A Multicenter Study Evaluating the Discontinuation of Eculizumab Therapy in Children with Atypical Hemolytic Uremic Syndrome

Saeed AlZabali 1,*, Sawsan AlBatati 1, Khawla Rahim 1, Hassan Faqeehi 1, Abubaker Osman 1, Abdulaziz Bamhraz 2, Mohammed A. Saleh 3, Jameela A. Kari 4, Majed Aloufi 5, Loai Eid 6, Haydar Nasser 7, Abubaker Imam 8, Entesar AlHammadi 9, Omar Alkandari 10, Mohammed Al Riyami 11, Sidharth Sethi 12, Christoph Lich 13, Khalid A. Alhasan 14,15 and Abdulkarim AlAnazi 1

Abstract: Background: Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening thrombotic microangiopathy (TMA), which has been treated successfully with eculizumab. The optimal duration of eculizumab in treating patients with aHUS remains poorly defined. Methods: We conducted a multicenter retrospective study in the Arabian Gulf region for children of less than 18 years of age who were diagnosed with aHUS and who discontinued eculizumab. The most common reason for the discontinuation of eculizumab in treating patients with aHUS remains poorly defined. Methods: We conducted a multicenter retrospective study in the Arabian Gulf region for children of less than 18 years of age who were diagnosed with aHUS and who discontinued eculizumab between June 2013 and June 2021 to assess the rate and risk factors of aHUS recurrence. Results: We analyzed 28 patients with a clinical diagnosis of aHUS who had discontinued eculizumab. The most common reason for the discontinuation of eculizumab was renal and hematological remission (71.4%), followed by negative genetic testing (28.6%). During a median follow-up period of 24 months after discontinuation, 8 patients (28.5%) experienced HUS relapse. The risk factors of recurrence were positive genetic mutations (p = 0.020). On the other hand, there was no significant relationship between the relapse and age of presentation, the need for acute dialysis, the duration of eculizumab therapy before discontinuation, or the timing of eculizumab after the presentation. Regarding the renal outcomes after discontinuation, 23 patients were in remission with normal renal function, while 4 patients had chronic kidney disease (CKD) (three of them had pre-existing chronic kidney disease (CKD) before discontinuation, and one case developed a new CKD after discontinuation) and one patient underwent transplantation. Conclusions: The discontinuation of eculizumab in patients with aHUS is not without risk; it can result in HUS recurrence. Eculizumab discontinuation can be performed with close monitoring of the patients. It is essential to assess risk the factors for relapse before eculizumab discontinuation, in particular in children with a positive complement variant and any degree of residual CKD, as HUS relapse may lead to additional loss of kidney function. Resuming eculizumab promptly after relapse is effective in most patients.
Keywords: atypical hemolytic uremic syndrome; discontinuation of eculizumab; thrombotic; microangiopathy; HUS relapse

1. Introduction

Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening form of TMA that is caused by uncontrolled complement activation in the alternative pathway (AP) [1,2]. It is characterized by microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and evidence of endothelial cell destruction, which result in ischemic end-organ damage [1,3,4]. Genetic defects in complement-related factors or acquired autoantibodies to complement factors have been found in 50 to 60% of patients [2]. The initial therapy for aHUS is supportive, focusing on managing acute kidney injury and systemic complications. Plasma exchange and eculizumab, a complement inhibitor, offer specific forms of treatment [5]. Eculizumab, the first approved treatment for patients with aHUS, is a humanized monoclonal complement inhibitor that binds to C5, preventing its cleavage to C5a and C5b and the subsequent activation of the complement terminal pathway (TP) and membrane attack complex (MAC) formation [6,7]. Eculizumab has profoundly changed the outcomes for patients with aHUS compared with other traditional options, such as plasma therapy [7]. The necessary duration of eculizumab treatment in aHUS is unknown [8]. The European Commission’s Summary of Product Characteristics and the Food and Drug Administration’s Full Prescribing Information, both released in 2014, do not take a stance on the treatment duration [9]. The reasons for discontinuing eculizumab therapy reported in the literature are the protection of patients from the risk of the potentially devastating side effects of meningococcal infection, the requirement for repeated infusions, and the high cost of treatment [1,10]. There is a substantial risk of relapse when stopping eculizumab in patients with aHUS. Selected groups of patients appear to discontinue eculizumab and remain relapse-free for several years [11]. This study aims to report the rate and risk factors for the relapse and renal outcome of the patients after discontinuation.

