Therapeutic Apheresis and Dialysis

Therapeutic Apheresis and Dialysis 2017
doi: 10.1111/1744-9987.12641

Atypical Hemolytic Uremic Syndrome: A Meta-Analysis of Case Reports Confirms the Prevalence of Genetic Mutations and the Shift of Treatment Regimens

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Abstract: Atypical hemolytic uremic syndrome (aHUS) is a rare life-threatening thrombotic microangiopathy (TMA) affecting multiple organ systems. Recently, aHUS has been shown to be associated with uncontrolled complement activation due to mutations in the alternative pathway of complement components paving the way for targeted drug therapy. By meta-analysis of case reports, we discuss the impact of new treatment strategies on the resolution time of aHUS symptoms and mortality, and the distribution of genetic mutations. A PubMed/Medline search was conducted for "atypical hemolytic uremic syndrome" case reports published between November 2005 and November 2015. R Version 3.2.2 was used to calculate descriptive statistics and perform univariate analyses. Wilcoxon rank-sum test was used to compare time to symptoms resolution, creatinine and platelet count normalization across the treatment and mutation carrier groups. A total of 259 aHUS patients were reported in 176 articles between 2005 and 2015. In the last 5-year period compared to the precedent, there was an increase in the number of aHUS cases reported (180 vs. 79 cases) and the use of eculizumab also increased (6.3% to 46.1%, $P < 0.000$), although plasma exchange usage did not change ($P = 0.281$). CFH antibodies were present in a significantly higher number of patients treated with plasma exchange therapy (19.1%, $P = 0.000$) while none of the non-plasma exchange therapy group had CFH antibodies. Most common mutation was CFH (50%, 69/139) followed by CFHR1 (35%, 30/85), MCP (22.8%, 23/101) and CFI (16.6%, 17/102). Time to symptoms resolution and serum creatinine or platelet count normalization were not significantly different between eculizumab and non-eculizumab group ($P = 0.166$, $P = 0.361$, $P = 0.834$), and between plasma exchange and non-plasma exchange group ($P = 0.150$, $P = 0.135$, $P = 0.784$). However, both eculizumab and plasma exchange groups had early platelet recovery (22 vs. 30 days and 25.5 vs. 32.5 days), faster creatinine normalization (27 vs. 30.5 days and 27 vs. 37 days) and interestingly, a longer period for symptoms resolution (45.5 vs. 21 days and 30 vs. 18.5 days) compared to non-eculizumab and non-plasma exchange groups. Mortality rate decreased with the use of eculizumab significantly ($P = 0.045$) compared to non-eculizumab group and there was no change in mortality rate with the use of plasma exchange therapy ($P = 0.760$) compared to non-plasma exchange group. Plasma exchange continues to be the initial treatment of choice for aHUS. Although significant reduction in the mortality rate was noted with the use of eculizumab, there were no differences in time to resolution of symptoms or serum creatinine or platelet normalization with the use of either eculizumab or plasma therapy. Atypical HUS is acute and life-threatening, so plasma exchange may be initiated before the confirmed diagnosis and in patients positive for CFH antibodies. Eculizumab therapy should be considered once aHUS is confirmed by genetic testing. **Key Words:** Alternative pathway of complement, Atypical hemolytic uremic syndrome, Eculizumab, Genetic mutations, Plasma exchange.

Received June 2017; revised August 2017; accepted September 2017.

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Atypical hemolytic uremic syndrome (aHUS) is a rare but life-threatening thrombotic microangiopathy (TMA) that affects multiple organ systems. Caused by uncontrolled activation of complement proteins, aHUS impacts renal function, leads to gastrointestinal disturbance, and also affects the central

nervous system (CNS). In addition to a wide distribution of effects, aHUS carries a mortality rate of 25% and progression to end-stage renal disease (ESRD) occurs in up to 50% of cases during the acute phase (1).

In the past, aHUS was a diagnosis of exclusion. Patients presenting without ADAMTS13 deficiency and without shiga-toxin *E. coli* infection, yet with symptoms of TMA were considered to have atypical disease. With a greater understanding of the complement system and its components, different mutations are now known to be involved in the pathophysiology of the disease. Moreover, many of these mutations have been specifically associated with the alternative pathway of complement, paving the way for corresponding targeted therapy. These include mutations in complement factor H (CFH), complement factor I (CFI), complement factor B (CFB), as well as membrane cofactor protein (MCP) which is encoded by CD46 gene (1q32). Recently, recessive mutations in DGKE (Diacylglycerol Kinase ε) have been shown to be associated with non-complement mediated aHUS (2). Anti-CFH antibodies are also implicated in the pathogenesis of aHUS particularly in patients with non-allelic homologous recombination of CFH related genes namely CFHR1/3 deletions.

Formerly, treatment was not disease-specific and was largely restricted to plasma exchange. Recently, a prospective phase 2 trial of eculizumab in 20 patients with aHUS demonstrated suppression of TMA in 80% of the patients at 26 weeks of therapy, with an increase in platelet count and eGFR observed in a separate arm of the same study (3). Eculizumab inhibits breakdown of compliment factor C5 to C5a and C5b by C5 convertase, thus preventing the formation of C5b-9 complexes (membrane attack complex, MAC) in the alternate complement pathway. C5a is a potent anaphylatoxin, and both C5a and C5b-9 are prothrombotic and proinflammatory, thereby eculizumab completely halts inappropriate systemic coagulation. It was approved in 2011 in the US and since then it has been used worldwide for the treatment of aHUS (4). Given that many aHUS patients have mutations in the alternative pathway of complement (APC) components, the specific approach of inhibition at the level of the terminal pathway of complement (TPC) is what makes eculizumab very effective. However, eculizumab use is limited by the cost of the treatment, which exceeds $300 000 (5).

