antibody that specifically targets dysregulated complement protein C5; thus it regulates an important complement protein that is active midway through the complement cascade.[7, 14]

The specific etiology of aHUS appears to be dysregulated C3 convertase activity.[15, 16, 17] While faulty C3 protein itself accounts for a small number of aHUS cases (~5%), there are a number of additional complement cascade components that appear to underlie the development of aHUS when they are abnormal.[13, 15, 16, 17]

Complement factor H glycoprotein (fH) coded by *CFH* is a major regulator of complement activity.[18] Located in close proximity to *CFH* on the long arm of chromosome 1, there are five genes that code for proteins that appear to control the activity of fH. These genes are known as complement factor H related genes; they are designated as *CFHR1-5*.[13, 15, 16, 17] The protein products of these factor H related genes show immunological cross-reactivity with one another and with factor H as well.[16] Rearrangements in the *CFH-CFHR1-5* gene cluster can result in the

of several pathologies; *CFHR1* and *CFHR3* mutations are especially implicated in the development of aHUS.[13, 15, 16, 17] *CFHR1* and *CFHR3* mutations are a common mutation and they increase one's risk of developing aHUS because these mutations appear to increase the likelihood of developing antibodies to the regulatory fH.[15, 16, 17, 18] If these autoantibodies develop, a loss of complement control is likely. In addition to the complement factor H related proteins, 5 additional complement proteins appear to contribute to the development of aHUS when their function is deviant. Complement factors B, H, and I, membrane cofactor protein, and thrombomodulin are coded for by *CFB, CFH, CFI MCP*, and *THBD* genes.[13] Mutations in complement factor H related genes and these specific complement protein genes collectively are believed to underlie nearly 50% of aHUS cases.[13]

SUMMARY/CONCLUSION

Thrombotic microangiopathies are hemolytic conditions caused by microcirculatory lesions. Diseases that lead to the development of TMA include antiphospholipid syndrome, disseminated intravascular coagulation (DIC), malignant hypertension, Hemolytic Uremic Syndrome (HUS), atypical HUS (aHUS), Thrombotic Thrombocytopenic Purpura (TTP), and a severe form of preeclampsia known as hemolysis, elevated liver enzymes, and low platelet count (HELLP). Our patient's inaugural CBC, BUN/Creatinine levels, and liver enzymes suggested that she was experiencing a TMA which is characterized by compromised red blood cell (RBC) and platelet parameters, compromised kidney function, and elevated liver enzymes. Further laboratory testing was directed towards diagnosing the exact nature of our patient's problem. Accordingly, additional coagulation tests, vision testing, a Urine C&S (data not shown), Shiga Toxin testing, C3 & C4 Complement, a Renal Biopsy, an ADAMTS13 activity assay, and B type natriuretic

peptide were ordered to clarify the exact nature of this patient's TMA. The results of these testing did not offer a clear cut interpretation (see LABORATORY FINDINGS) but considering the patients' clinical history of a current pregnancy, it was decided that the patient most likely suffered from HELLP syndrome – possibly complicated by TTP. The patient was started on plasma exchange therapy although her ADAMTS13 activity was not as low as is generally seen in classical TTP. After a week of plasma exchange, the patient's laboratory values did not improve satisfactorily, and she was still experiencing disturbing clinical symptoms. At this time an aHUS susceptibility panel was ordered on our patient with results that suggested that her problem was aHUS even though the typical decreased levels of C3 were not present. The patient's therapy was changed to a regimen of Eculizumab and her problems were resolved. This outcome was supportive of a diagnosis of aHUS.

REFERENCES

1. Kappler S, Ronan-Bentle S, Graham A. Thrombotic Microangiopathies (TTP, HUS, aHUS, HELLP). *Hematol Oncol Clin North Am*, 2017;31(6):1081-1103.
2. Kottke-Marchant K. Diagnostic approach to Microangiopathic hemolytic disorders. *Int J Lab Hematol*, 2017;39(S1):69-75.
3. Smith, LA (2015). Hemolytic Anemia: Nonimmune Defects. In SB McKenzie (Ed.), *Clinical Laboratory Hematology* (3rd ed., pp372-387). Upper Saddle River, NJ: Pearson.
4. Shen YM. Clinical evaluation of thrombotic microangiopathy: identification of patients with suspected atypical hemolytic uremic syndrome. *Thromb J*, 2016:14(1):19.
5. Polito MG, Kirsztajn GM. Thrombotic microangiopathies: thrombotic thrombocytopenic purpura / hemolytic uremic syndrome. *J Bras Nefrol*, 2010:32(3):303-15.