2. Materials and Methods

A retrospective multicenter study for the period from June 2013 to June 2021 was carried out across five countries in the Arabian Gulf region, in the Kingdom of Saudi Arabia (5 centers) and the United Arab Emirates (2 centers), and in one center in each of Oman, Qatar, and Kuwait. Patients were included in the study if they were under 18 years of age, were diagnosed with aHUS (first episode or relapse), received at least two months of eculizumab treatment before discontinuation, and had at least six months of follow-up after the initial eculizumab discontinuation unless HUS recurred sooner. Patients on chronic dialysis (defined as three months’ of dialysis) at the time of discontinuation and patients with other causes of thrombotic microangiopathy, including infection with Shiga-toxin-producing bacteria; a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) deficiency; and conditions associated with secondary HUS (malignancy, malignant hypertension, the use of drugs, autoimmune diseases, infections, cobalamin C deficiency, transplantation, and pregnancy) were excluded. Primary aHUS was defined [10] by at least two of the following criteria: (i) thrombocytopenia (platelet count < 150,000 9/L); (ii) mechanical hemolytic anemia (Hb < 10 g/dL, lactate dehydrogenase (LDH) serum level > upper limit of normal, low haptoglobin, and the presence of schistocytes on blood smear); (iii) acute kidney injury (serum creatinine upper limit of normal for age or increased >25% compared to baseline).

The diagnosis of relapse was based on the criteria of the initial diagnosis of aHUS. Remission (at the time of eculizumab discontinuation) was defined as lactate dehydrogenase (LDH) < 1.5 upper limit of normal, the absence of hemolysis (hemoglobin started to increase or returned to baseline), platelet count > 150,000 9/L, and serum creatinine decreased by 25% compared with baseline. The data collected were on the demographic
information, clinical history, laboratory investigations (hemoglobin, platelets, reticulocytes count, blood smear, LDH, haptoglobin, serum creatinine, C3, C4, complement factor H, I, B, CH50/AHA50 level, blood culture, urine culture, urine for protein/creatinine ratio), renal biopsy findings, genetic studies, the timing of eculizumab (after presentation), requirement of acute dialysis before eculizumab therapy, the duration of eculizumab before discontinuation, reason for the discontinuation of eculizumab therapy, follow-up time after discontinuation, laboratory results at the time of the discontinuation of eculizumab and thereafter, estimated glomerular filtration rate (eGFR) at the last visit after discontinuation, relapse after discontinuation, possible trigger for new HUS recurrence episode, timing of starting eculizumab after relapse, trial of discontinuation after treatment relapse episode, and outcome after discontinuation. The CKD was defined based on the KDIGO definition 2012.

The data were saved in a password-protected Excel folder, with only the investigators having access to the data. The patient information remained confidential, with all identifiers removed from the stored data. IRB approval was obtained from all contributing centers.

Statistical Analysis

All data analyses were performed using Statistical Packages for Software Sciences (SPSS) version 21 (Armonk, New York, NY, USA, IBM Corporation). The descriptive statistics are presented using counts and proportions (%). A p-value cutoff point of 0.05 at 95% CI was used to determine the statistical significance. The research team performed the statistical association and calculation process; special software may have been used during the analysis. To compare patients with vs. without relapse, continuous variables were tested using the Wilcoxon signed-rank test, and categorical variables were tested using Fisher’s exact test. A Kaplan–Meier analysis of relapse-free survival was performed.

3. Results

3.1. Patient Cohort

Twenty-eight patients were included in this study. The mean age of the patients was 3.47 years (SD ± 2.96), with the majority being males (70.4%). Eleven patients (39.2%) had a positive family history of the disease, while twenty (71.4%) patients had positive consanguinity. The mean values for the presentation of hemoglobin, platelets, and LDH were 8.1 g/dL, 84.5 10⁹ /L, and 2106 U/L, respectively. Almost 50% of patients received eculizumab during the first four days of presentation, while 35.7% of patients received eculizumab after four days. Acute dialysis was required in 64.3% of the children before the initiation of eculizumab. After receiving eculizumab, 82.1% (n = 23) showed complete remission. The median duration of eculizumab treatment before discontinuation was 12 months. Seven (25%) patients had received eculizumab therapy for 12 to 24 months, while 6 (21.4%) patients had received eculizumab for 24–48 months and the same percentage had received eculizumab for >3–6 months. The remaining patients had variable durations (Table 1).
Table 1. Summary of the baseline general characteristics of the patients and laboratory tests at the first presentation of the patients.