The rarity of aHUS leads many clinicians to offer case reports. With the recent development of eculizumab as a highly effective treatment, many case studies now offer information on the efficacy of complement pathway inhibition in aHUS. This study seeks to assimilate knowledge from the case reports submitted over the 10-year period with the aim of using a meta-analysis approach to understand how new treatments have impacted the time to resolution of aHUS symptoms and the distribution of complement pathway genetic mutations that lead to aHUS.

METHODS

A PubMed/MEDLINE search for the terms "atypical hemolytic uremic syndrome" was performed, with filters applied for case reports and dates between November 2005 and November 2015. Case reports were sought because the rarity of aHUS prevents its extensive study; chronology was restricted to the 10-year period to minimize era effects.

Articles were included if the subject was diagnosed with aHUS consistent with lack of ADAMTS13 deficiency and negative *E. coli* O157: H7/stool culture. Studies that focused on genetics, as opposed to patient presentation or outcomes, were excluded. Selected articles were reviewed by three independent reviewers, and information regarding demographics, complement pathway genetics, disease, and outcomes were tabulated. Two primary endpoints were selected for review: mortality and a composite of time to lab value normalization or symptoms resolution. Patients whose lab values or symptoms did not resolve within 180 days of starting treatment were considered non-responders to therapy. For lab parameters, only the lowest or highest reported values were collected if the case report has more than one value for each variable. Patients in whom aHUS recurred were excluded from time to resolution analysis. Time to resolution of symptoms was defined as complete resolution of proteinuria, hematuria, hypertension and cardiac or neurological symptoms.

Statistical analysis

R version 3.2.2 was used to calculate descriptive statistics and perform univariate analyses across interval and treatment strata. Wilcoxon rank-sum tests were performed to compare time to symptom resolution, creatinine normalization, and platelet count normalization across treatment and mutation carrier groups. For a comparison of the means of the continuously measured characteristics across interval and treatment strata, a difference of means *t*-test was

conducted. The *P*-values for the other patient characteristics were calculated from a test of the equality of two binomial proportions across interval and treatment strata, which is equivalent to a χ^2 test for categorical data from a 2×2 table. However, if the observed frequency was less than five for one of the cells that comprised the 2×2 table, the *P*-values were calculated using a Fisher's exact test.

RESULTS

A PubMed search returned 695 results, of which 191 articles were selected for full text review (Fig. 1). Three articles were excluded for a lack of information regarding pre-specified variables, seven were excluded due to description of genetic characteristics rather than clinical characteristics, and five articles

