Complement Regulatory Genetic Mutations in the Setting of Autoimmune Thrombotic Thrombocytopenic Purpura: A Case Series

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Abstract

Objective: To explore the benefits of adding eculizumab for the treatment of refractory autoimmune thrombotic thrombocytopenic purpura (iTTP) with complement dysregulation.

Patients and Methods: From January 1, 2014, through July 1, 2017, we identified patients with iTTP defined by ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) levels less than 5% and the presence of ADAMTS13 inhibitor. Patients who progressed after receiving standard of care management for iTTP were subjected to a comprehensive evaluation to look for evidence of complement activation. Herein, we share our single-institute experience regarding the clinical course and treatment algorithm for 3 patients with refractory iTTP.

Results: All the patients had clinical deterioration despite treatment with plasma exchange, corticosteroids, rituximab, and vincristine, which prompted us to look for evidence of complement activation and associated genetic mutations. Complement-related genetic aberrations were present in all 3 patients, who had different degrees of complement activation. The first 2 patients did not benefit from eculizumab when treatment was started before complete clearance of inhibitors to ADAMTS13. However, they had durable remissions when eculizumab was introduced after clearance of ADAMTS13 inhibitors. The third patient started eculizumab therapy after inhibitor levels were undetectable.

Conclusion: We found eculizumab therapy to be effective in all 3 patients. However, its efficacy was prominent only after clearance of antibodies against ADAMTS13 via therapeutic plasma exchange.

Thrombotic microangiopathies (TMAs) are a diverse group of disorders presenting with non–immune-mediated hemolytic anemia and thrombocytopenia. Treatment of the underlying cause in such cases is crucial for controlling the TMA. However, once all other causes are excluded, physicians face the diagnostic challenge of determining which one of the life-threatening TMAs it is: hereditary or autoimmune thrombotic thrombocytopenic purpura (iTTP), Escherichia coli–induced hemolytic uremic syndrome (HUS), or atypical HUS (aHUS).

Classic HUS is caused by Shiga toxin–producing organisms; aHUS is associated with complement dysregulation due to mutations in CD46, complement factor (CF) I, CFb, complement component 3, CFH-related (CFHR) 5, CFH, and thrombomodulin or secondary to CFH autoantibodies. On the other hand, TTP is characterized by congenital or autoimmune-related deficiency of the von Willebrand factor cleaving protein ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13).

The case series reported herein illustrates an unusual clinical entity in 3 patients with an acute TMA syndrome with clinical and laboratory features of refractory iTTP associated with genetic mutations typically seen in aHUS as well. All the genetic mutation tests were performed by Machaon Diagnostics.

CASE REPORTS

Patient 1
A 24-year-old African American man with Klinefelter syndrome had recently started
working at a new job in a poultry processing facility, where he was in direct contact with meat. He initially presented with bloody diarrhea, diffuse abdominal pain, and acute renal failure, with a creatinine level up to 7 mg/dL (to convert to μmol/L, multiply by 88.4). An *E coli* infection was ruled out. ADAMTS13 activity was less than 5%, with inhibitor titers of 5 Bethesda units (BU). Treatment of TTP is outlined in Figure 1. Initially, the patient had a good response to treatment, and his creatinine level improved, down to 3 mg/dL, for a few weeks only and thereafter started to climb again to the point where hemodialysis was required. As a result of the severe renal injury and lack of lasting response to therapeutic plasma exchange (TPE), corticosteroids, rituximab, cyclophosphamide, and vincristine, aHUS genetic mutation testing was performed. He was found to have a large homozygous deletion in *CFHR1* and *CFHR3* genes. Skin biopsy performed at this time showed focal C5b-9 deposition within vessels, including the superficial vascular plexus (Figure 2). Antibodies against CFH were detectable. Therefore, eculizumab treatment was added to TPE (Figure 1). We noted the inadequate response to eculizumab, without clearance of ADAMTS13 inhibitor, and so eculizumab administration was with held until serial testing showed loss of the inhibitor. Reinstitution of eculizumab after clearance of the ADAMTS13 inhibitor led to improvement in renal function such that dialysis could be stopped and he maintained his platelet count in the reference range.

**Patient 2**

A 35-year-old African American woman presented with slurred speech and left arm weakness. ADAMTS13 activity was less than 5%, with an inhibitor level of 1.1 BU. Renal function was mildly decreased (creatinine clearance, 30 mL/min per 1.73 m² [to convert to mL/s per m², multiply by 0.0167]). Daily TPE and corticosteroid therapy were initiated for acquired TTP. This resulted in complete clearance of the ADAMTS13 inhibitor; however, her condition continued to deteriorate and right-sided weakness, bilateral cerebral infarcts on magnetic resonance imaging, and seizure-like activity developed. Hence, complement-related genetic testing was ordered and showed a large homozygous deletion in *CFHR1-CFHR3*. Treatment with eculizumab was instituted, and an improvement in the platelet count was seen after the first dose (Figure 2). Six months after initiating treatment with eculizumab, treatment was discontinued. Two years later, she has not had a recurrence of her TMA. Further details about this patient can be found in a recently published report. Baseline testing for CFH antibodies at diagnosis is not available. However, antibodies to CFH were undetectable during treatment with eculizumab.