| Pt # | Sex | Age at Onset | Familial Consanguinity | Family history of aHUS | Hemoglobin on Presentation (g/dL) | Platelets on Presentation (10⁹/L) | LDH on Presentation (U/L) | Creatinine on Presentation (µM/L) | GFR mL/min/1.73 m² on Presentation | C3 Level on Presentation | Hypertensive Timing of Eculizumab (After Presentation) | Acute Dialysis on Presentation | Duration While on Eculizumab (Months) before Discontinuation |
|------|-----|--------------|------------------------|------------------------|----------------------------------|----------------------------------|-------------------------|-------------------------------|----------------------------------|-----------------------------|-----------------------------------------------|---------------------------|--------------------------------------------------|
| 1    | M   | 54 m         | No                     | Negative               | 7.00                             | 86,000                           | 3539                    | 174                           | 45                               | Normal                      | No                                    | 1 day                      | Yes                                                     | 2                          |
| 2    | F   | 6 m          | No                     | Sibling                | 7.60                             | 86,000                           | 3616                    | 278                           | 35                               | Normal                      | Yes                                   | 2 days                     | Yes                                                     | 3                          |
| 3    | M   | 6 y          | Yes                    | Cousin                 | 10.00                            | 73,000                           | 2008                    | 191                           | 40                               | Low                         | Yes                                   | 1 day                      | No                                                     | 3                          |
| 4    | M   | 6 y          | Yes                    | Cousin                 | 8.90                             | 50,000                           | 3770                    | 282                           | 20                               | Low                         | Yes                                   | 1 day                      | Yes                                                     | 6                          |
| 5    | M   | 4 y          | Yes                    | Cousin                 | 7.80                             | 35,000                           | 2182                    | 169                           | 25                               | Low                         | No                                    | 2 days                     | Yes                                                     | 2                          |
| 6    | F   | 6 y          | Yes                    | Negative               | 10.00                            | 71,000                           | 1780                    | 38                            | 100                              | Normal                      | No                                    | 1 day                      | No                                                     | 10                         |
| 7    | F   | 20 m         | Yes                    | Negative               | 8.00                             | 41,000                           | 2991                    | 202                           | 35                               | Normal                      | Yes                                   | 30 days                    | Yes                                                     | 12                         |
| 8    | F   | 2 y          | Yes                    | Negative               | 7.80                             | 77,000                           | 2356                    | 275                           | 20                               | Normal                      | No                                    | 2 days                     | Yes                                                     | 6                          |
| 9    | M   | 14 m         | No                     | Negative               | 5.00                             | 12,000                           | 3531                    | 137                           | 22                               | Low                         | No                                    | 1 day                      | Yes                                                     | 19                         |
| 10   | M   | 9 m          | Yes                    | Negative               | 8.10                             | 167,000                          | 951                     | 97                            | 95                               | Normal                      | Yes                                   | NA                        | No                                                     | 2                          |
| 11   | F   | 3 y          | No                     | Negative               | 10.60                            | 44,000                           | 2242                    | 164                           | 27                               | Low                         | No                                    | 3 days                     | No                                                     | 12                         |
| 12   | M   | 3 m          | Yes                    | Cousin                 | 5.20                             | 53,000                           | 1840                    | 300                           | 18                               | Normal                      | Yes                                   | 9 years                    | Yes                                                     | 15                         |
| 13   | F   | 3 m          | Yes                    | Cousin                 | 8.60                             | 92,000                           | 1760                    | 300                           | 19                               | Normal                      | Yes                                   | 5 years                    | Yes                                                     | 15                         |