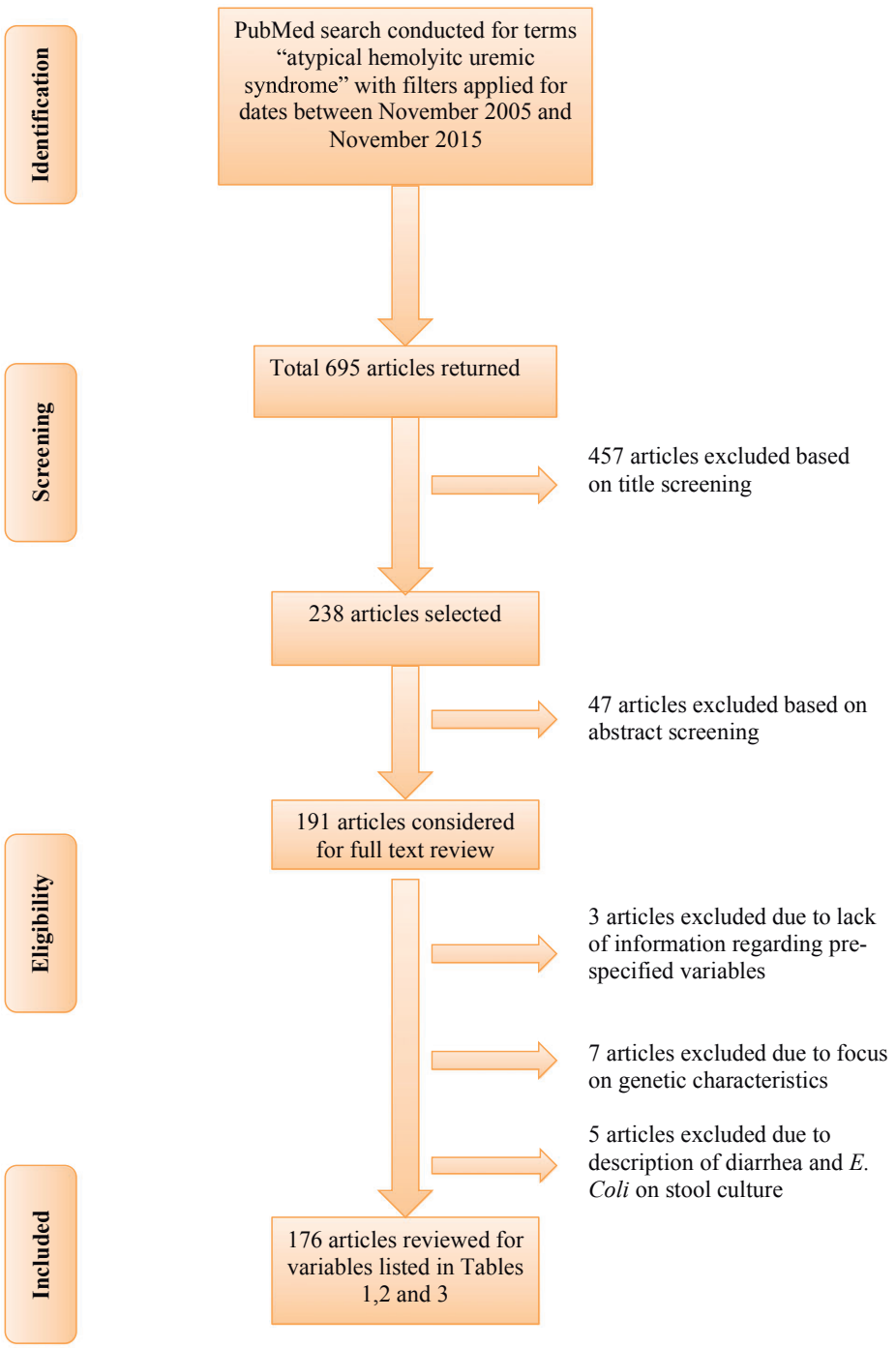

FIG. 1. Literature search flow chart.

TABLE 1. *Characteristics of aHUS patients, stratified by 5-year interval*

	2005–2010	2011–2015	*P*
No. of patients	79	180	
Age, years (mean (sd))	13.74 (14.88)	15.32 (17.42)	0.485
Sex = M % (*N*)	57 (45/79)	45 (81/180)	0.076
Pre-aHUS Conditions % (*N*)			
Dialysis dependence	3.8 (3/79)	16.7 (30/180)	0.004
CKD	1.3 (1/79)	6.7 (12/180)	0.067
ESRD	10.1 (8/79)	20 (36/180)	0.051
Prior transplantation	6.3 (5/79)	12.2 (22/180)	0.153
aHUS symptoms % (*N*)			
Hypertension	53.2 (42/79)	41.7 (75/180)	0.087
Proteinuria	48.1 (38/79)	56.7 (102/180)	0.203
Hematuria	21.5 (17/79)	31.1 (56/180)	0.114
Cardiac	10.1 (8/79)	15.6 (28/180)	0.245
Neurological	19 (15/79)	20.6 (37/180)	0.772
Treatment strategies % (*N*)			
Eculizumab	6.3 (5/79)	46.1 (83/180)	0.000
Plasma exchange	73.4 (58/79)	66.7 (120/180)	0.281
Dialysis	60.8 (48/79)	63.3 (114/180)	0.694
Laboratory values			
CFH Ab % (*N*)	10.1 (8/79)	18.9 (34/180)	0.078
C3 mg/dL (mean (sd))	54.84 (32.01)	40.84 (24.00)	0.000
C4 mg/dL (mean (sd))	35.12 (16.89)	35.35 (18.82)	0.926
Peak creatinine mg/dL (mean (sd))	5.17 (4.31)	4.14 (3.21)	0.036
Peak BUN mmol/L (mean (sd))	9.10 (2.45)	NA	
Lowest platelets $\times 10^3/\mu L$ (mean (sd))	54.81 (32.56)	49.85 (27.22)	0.208
aHUS Outcomes			
Time to resolve, days median {IQR}	34.00 {14.00, 130.00}	24.50 {12.50, 90.00}	0.401
Time for creatinine normalization median {IQR}	38.50 {20.00, 55.00}	26.00 {15.00, 46.00}	0.077
Time to platelet normalization median {IQR}	29.50 {12.50, 40.00}	25.00 {14.50, 40.00}	0.935
Mortality % (*N*)	7.6 (6/79)	6.1 (11/180)	0.657
Mutations			
CFH % (*N* positive/*N* tested)	60.9 (28/46)	44.1 (41/93)	0.063
Heterozygous	52.2 (24/46)	36.6 (34/93)	
Homozygous	8.7 (4/46)	7.5 (7/93)	
None	39.1 (18/46)	55.9 (52/93)	
CFI % (*N* positive/*N* tested)	28.6 (8/28)	12.2 (9/74)	0.047
Heterozygous	21.4 (6/28)	12.2 (9/74)	
Homozygous	7.1 (2/28)	0.0 (0/74)	
None	71.4 (20/28)	87.8 (65/74)	
CFHR1% (*N* positive/*N* tested)	36.4 (8/22)	34.9 (22/63)	0.903
Heterozygous	9.1 (2/22)	15.9 (10/63)	
Homozygous	27.3 (6/22)	19 (12/63)	
None	63.6 (14/22)	65.1 (41/63)	
MCP % (*N* positive/*N* tested)	43.8 (14/32)	13 (9/69)	0.001
Heterozygous	34.4 (11/32)	13 (9/69)	
Homozygous	9.4 (3/32)	0 (0/69)	
None	56.2 (18/32)	87 (60/69)	

Ab, antibody; aHUS, atypical hemolytic uremic syndrome; CFH, complement factor H; CFHR1, complement factor H-related protein 1; CFI, complement factor I; CKD, chronic kidney disease; ESRD, end-stage renal disease; IQR, interquartile range; M, male; MCP, membrane cofactor protein; n, number; sd, standard deviation.

were excluded due to description of diarrhea and *E. coli* on stool culture. The remaining 176 articles were reviewed for information regarding the variables listed in Tables 1–3. Descriptive statistics for all collected variables, across time intervals and treatment category are presented in Tables 1–3.