6. Winter WE, Flax SD, Harris NS. Coagulation testing in the core laboratory. *Lab Med*, 2017:*48*(4): 295-313.

7. Abe T, Sasaki A, Ueda T, Miyakawa Y, Ochiai H. Complement-mediated thrombotic microangiopathy secondary to sepsis-induced disseminated intravascular coagulation successfully treated with eculizumab: A case report. *Medicine (Baltimore)*, 2017:96(6):e6056.

8. Thayu M, Chandler WL, Jelacic S, Gordon CA, Rosenthal GL, Tarr PI. Cardiac ischemia during hemolytic uremic syndrome. *Pediatr Nephrol*, 2003:18(3):286-9.

9. Mathew RO, Nayer A, Asif A. The endothelium as the common denominator in malignant hypertension and thrombotic microangiopathy. *J Am Soc Hypertens*, 2016:10(4):352-9.

10. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. *Blood*, 2017:129(21):2836-2846.

11. Sacerdoti F, Scalise ML, Burdet J, Amaral MM, Franchi AM, Ibarra C. Shiga Toxin-Producing *Escherichia coli* Infections during Pregnancy. *Microorganisms*, 2018:6(4):111.

12. Cabo Fustaret MC FMC, Escobar A, Illia R, Imaz MU, Rivas C, Lobenstein G, Olejnik P, Mayer H, Anchorena MD, Strika M, Climenti B. PP050. NT-Pro-BNP: Correlation with adverse outcome markers in hypertensive gestational syndromes. *Pregnancy Hypertens*, 2013:3(2):85.

13. Noris M, Bresin E, Mele C, Remuzzi G. Genetic Atypical Hemolytic-Uremic Syndrome. 2007 Nov 16 [Updated 2016 Jun 9]. In: MP Adam, HH Ardinger, RA Pagon, SE Wallace, LJH Bean, K Stephens, A Amemiya, editors. *Gene Reviews* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1367/

14. Fakhouri F, Loirat C. Anticomplement Treatment in Atypical and Typical Hemolytic Uremic Syndrome. *Semin Hematol*, 2018:55(3):150-158.

15. Zipfel PF, Edey M, Hainen S, Jozsi M, Richter H, Misselwitz J, Hoppe B, Rutledge S, Strain L, Highes AE, Goodship JA, Lict C, Goodship THJ, Skerka C. Deletion of Complement Factor H-Related Genes CFHR1 and CFHR3 is Associated with Atypical Hemolytic Uremic Syndrome. *PLoS Genetics*, 2007:3(3):e41.

16. Skerka C, Chen Q, Fremeauz-Bacchi V, Roumenina LT. Complement factor H related proteins (CFHRs). *Molecular Immunology*, 2013:56(3):170-180.

17. Abarrategui-Garrido C, Martinez-Barricarte R, Lopez-Trascasa M, de Cordoba SR, Sanchez-Corral P. Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood*, 2009:114(19):4261-4271.

18. Lee BH, Kwak SH, Shin JI, Lee SH, Choi HJ, Kang HG, Ha IS, Lee JS, Dragon-Durey MA, Choi Y, Cheong HI. Atypical hemolytic uremic syndrome associated with complement factor H autoantibodies and CFHR1/CFHR3 deficiency. *Pediatr Res*, 2009:66(3):336-40.

Figure 1

Peripheral blood displaying a significantly low platelet count (42,000/uL) and shistocyes.

100X magnification.