| 14   | M   | 10 m         | Yes                    | Negative               | 7.50                             | 200,000                          | 400                     | 252                           | 20                               | Normal                      | Yes                                   | 5 days                     | No                                                     | 7                          |
| 15   | M   | 5 y          | Yes                    | Negative               | 12.00                            | 282,000                          | 379                     | 160                           | 25                               | Low                         | Yes                                   | 3 months                   | Yes                                                     | 12                         |
| 16   | M   | 5 y          | No                     | Negative               | 9.10                             | 96,000                           | 391                     | 205                           | 28                               | Low                         | Yes                                   | 7 days                     | No                                                     | 11                         |
| 17   | M   | 7 y          | Yes                    | Negative               | 8.20                             | 13,000                           | 4202                    | 195                           | 24                               | Low                         | Yes                                   | 7 days                     | Yes                                                     | 12                         |
| 18   | M   | 2 y          | No                     | Negative               | 8.70                             | 6000                             | 4484                    | 393                           | 20                               | Normal                      | No                                    | 5 days                     | Yes                                                     | 5                          |
| 19   | M   | 4 y          | Yes                    | Negative               | 9.30                             | 121,000                          | 631                     | 291                           | 16                               | Low                         | No                                    | 1 week                     | No                                                     | 4                          |
| 20   | M   | 1 m          | Yes                    | Sibling                | 6.20                             | 32,000                           | 1118                    | 709                           | 12                               | Low                         | Yes                                   | 7 days                     | No                                                     | 12                         |
| 21   | M   | 9 y          | Yes                    | Negative               | 5.80                             | 81,000                           | 776                     | 511                           | 16                               | Low                         | No                                    | 3 days                     | Yes                                                     | 20                         |
| 22   | M   | 4 y          | Yes                    | Negative               | 8.10                             | 59,000                           | 3274                    | 904                           | 17                               | Low                         | Yes                                   | 72 h                      | Yes                                                     | 48                         |
| 23   | M   | 9 m          | Yes                    | Sibling                | 7.50                             | 47,000                           | 1023                    | 80                            | 16                               | Normal                      | Yes                                   | 7 days                     | Yes                                                     | 51                         |
| 24   | M   | 20 m         | Yes                    | Cousin                 | 7.70                             | 321,000                          | 433                     | 165                           | 24                               | Low                         | Yes                                   | 4 months                   | No                                                     | 48                         |
| 25   | M   | 10 m         | Yes                    | Sibling                | 6.10                             | 70,000                           | 519                     | 101                           | 29                               | Low                         | Yes                                   | 4 days                     | Yes                                                     | 36                         |
| 26   | M   | 4 y          | No                     | Negative               | 8.60                             | 23,000                           | 1896                    | 182                           | 20                               | Low                         | Yes                                   | 4 days                     | Yes                                                     | 4                          |
| 27   | F   | 2 m          | Yes                    | Negative               | 7.70                             | 104,000                          | 4904                    | 80                            | 23                               | Normal                      | No                                    | 59 days                    | Yes                                                     | 5                          |