A total of 259 aHUS patients were reported in 176 articles during the 10-year period. There has been an increase in the number of aHUS patients reported (180 vs. 79 cases) as well an increase in the use of eculizumab from 6.3% to 46.1% ($P < 0.000$) during the last 5-year period compared to the precedent. The rates of plasma exchange and dialysis usage for aHUS did not change significantly during the same time periods ($P = 0.281$, $P = 0.694$). During the last 5-year period compared to the precedent, greater proportions of patients had chronic kidney disease (CKD), ESRD, transplantation and were dialysis-dependent before the diagnosis of aHUS; however, only ESRD and dialysis dependence were statistically significant ($P = 0.051$, $P = 0.004$). Mean C3 and serum

TABLE 2. *Characteristics of patients treated with eculizumab*

	No	Yes	*P*
No. patients	171	88	
Age, years (mean(sd))	12.6 (15.62)	19.1 (18.48)	0.003
Sex = M % (*N*)	50.9 (87/171)	34.1 (30/88)	0.010
Pre-aHUS conditions % (*N*)			
Dialysis dependence	7.6 (13/171)	20.45 (18/88)	0.003
CKD	2.9 (5/171)	9.1 (8/88)	0.031
ESRD	8.8 (15/171)	29.5 (26/88)	0.000
Prior transplantation	4.1 (7/171)	20.5 (18/88)	0.000
aHUS symptoms % (*N*)			
Hypertension	38 (65/171)	38.6 (34/88)	0.922
Hematuria	50.3 (86/171)	39.8 (35/88)	0.108
Proteinuria	25.1 (43/171)	15.9 (14/88)	0.089
Cardiac	8.8 (15/171)	13.6 (12/88)	0.225
Neurological	13.5 (23/171)	17 (15/88)	0.439
Treatment strategies % (*N*)			
Plasma exchange	68.4 (117/171)	69.3 (61/88)	0.883
Dialysis	62.5 (107/171)	62.5 (55/88)	0.991
Laboratory values			
CFH Ab % (*N*)	19.9 (34/171)	9.1 (8/88)	0.026
C 3 mg/dL (mean (sd))	46.34 (29.77)	45.54 (24.08)	0.828
C 4 mg/dL (mean (sd))	36.68 (16.40)	34.03 (19.93)	0.255
Peak creatinine mg/dL (mean (sd))	4.45 (3.34)	4.53 (4.28)	0.869
Peak BUN mmol/L (mean (sd))	9.10 (2.45)	NA	
Lowest platelets $\times 10^3/\mu L$ (mean (sd))	54.61 (30.56)	47.08 (25.89)	0.050
aHUS outcomes			
Time to resolve, days median {IQR}	21.00 {11.00, 90.00}	45.50 {15.50, 101.25}	0.166
Time to creatinine normalization median {IQR}	30.50 {15.25, 49.00}	27.00 {14.00, 44.00}	0.361
Time to platelet normalization median {IQR}	30.00 {13.25, 40.00}	22.00 {15.00, 39.75}	0.834
Mortality % (*N*)	8.8 (15/171)	2.3 (2/88)	0.045
Mutations			
CFH % (*N* positive/*N* tested)	50.6 (41/81)	48.3 (28/58)	0.785
Heterozygous	42 (34/81)	41.4 (24/58)	
Homozygous	8.6 (7/81)	6.9 (4/58)	
None	49.4 (40/81)	51.7 (30/58)	
CFI % (*N* positive/*N* tested)	16.7 (9/54)	16.7 (8/48)	1.000
Heterozygous	13 (7/54)	16.7 (8/48)	
Homozygous	3.7 (2/54)	0 (0/48)	
None	83.3 (45/54)	83.3 (40/48)	
CFHR 1% (*N* positive/*N* tested)	37.5 (15/40)	33.3 (15/45)	0.688
Heterozygous	17.5 (7/40)	11.1 (5/45)	
Homozygous	20 (8/40)	22.2 (10/45)	
None	62.5 (25/40)	66.7 (30/45)	
MCP % (*N* positive/*N* tested)	29.1 (16/55)	15.2 (7/46)	0.098
Heterozygous	23.6 (13/55)	15.2 (7/46)	
Homozygous	5.5 (3/55)	0 (0/46)	
None	70.9 (39/55)	84.8 (39/46)	

Ab, antibody; aHUS, atypical hemolytic uremic syndrome; CFH, complement factor H; CFHR1, complement factor H-related protein 1; CFI, complement factor I; CKD, chronic kidney disease; ESRD, end-stage renal disease; IQR, interquartile range; M, male; MCP, membrane cofactor protein; *N*, number; sd, standard deviation.

creatinine levels were observed to be low during the last 5-year period ($P = 0.000$, $P = 0.036$). Similarly, the number of patients with mutations was lower during the last 5-year period compared to the precedent with significantly decreased numbers noted among CFI ($P = 0.047$) and MCP ($P = 0.001$) mutations. However, there were no statistically significant differences in time to symptoms resolution or creatinine or platelet normalization between the two 5-year intervals ($P = 0.401$, $P = 0.077$, $P = 0.935$).