**DISCUSSION**

Severe ADAMTS13 deficiency has been considered the unique pathognomonic event of TTP. Initially, Furlan et al reported normal ADAMTS13 activity in approximately 85% of patients with HUS. The finding that patients with HUS have normal ADAMTS13 activity was classically used as the definitive criteria to differentiate patients with HUS from those with TTP. However, these findings have been challenged by reports of the coexistence of iTTP and aHUS. The first report described a patient with clinical deterioration despite TPE; a skin biopsy confirmed the presence of heavy perivascular CF depositions and triggered
FIGURE 1. Treatment timelines for the 3 study patients with thrombotic thrombocytopenic purpura showing ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity and inhibitor levels. The first 2 patients illustrate the importance of eliminating the ADAMTS13 inhibitor before starting treatment with eculizumab. The ADAMTS13 test is sent to an outside laboratory and has a few days’ turnaround time. This principle was applied to the treatment of the third patient with good clinical response. BU = Bethesda units; CFHR1-3 = complement factor H–related 1-3; K = thousand; MRI = magnetic resonance imaging; TPE = therapeutic plasma exchange.
successful treatment with eculizumab. The second report described 3 of 4 patients with TMA due to ADAMTS13 inhibitors approximately 2.4 to 4 weeks after starting treatment with ticlopidine. Renal disease in these patients responded poorly to TPE, although hematologic parameters sometimes normalized. Patients had CFH mutations. Complement regulatory factor mutations represent another independent susceptibility factor, and their presence in addition to ADAMTS13 deficiency may be required for the onset of disease.

The lack of clinical recovery in the present patients prompted us to look for complement pathway defects. The aHUS mutation testing in the first and third patients showed CFHR1-CFHR3 homozygous deletion. Despite the fact that CFHR1-3 deletions are seen in approximately 33% of the healthy African population, homozygous deletions in CFHR1-CFHR3 have been reported to be strongly associated with the presence of CFH autoantibodies. However, only 4% to 14% of patients with aHUS may have CFH autoantibodies. In fact, CFH antibodies in patient 1 were within the reference range.

A random skin biopsy may be helpful in demonstrating the presence of extensive microvascular deposition of C5b-9, which supports the diagnosis of aHUS or a subset of TTP with concomitant complement dysregulation. Such depositions are not diagnostic of aHUS. However, in confirmed cases of aHUS, the presence of significant vascular C5b-9 deposition may predict clinical responsiveness to eculizumab.

The second patient has multiple genetic mutations (Table). The lack of response to traditional TTP treatment in patients 2 and 3 prompted us to look for complement dysregulation despite the absence of severe renal injury. Both patients

![FIGURE 2. A biopsy sample of the normal skin did not show any conspicuous light microscopic abnormalities (hematoxylin-eosin, x400). B, Although the routine hematoxylin-eosin–stained material was unremarkable, fairly striking deposits of C5b-9 were noted in the capillaries and venules of the skin, corroborating the diagnosis of atypical hemolytic uremic syndrome (C5b-9 diaminobenzidine, x400).](image)

| Mutation                      | Location       | Significance, frequency (1000 Genomes Project database) |
|-------------------------------|----------------|--------------------------------------------------------|
| Heterozygous missense variant | Exon 11 of CFI | Linked to aHUS, 0.0028                                  |
| (c.1246A>G, p.Lle416Leu)      |                |                                                        |
| (c.1135G>C, p.Val379Leu)      | Exon 7 of CFHR5 | Linked to aHUS, 0.005                                  |
| (c.3019G>T, p.Val1007Leu)     | Exon 19 of CFH | Linked to aHUS, 0.2553 in AA population               |
| (c.2669G>T, p.Ser890Ile)      | Exon 17 of CFH | Benign mutation, 0.1893 in AA population              |
| (c.3207T>C, p.Ser1069Ser)     | Exon 20 or CFH | Unknown significant, 0.0018                            |
| Intronic deletion             | Upstream of exon 4 of MCP/CD46 | Unknown significant, 0.0042 |
| (chr1:207932961, T A>T)       |                |                                                        |
| (c.40G>A, p.Gly14Ser)         | Exon 1 of THBD | Unknown significant, 0.0068                            |
| Nonsense variant              | Exon 10 of CFHR5 | Unknown significant, 0.01                           |
| (c.1704T>A, p.Cys568Stop)     |                |                                                        |
| Heterozygous polymorphism     | Intron in MCP/CD46 | Common in the AA population up to 44% |
| (IVS9-78 G>A)                 |                |                                                        |
| Heterozygous large deletion   | CFHR1-CFHR3    | Only homozygous deletion is strongly associated with CFH antibodies Found in up to 33% in the AA population |

AA = African American; aHUS = atypical hemolytic uremic syndrome; CF = complement factor; CFHR = complement factor H–related.
had an excellent response to eculizumab treatment. This finding is consistent with previous reports on the use of eculizumab for refractory TTP.\textsuperscript{7}

**CONCLUSION**

Herein we presented our experience in treating 3 African American patients with diagnostic features of iTTP and genetic mutations consistent with complement dysregulation. The simultaneous presence of inhibitor-related ADAMTS13 deficiency and complement dysregulation may blur the distinction between aHUS and TTP. Finally, response to treatment was facilitated by clearance of ADAMTS13 inhibitor levels before the institution of anticomplement therapy.

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**Abbreviations and Acronyms:** ADAMTS13 = disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aHUS = atypical hemolytic uremic syndrome; BU = Bethesda units; CF = complement factor; CFHR = complement factor H related; HUS = hemolytic uremic syndrome; iTTP = autoimmune thrombotic thrombocytopenic purpura; TMA = thrombotic microangiopathy; TPE = therapeutic plasma exchange; TTP = thrombotic thrombocytopenic purpura.

**Potential Competing Interests:** The authors report no competing interests.

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