M: male; F: female.
The most common reason for the discontinuation of eculizumab therapy was that the patient was in remission (71.4%), followed by negative genetic testing (28.6%). Other reasons included family preference (10.7%), DGKE mutation (10.7%), and loss to follow-up (7.4%). The median follow-up after discontinuation was 24 months.

3.2. Gene Mutation and Relapse

Of the 28 patients, sixteen (57.2%) showed positive genetic testing results. Eleven (39.2%) patients showed no genetic mutation, and one (3.6%) had no genetic testing done (Table 2).

Table 2. Genetic variants of the patients.

| Pt # | Genetic Testing Result | Variant Nomenclature | Zygosity | Variant Classification | Relapse | Outcome |
|------|------------------------|----------------------|----------|------------------------|---------|---------|
| 1    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 2    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 3    | MCP/CD46, CFHR1, CFHR3 mutation | c.736T > A; p.Phe246Ile | Homozygous | VOUS               | No      | In remission |
| 4    | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | No      | In remission |
| 5    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 6    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 7    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 8    | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 9    | Not done               | NA                   | NA       | NA                     | No      | In remission |
| 10   | CFH mutation           | c.2195C > T; p.Thr732Met | Heterozygous | VOUS       | No      | In remission |
| 11   | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | No      | In remission |
| 12   | CFHR1-CFHR3 mutation  | c.350_351dup;p.Glu118Thrfs17 | Likely pathogenic | VOUS       | Yes    | In remission |
| 13   | DGKE mutation          | No                   | NA       | NA                     | No      | CKD      |
| 14   | DGKE mutation          | No                   | NA       | NA                     | No      | CKD      |
| 15   | CFH mutation           | c.3545G > T; p.Arg181Met | Heterozygous | VOUS       | Yes    | Transplant |
| 16   | CFHR1, CFHR3 mutation | Whole genes deletion | Heterozygous | VOUS       | No      | In remission |
| 17   | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 18   | MCP/CD46 mutation      | c.608T > C; p.Ile203Thr | Homozygous | VOUS       | Yes    | In remission |
| 19   | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | No      | In remission |
| 20   | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | No      | In remission |
| 21   | DGKE                   | c.413 G > A (p.Cys138Thr) | Homozygous | Likely pathogenic | Yes    | CKD      |
| 22   | No mutation            | NA                   | NA       | NA                     | No      | In remission |
| 23   | MCP/CD46               | c.736T > A (p.Phe246Ile) | Homozygous | VOUS               | No      | In remission |
| 24   | C3 mutation            | c.3326 T > G (p.Leu1109Arg) | Homozygous | Likely pathogenic | Yes    | In remission |
| 25   | CFI                    | c.1246A > C (p.Ile416Leu) | Homozygous | Pathogenic         | Yes    | In remission |
| 26   | No mutation            | NA                   | NA       | NA                     | Yes    | In remission |
| 27   | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | Yes    | In remission |
| 28   | CFHR1, CFHR3 mutation | Whole genes deletion | Homozygous | VOUS               | No      | In remission |

CFH: complement factor H; CFB: complement factor B; CFI: complement factor I; MCP: membrane cofactor protein; CFHR1, CFHR3: complement factor H-related 1, -3; VOUS: variant of uncertain significance; NA: not available; DGKE: diacylglycerol kinase epsilon; CKD: chronic kidney disease.
CFHR1 and CFHR3 mutations were reported in 5 patients (17.8%). Only one of them relapsed after 12 months of discontinuation. Three patients (10.7%) had DGKE mutations, and one of them relapsed after 12 months of discontinuation. Two patients (7.1%) had combined mutations, one of them had combined MCP/CD46 and CFHR1, CFHR3 mutations, the other patient had CFI and CFB mutations; both relapsed after 3 months of discontinuation. MCP/CD46 mutations were reported in two patients (7.1%); one experienced a relapse after 15 months of eculizumab discontinuation. CFH mutations were reported in two patients (7.1%); one had a relapse after 2 months of discontinuation. The only patient with a C3 mutation (3.5%) relapsed after 3 months of discontinuation. Eleven patients (39.2%) had no detected mutations; one of them had a relapse after 2 months of discontinuation. One patient underwent no genetic testing without a history of relapse (Figure 1).

3.3. Relapse of aHUS

Eight (28.5%) patients experienced HUS relapse after the discontinuation of eculizumab. Seven of them (25%) had a detected gene mutation. During a median follow-up period of 24 months after discontinuation, most of the patients (75%) restarted eculizumab within 24–48 h. The median time to HUS relapse after discontinuation was 3 months. We observed that 5 patients experienced a relapse within 1 to 3 months, with upper respiratory tract infection (URTI) being the most common triggering factor. None of the patients required dialysis at the time of relapse. All of the patient’s hematological and renal functions returned to pre-cessation values following the resumption of eculizumab after HUS relapse. The prevalence of no relapse was significantly more common among those with no mutation ($p = 0.020$). On the other hand, there was no significant relationship between relapse and the need for acute dialysis ($p$ value 0.669), the duration of eculizumab therapy before discontinuation ($p$ value 0.230), the timing of eculizumab after the presentation ($p$ value 0.252), the GFR ($p$ value 0.43), the protein/creatinine ratio ($p$ value 0.700), or the C3 level at the time of discontinuation ($p$ value 0.678) (Table 3).

There were no significant differences in the rates of relapse according to the types of gene mutations ($p > 0.05$) (Table 4).

Additionally, there were no significant differences in the relapse times between males and females (mean relapse time for males was 7.84 months, compared to 8.50 months for females). The overall mean time to relapse was 8.04 months. According to the log-rank, it was revealed that $X^2 = 0.143, p = 0.705$ (Figure 2).