Patients treated with eculizumab were older, more likely to be females, more often dialysis-dependent, or had CKD or ESRD or prior transplantation. The average age of patients treated with eculizumab was 19.1 years compared to the non-eculizumab group who were 12.6 years. Lab parameters were not significantly different between the eculizumab and non-eculizumab groups, except for CFH antibodies and platelet count. CFH antibodies and mean platelet count were significantly lower in patients treated with eculizumab compared to non-eculizumab group

TABLE 3. *Characteristics of patients treated with plasma exchange*

	No	Yes	*P*
No. of patients	81	178	
Age, years (mean (sd))	14.49 (18.46)	15.47 (16.51)	0.671
Sex = M % (*N*)	49.4 (40/81)	46.6 (83/178)	0.681
Pre-aHUS conditions % (*N*)			
Dialysis dependence	6.2 (5/81)	15.2 (27/178)	0.041
CKD	2.5 (2/81)	5.6 (10/178)	0.351
ESRD	7.4 (6/81)	19.1 (34/178)	0.016
Prior transplantation	4.9 (4/81)	12.4 (22/178)	0.076
aHUS symptoms % (*N*)			
Hypertension	16 (13/81)	45.5 (81/178)	0.000
Proteinuria	28.4 (23/81)	53.4 (95/178)	0.000
Hematuria	22.2 (18/81)	20.8 (37/178)	0.793
Cardiac	4.9 (4/81)	9.6 (17/178)	0.325
Neurological	9.9 (8/81)	18 (32/178)	0.094
Treatment strategies % (*N*)			
Eculizumab	35.8 (29/81)	33.14 (59/178)	0.676
Dialysis	45.7 (37/81)	70.2 (125/178)	0.000
Laboratory values			
CFH Ab % (*N*)	0 (0/81)	19.1 (34/178)	0.000
C 3 mg/dL (mean (sd))	44.11 (27.65)	48.08 (28.17)	0.293
C 4 mg/dL (mean (sd))	36.52 (16.46)	34.54 (18.30)	0.408
Peak creatinine mg/dL (mean (sd))	2.87 (2.42)	5.32 (3.84)	0.000
Peak BUN mmol/L (mean (sd))	7.47 (NA)	9.64 (2.69)	
Lowest platelets $\times 10^3/\mu L$ (mean (sd))	48.11 (29.69)	53.56 (30.17)	0.179
aHUS outcomes			
Time to resolve, days (median {IQR})	18.50 {8.00, 60.00}	30.00 {14.00, 112.00}	0.150
Time to creatinine normalization (median {IQR})	37.00 {21.25, 49.00}	27.00 {13.00, 47.50}	0.135
Time to platelet normalization (median {IQR})	32.50 {13.75, 40.00}	25.50 {14.00, 39.25}	0.784
Mortality % (*N*)	3.7 (3/81)	5.6 (10/178)	0.760
Mutations			
CFH % (*N* positive/*N* tested)	37.9 (11/29)	52.7 (58/110)	0.156
Heterozygous	24.1 (7/29)	46.4 (51/110)	
Homozygous	13.8 (4/29)	6.4 (7/110)	
None	62.1 (18/29)	47.3 (52/110)	
CFI %(*N* positive/*N* tested)	0 (0/23)	21.5 (17/79)	0.011
Heterozygous	0 (0/23)	19 (15/79)	
Homozygous	0 (0/23)	2.5 (2/79)	
None	100 (23/23)	78.5 (62/79)	
CFHR 1%(*N* positive/*N* tested)	24 (6/25)	40 (24/60)	0.160
Heterozygous	16 (4/25)	13.3 (8/60)	
Homozygous	8 (2/25)	26.7 (16/60)	
None	76 (19/25)	60 (36/60)	
MCP % (*N* positive/*N* tested)	20 (5/25)	23.7 (18/76)	0.703
Heterozygous	20 (5/25)	19.7 (15/76)	
Homozygous	0 (0/25)	3.9 (3/76)	
None	80 (20/25)	76.3 (58/76)	

Ab, antibody; aHUS, atypical hemolytic uremic syndrome; CFH, complement factor H; CFHR1, complement factor H-related protein 1; CFI, complement factor I; CKD, chronic kidney disease; ESRD, end-stage renal disease; IQR, interquartile range; M, male; MCP, membrane cofactor protein; *N*, number; sd, standard deviation.

($P = 0.026$, $P = 0.050$). There was no statistically significant difference in mutations between the eculizumab and non-eculizumab group. Interestingly, in eculizumab group, MCP mutations were present in only 15.2% of patients who had genetic testing compared to 29.1% in the non-eculizumab group.