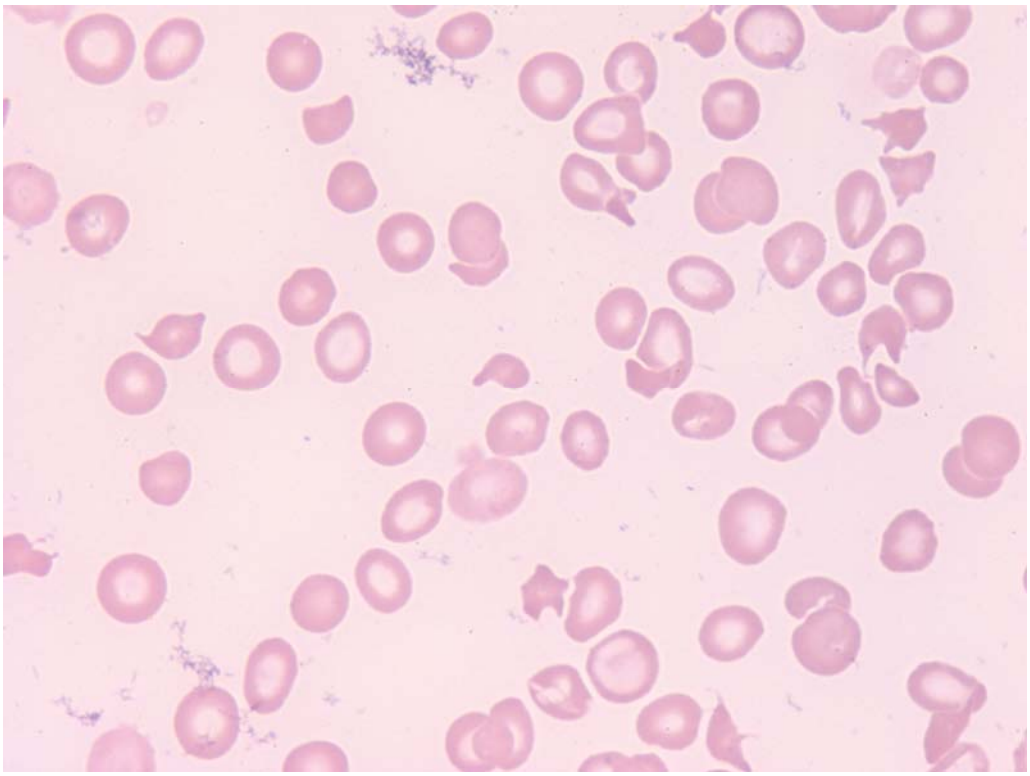

Table 1. Diagnostic Timeline. Yellow = TMA suggested; Fuchsia = DIC ruled out; Light Blue = HUS ruled out; Mint = Antiphospholipid antibody syndrome ruled out; Light Pink = aHUS falsely ruled out; Purple = TTP falsely suggested

Test	Patient Result						Reference Range
	Day 1	Day 5	Day 10	Day 20	Day 30	Day 50	
WBC	20.2	14.5	9.6	6.5	8.6	9.0	4.5-11 X 10^3/uL
RBC	2.48	2.18	2.44	2.53	2.64	2.86	4.0-5.4 X 10^6/uL
HGB	7.6	6.5	7.5	8.2	8.5	9.2	12.0-16.0 g/dL
HCT	24.0	19.6	23.2	26.2	27.9	29.6	36.0-47.0%
PLT	46	52	121	143	156	186	150-450 X 10^3/uL
DIFF	↑ PMN	↑ PMN	↑ PMN 1 meta, 1 myelo				
RBC Morphology	1+ poly 1+ decryo Occ. shisto	2+ aniso 1+ poly Occ. shisto	2+ aniso 1+ poly Occ. shisto	3+ aniso 1+ macro 1+ stomato 1+ decryo Occ. shisto	3+ aniso 1+ macro Occ. shisto		
INR	0.9	1.3	1.3				1.0
APTT	23.5	33.2	33.2				25.1-36.5 seconds
FIB	229	292	635				200-400 mg/dL
FDP	> 5, < 20						< 5 ug/mL
BUN	31	69	20	29	9	20	7-18 mg/dL
CR	1.9	3.5	2.6	7.2	4.2	6.5	0.6-1.3 mg/dL
ALT	79	28	19	13	12		13-56 U/L
AST	257	59	28	22	23		15-37 U/L
LDH	746		778	439	414		84-246 U/L
CK-MB		Low MI Probability		No evidence of acute MI			
Troponin I		Low MI Probability		Developing or subclinical MI			
B type Natriuretic Peptide			991		2744		< 125 pg.mL
C3 Complement		107.0		84.4			90-180 mg/dL
C4 Complement		25.7		11.4			10-40 mg/dL
Haptoglobin			8				40-240 mg/dL
Shiga Toxin I		Neg					Negative
Shiga Toxin 2		Neg					Negative
Cl. Diff.		Neg					Negative
ADAMTS13		20					> 60%
Renal biopsy			Changes consistent with toxemia of pregnancy with progression to frank thrombotic microangiopathy. Given history of recent pregnancy, this most likely represents HELLP syndrome. However HUS, aHUS, and TTP cannot be excluded.				

Table 2. aHUS Susceptibility Panel. Deletion of CFHR3-CFHR1 on allele 1 suggestive of aHUS.

Test	Patient Result, Day 10	Reference Range
aHUS susceptibility panel C3, CFB, CFH, CFHR1, CFHR3, CFHR5, CFI, MCP, THBD deletion analysis	Allele 1: Deletion of CFHR3-CFHR1 Allele 2: No deletion detected	No deletions detected