Figure 1. Durations of eculizumab (months) before and after relapse according to the type of gene mutation.
Table 3. Characteristics of patients who relapsed and patients who did not relapse before eculizumab discontinuation.

| Factor                              | With Relapse | No Relapse | p-Value |
|-------------------------------------|--------------|------------|---------|
|                                     | (n = 8)      | (n = 20)   |         |
| Age at onset HUS                    |              |            |         |
| • <1 year                           | 04 (50.0%)   | 04 (20.0%) | 0.505   |
| • 1–3 years                         | 02 (25.0%)   | 04 (20.0%) |         |
| • 4–5 years                         | 01 (12.5%)   | 05 (25.0%) |         |
| • 6–8 years                         | 01 (12.5%)   | 03 (15.0%) |         |
| • 9–12 years                        | 0            | 04 (20.0%) |         |
| Creatinine at presentation, micromole/L (mean ± SD)| 231.0 ± 200.4 | 251.6 ± 133.1 | 0.753 |
| C3 Level at the presentation        |              |            |         |
| • Low                               | 05 (62.5%)   | 09 (45.0%) | 0.678   |
| • Normal                            | 03 (37.5%)   | 11 (55.0%) |         |
| Timing of eculizumab after the presentation |              |            |         |
| • <2 days                           | 0            | 07 (35.0%) |         |
| • 2–4 days                          | 03 (37.5%)   | 04 (20.0%) |         |
| • >4 days                           | 04 (50.0%)   | 06 (30.0%) |         |
| • Unknown                           | 01 (12.5%)   | 03 (15.0%) |         |
| Acute dialysis before eculizumab    |              |            |         |
| • Yes                               | 06 (75.0%)   | 12 (60.0%) | 0.669   |
| • No                                | 02 (25.0%)   | 08 (40.0%) |         |
| Duration while on eculizumab (months) |            |            |         |
| • 1–3                               | 0            | 04 (20.0%) |         |
| • >3–6                              | 01 (12.5%)   | 05 (25.0%) |         |
| • >6–12                             | 02 (25.0%)   | 02 (10.0%) |         |
| • >12–24                            | 01 (12.5%)   | 06 (30.0%) | 0.23    |
| • >24–48                            | 03 (37.5%)   | 3 (5.0%)   |         |
| • >48–60                            | 01 (12.5%)   | 0          |         |
| Complement gene variants            |              |            |         |
| • CFH mutation                      | 01 (12.5%)   | 01 (05.0%) | 0.497   |
| • MCP/CD46 mutation                 | 01 (12.5%)   | 01 (05.0%) | 0.497   |
| • C3 mutation                       | 01 (12.5%)   | 0          | 0.286   |
| • DGKE mutation                     | 01 (12.5%)   | 02 (10.0%) | 1.000   |
| • CFHR1, CFHR3 mutation             | 01 (12.5%)   | 04 (20.0%) | 1.000   |
| • MCP/CD46 and CFHR1, CFHR3 mutation| 01 (12.5%)   | 01 (05.0%) | 0.497   |
| • CFI, CFB mutation                 | 01 (12.5%)   | 0          | 0.286   |
| • No mutation                       | 01 (12.5%)   | 10 (50.0%) | 0.888   |
| Creatinine at time discontinuation of eculizumab | 63.7 ± 76.6 | 43.2 ± 19.9 | 0.267 |
| GFR at time of discontinuation of eculizumab (mean ± SD) | 101.9 ± 32.1 | 109.1 ± 16.5 | 0.700 |
| Protein/creatinine ratio (g/mmol) at time of discontinuation of eculizumab (median (min–max)) (mean ± SD) | 0.45 (0.01–1.90) | 0.50 (0.01–15) | 0.439 |
| Durations of eculizumab treatment in months, median (min–max) | 12.0 (4.0–51.0) | 10.5 (2.0–48.0) | 0.165 |
| GFR (mL/min/1.73 m²) at the last visit after discontinuation |            |            |         |
| • ≥90                               | 06 (75.0%)   | 16 (80.0%) |         |
| • 60–89                             | 01 (12.5%)   | 02 (10.0%) |         |
| • 30–59                             | 01 (12.5%)   | 02 (10.0%) |         |

Table 4. Rates of relapse according to gene mutations.