There were no significant differences in age or sex between plasma exchange therapy group and non-plasma exchange therapy group. However, the plasma exchange therapy group had a significantly higher number of people with hypertension and proteinuria ($P = 0.000$, $P = 0.000$), had ESRD and were dialysis dependent ($P = 0.016$, $P = 0.041$), and were overall more likely to be treated with dialysis ($P = 0.000$). CFH antibodies were present in a significantly higher number of patients treated with plasma exchange therapy (19.1%, $P = 0.000$) while none of the non-plasma exchange therapy group had CFH antibodies. Mean serum creatinine levels were observed to be higher in patients treated with plasma exchange compared with the non-plasma exchange group (5.32 mg/dL vs. 2.87 mg/dL, $P < 0.000$). Patients treated with plasma exchange therapy showed significant association with CFI

mutation ($P = 0.011$), and associations with CFH, CFHR1and MCP mutations were insignificant ($P = 0.156$, $P = 0.160$, $P = 0.703$), although they were more frequently associated.

Mortality rate decreased with the use of eculizumab significantly ($P = 0.045$) and there was no significant change in mortality rate with the use of plasma exchange therapy ($P = 0.760$) compared to non-eculizumab and non-plasma exchange groups, respectively. Furthermore, mortality rates did not change significantly during the last 5-year period where eculizumab use was noted to be high compared to the precedent 5-year period ($P = 0.657$). Time to symptoms resolution and serum creatinine or platelet count normalization were not statistically significant between the eculizumab and non-eculizumab group ($P = 0.166$, $P = 0.361$, $P = 0.834$), and between plasma exchange and non-plasma exchange group ($P = 0.150$, $P = 0.135$, $P = 0.784$). However, both the eculizumab and plasma exchange groups had early platelet recovery (22 vs. 30 days & 25.5 vs. 32.5 days), faster creatinine normalization (27 vs. 30.5 days and 27 vs. 37 days) and interestingly, longer period for symptoms resolution (45.5 vs. 21 days and 30 vs. 18.5 days) compared to non-eculizumab and non-plasma exchange groups.

The most common mutation noted was CFH followed by CFHR1, MCP and CFI. Of the patients ($N = 139$) who were screened for CFH genetic mutations, 50% (69/139) had mutation in the CFH gene with 15.9% (11/69) being homozygous and 84.1% (58/69) being heterozygous for the mutation (Table 4). CFH receptor 1 (CFHR1) mutations were observed to be less frequent (35%, 30/85) with homozygous mutation in 60% (18/30) and heterozygous mutation in 40% (12/30) of the patients. Of the patients ($N = 102$) who were screened for CFI mutations, only 16.6% (17/102) had CFI mutation with the majority being heterozygous (88.2%, 15/17) and the remaining were homozygous (11.8%, 2/17) for the mutation. MCP mutations were prevalent in 22.8% of patients tested (23/101) with majority of them being heterozygous (87%, 20/23) and remaining being homozygous (13%, 3/23) for the mutation.

Mortality and time to resolution of symptoms or normalization of lab values were stratified by mutation type and treatment (Table 5). Mortality was infrequent among treatment strata, but the frequency was greater in patients treated with plasma exchange therapy than with eculizumab.

DISCUSSION

The use of aggregate information from case studies is subject to potential biases. Only noteworthy cases are considered for analysis after rigorous screening by three independent reviewers. Still, given the exceeding rarity of aHUS, the sum of knowledge from these experiences may offer worthwhile lessons.

Regarding chronological trends, the first notable feature is an increase in the number of aHUS cases reported during the last 5-year period compared to the precedent (180 vs. 79 cases). This may be attributed to increasing rates of publication, or may

TABLE 4. *Distribution of genotypes across patients*

	Patients with no mutations	Patients with heterozygous mutations	Patients with homozygous mutations	Total number of patients with mutation
CFH Mutation	70	84.1% (58/69)	15.9% (11/69)	50% (69/139)
CFHR 1 Mutation	55	40% (12/30)	60% (18/30)	35% (30/85)
CFI Mutation	85	88.2% (15/17)	11.8% (2/17)	16.6% (17/102)
MCP Mutation	78	87% (20/23)	13% (3/23)	22.8% (23/101)

CFH, complement factor H; CFHR1, complement factor H-related protein 1; CFI, complement factor I; MCP, membrane cofactor protein.

TABLE 5. *Outcomes across genotypes*

		CFH Mutation	CFHR 1 Mutation	CFI Mutation	MCP Mutation
Eculizumab	Mortality (*N* (%))	0 (0%)	0 (0%)	0 (0%)	1 (25%)
	Time to resolve (days (sd))	72.2 (81.6)	51.0 (49.3)	35 (19.8)	16.33 (16.2)
	Total patients	25	13	8	4
Plasma exchange	Mortality (*N* (%))	4 (7.7%)	2 (9.1%)	0 (0%)	1 (6.3%)
	Time to resolve (days (sd))	80.6 (86.9)	105.9 (96.8)	123.3 (153.6)	45.5 (55.13)
	Total	52	22	12	16

CFH, complement factor H; CFHR1, complement factor H-related protein 1; CFI, complement factor I; MCP, membrane cofactor protein; *N*, number; sd, standard deviation.

reflect growing interest in aHUS and complement disorder treatment with the terminal complement inhibitor, eculizumab following its approval by FDA and EMA (European Medicines Agency) in 2011. Plasma exchange is usually considered as the first line treatment for aHUS. Our analysis confirmed that the plasma exchange remained as the most used initial treatment modality for aHUS with no significant difference in its use in the last 5-year period compared to the precedent (66.7% vs. 73.4%, $P = 0.281$). Plasma exchange therapy for aHUS consists of removing pathologic mutated factors, antibodies, immune complexes and cytokines to restore endothelial function and prevent platelet aggregation (6,7). Effective aHUS treatment with plasma exchange is generally defined as increase in platelet count and cessation of hemolysis (7). Of the 259 cases we analyzed, plasma exchange was used in 68.7% (178/259) of the patients. CFH antibodies were present in 19.1% (34/178) of patients who had plasma exchange therapy compared to none in non-plasma exchange group, reflecting plasma exchange to be effective and the main treatment modality in antibody positive cases. Furthermore, expensive eculizumab therapy was used in only 9.1% of CFH antibody positive cases.

The majority of patients who underwent plasma exchange had dialysis (125/178, 70.2%, $P = 0.000$), and had significantly higher levels of mean serum creatinine (5.32 vs. 2.87 mg/dL, $P = 0.000$), and a higher number of patients with hypertension and proteinuria (45.5 vs. 16%, $P = 0.000$ and 53.4 vs. 28.4%, $P = 0.000$). These associations may be attributed to the fact that plasma exchange is initiated as an acute treatment when aHUS is suspected and dialysis treatment yet to be started. Furthermore, there was no significant difference in the time to resolution of symptoms, normalization of serum creatinine or platelets between plasma exchange and non-plasma exchange groups. Similarly, no significant mortality difference was observed between the two groups, although there was a slight increase in the number of deaths in the plasma exchange group, which may be attributed to the severity of the aHUS in this sub-group. Interestingly, genetic mutations were found to be higher in patients who had plasma exchange compared to the non-plasma exchange group, although it was not statistically significant except for CFI (52.7% vs. 37.9% and $P = 0.156$ for CFH, 21.5% vs. 0% and $P = 0.011$ for CFI, 40% vs. 24% and $P = 0.160$ for CFHR1, 23.7% vs. 20% and $P = 0.703$ for MCP).

Many studies reported the use of eculizumab as a treatment modality to replace plasma exchange or to treat plasma exchange resistant aHUS (8–10). During the last 5-year period, there has been a significant increase in the use of eculizumab for aHUS treatment. This may be partly attributed to its approval by FDA and EMA (European Medicines Agency) in 2011. Eculizumab, a monoclonal antibody, binds to C5 to prevent its cleavage into C5a and C5b effector molecules; this results in blockade of the pro-inflammatory, pro-thrombotic and lytic functions of complement (11). Several studies have described the use of eculizumab in the treatment of aHUS. Ohanian et al. described the long-term use of eculizumab to maintain and improve renal function (12). Other case studies, such as Mache et al., Chatelet et al., and Zuber et al., discuss the use of a maintenance dose of eculizumab to prevent aHUS relapse, which was described as recurrent hemolysis and deterioration of renal function (13–15). Eculizumab has also been recently used in the treatment of gemcitabine induced HUS (16). Fakhouri et al. described a randomized clinical trial in which eculizumab was used to treat adult patients with severe aHUS and was found to benefit approximately 73% of the patients following 26 weeks of treatment (17). Once remission is achieved, discontinuation of eculizumab is encouraged owing to its side-effects. It has been shown to be associated with maintenance of remission in a majority of cases; however, relapses were reported in selected cases (18). Our analysis showed more than half of the cases (51.4%, 88/171) had eculizumab treatment and a significant proportion of the eculizumab treatment group had pre-morbidities such as CKD, prior transplantation, ESRD and dialysis dependence ($P = 0.031$, $P = 0.000$, $P = 0.000$, $P = 0.003$) compared to the non-eculizumab group. Interestingly, lower numbers of CFH, CFHR1 and MCP mutations were observed in eculizumab group compared to non-eculizumab group, although, there were no significant differences observed between the two groups (48.3% vs. 50.6% and $P = 0.785$ for CFH, 16.7% vs. 16.7% and $P = 1$ for CFI, 33.3% vs. 37.5% and $P = 0.688$ for CFHR1 and 15.2% vs. 29.1% and $P = 0.098$ for MCP). Although patients who were treated with eculizumab had a higher number of premorbid conditions, the mortality rate with the use of eculizumab was significantly low (2.3% compared to 8.8%, $P = 0.045$) compared to the non-eculizumab group.

A study by Bresin et al. involving 794 patients with aHUS revealed mutations in CFH, CFI, C3, CFB and CD46 in 41% of patients (19). A majority of the aHUS cases analyzed reported mutations occurring in key complement components and regulators such as

CFH, CFHR1, MCP, and CFI. These mutations results in unregulated activation of complement cascade predominantly in glomerular capillary bed causing thrombotic microangiopathy and renal failure—a hallmark of aHUS (20). CFH gene mutation results in functional disruption of the CFH protein, an important fluid-phase regulator of alternative pathway causing thrombotic microangiopathy seen in aHUS (21). More than half of the cases linked to the CFH mutation were related to the homozygous deletion of four nucleotides located at the end of CFH (22). Of the 139 cases analyzed for CFH gene mutation, 41.7% (58/139) of cases were heterozygous and 7.9% of cases were homozygous (11/139). CFI mutations are less common in aHUS and were reported in 16% (17/102) of patients analyzed for CFI abnormality. Mutations in MCP gene accounted for 22.8% of aHUS patients analyzed for MCP abnormality. CFI is a serine protease that downregulates both alternative and classical complement pathways in the presence of its cofactors such as MCP proteins. MCP serves as a cofactor for factor I and plays a role to protect host cells from complement attack (21). A study by Caprioli et al. involving patients with MCP mutations demonstrated reduced protein expression and better prognosis with almost 90% remission rates compared to CFH-mutated patients (1). Similarly, Noris et al. studied 273 patients and reported MCP mutations to be associated with the best prognosis compared to other mutations (23). Bresin et al. studied the above genetics of aHUS in relation to the risk of recurrence after renal transplant and found that patients with mutations in either CFI or CFH had a higher risk of recurrence compared MCP gene mutations (24). Comparatively, CFH mutation was shown to be associated with poor outcome and increased recurrence (25). Our analysis revealed that there was no significant difference in MCP mutations between the plasma exchange group and non-plasma exchange group. However, there was a higher number of CFH mutations noted in patients who had plasma exchange compared to non-plasma exchange group, although not statistically significant (52.7% vs. 37.9%, $P = 0.156$). The time to resolution of symptoms was longer in this group. Though plasma exchange is routinely used as a treatment option for aHUS, genotype might contribute to varying outcomes.

Strengths and limitations of this study

Unlike many other literature reviews, our work is the result of aggregated case studies. The case study is considered as the least significant source of evidence due to its sample size and may have potential for bias due to reporting of only selected information. Yet, with a lack of studies and rarity of a disease like aHUS, it is possible that aggregate results of case studies represent the "best available evidence". Currently, high-quality evidence is largely limited to studies whose populations are less than the number of patients studied here (3,26,27). To minimize the possibility of bias as a result of using case studies, each of the selected patient characteristics was carefully chosen before retrieving any information. Further, data were retrieved by several people, to help minimize individual reviewer bias.

Atypical HUS was not an established medical term a decade ago according to published literature (28). The definition of aHUS has evolved during recent years to indicate more specific etiology that includes anti-CFH antibodies and genetic mutations in the components of complement system and their regulatory genes (29). Hence, the key term "atypical hemolytic uremic syndrome" might indicate different diseases in the case reports published during the last 5-year period from the precedent. The second limitation of this study was lack of proper reporting about symptoms resolution and normalization of laboratory values especially renal functions in some of the case studies, which would have affected the results. It is known that antibody-mediated aHUS (anti-CFH antibody) responds well to plasma exchange therapy, but usually, aHUS is acute and life-threatening, so plasma exchange may be selected in the majority of cases before the diagnosis and initiation of eculizumab therapy. This may be the third limitation of our study to verify the contribution of eculizumab.

Given the heterogeneity inherent in many different case reports, only univariate methods were used. For those patients who were treated with eculizumab, it appears that their pre-aHUS state was of lower health than those patients not treated with eculizumab—patients were older, and more likely to have ESRD. Similarly, creatinine values were significantly higher for patients treated with plasma exchange, as was the proportion of patients presenting with hypertension and proteinuria. These associations may be attributed to the fact that plasma exchange is initiated as acute treatment when aHUS is suspected, and eculizumab therapy is considered at a later stage after confirmed diagnosis, perhaps after several episodes of aHUS resulting in the development of ESRD. Furthermore, low levels of serum creatinine in eculizumab therapy group may be attributed to the fact that these patients are more likely to be on hemodialysis compared to plasma exchange group where it was initiated as acute treatment.

CONCLUSIONS

Despite the limitations associated with meta-analysis of case studies, this study provides enough atypical hemolytic uremic syndrome cases to draw conclusions cautiously. Reports of aHUS cases and use of eculizumab significantly increased during the last 5-year period which may be due to its approval by FDA and EMA in 2011 or may reflect growing interest in aHUS resulting in increased rates of publication. Plasma exchange remained as the most used initial treatment modality for aHUS as the majority of analyzed patients and most CFH antibody positive cases in our study underwent plasma exchange. There was a decrease in CFH, CFI and MCP mutations in the last 5-year period compared to the precedent for unknown reasons. Although significant reduction in the mortality rate was observed with the use of eculizumab, there were no differences in time to resolution of symptoms or serum creatinine or platelet normalization with the use of eculizumab or plasma therapy. Atypical HUS is acute and life-threatening, so plasma exchange may be initiated before the confirmed diagnosis and in patients positive for CFH antibodies. Eculizumab therapy should be considered once aHUS is confirmed by genetic testing. Atypical HUS was a disease of exclusion, but now has been shown to be associated with mutations. More registry programs and clinical trials are warranted to better understand the impact of various treatment modalities on aHUS outcome.

Acknowledgments: We would like to thank Mr. Mustafa S Ascha for his assistance in literature search, data collection and manuscript preparation. The authors thank Dr. Ravkiran Khurana for her assistance in data collection.

Conflict of Interest: Authors have no conflict of interest to declare.

Financial Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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