| Gene Mutations                  | Rate of Relapse | Rate of Relapse | Rate of Relapse | Rate of Relapse | Rate of Relapse |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | One N (%)       | Two N (%)       | Three N (%)     | p-Value         |                 |
| CFH mutation                   | 01 (20.0%)      | 0               | 01 (100%)       | 1.000           |                 |
| MCP/CD46 mutation              | 0               | 0               | 01 (100%)       | 1.000           |                 |
| C3 mutation                    | 01 (20.0%)      | 0               | 0               | 1.000           |                 |
| DGKE mutation                  | 0               | 01 (50.0%)      | 0               | 0.375           |                 |
| CFHR1, CFHR3 mutation          | 01 (20.0%)      | 0               | 0               | 1.000           |                 |
| MCP/CD46 and CFHR1, CFHR3 mutation | 01 (20.0%) | 0               | 0               | 1.000           |                 |
| CFI, CFB mutation              | 0               | 01 (50.0%)      | 0               | 0.375           |                 |
| No mutation                    | 01 (20.0%)      | 0               | 0               | 1.000           |                 |

Note: *p-value calculated using Fischer’s exact test.
Durations of eculizumab treatment in months, median (min–max) 12.0 (4.0–51.0) 10.5 (2.0–48.0) 0.165

GFR (mL/min/1.73 m²) at the last visit after discontinuation

- ≥90: 06 (75.0%) 16 (80.0%)
- 60–89: 01 (12.5%) 02 (10.0%)
- 30–59: 01 (12.5%) 02 (10.0%)

There were no significant differences in the rates of relapse according to the types of gene mutations (p > 0.05) (Table 4).

Table 4. Rates of relapse according to gene mutations.

| Gene Mutations                          | Rate of Relapse | p-Value |
|----------------------------------------|-----------------|---------|
| CFH mutation                           | 01 (20.0%)      | 1.000   |
| MCP/CD46 mutation                      | 0               | 1.000   |
| C3 mutation                            | 01 (20.0%)      | 1.000   |
| DGKE mutation                          | 0               | 0.375   |
| CFHR1, CFHR3 mutation                  | 0               | 1.000   |
| MCP/CD46 and CFHR1, CFHR3 mutation     | 0               | 1.000   |
| CFI, CFB mutation                      | 0               | 0.375   |
| No mutation                            | 0               | 1.000   |

Note: * p-value calculated using Fischer’s exact test.

Additionally, there were no significant differences in the relapse times between males and females (mean relapse time for males was 7.84 months, compared to 8.50 months for females). The overall mean time to relapse was 8.04 months. According to the log-rank, it was revealed that X² = 0.143, p = 0.705 (Figure 2).

Figure 2. Survival plot for the times to relapse between males and females.

3.4. Outcomes

Following discontinuation, all patients who relapsed achieved remission after resuming eculizumab, and none of them progressed to end-stage renal disease (ESRD) (Table 5).

Table 5. The individual characteristics and outcomes of 8 patients who experienced aHUS relapse.

| Patient  | Age at Initial Eculizumab Treatment (Years) | Mutation                      | Reason for Discontinuation Time | Time of Eculizumab before Discontinuation | Time of Relapse | Restarted Eculizumab | Outcome | Duration of Follow Up Months |
|----------|--------------------------------------------|-------------------------------|---------------------------------|------------------------------------------|-----------------|----------------------|----------|-------------------------|
| Patient 12 | 6 years                                   | MCP/CD46, CFHR1, CFHR3 mutation | Family preference                | 12 months                                | 3 months        | Yes                   | Stable (in remission) | 15          |
| Patient 15 | 6 years                                   | CFH mutation                  | Stable patient and complete remission | 7 months                                | 3 months        | Yes                   | Transplanted         | 16          |
| Patient 18 | 7 years                                   | MCP/CD46 mutation             | Stable patient and complete remission | 12 months                               | 15 months       | Yes                   | Stable (in remission) | 48          |
| Patient 21 | 14 months                                 | DGKE mutation                 | DKGE mutation                   | 12 months                                | 12 months       | Yes                   | CKD                  | 60          |
| Patient 24 | 3 months                                   | C3 mutation                   | Genetic testing showed DKGE mutation | 51 months                               | 3 months        | Yes                   | Stable (in remission) | 30          |
| Patient 25 | 3 months                                   | CFI, CFB mutation             | Genetic testing showed DKGE mutation | 48 months                               | 3 months        | Yes                   | Stable (in remission) | 17          |
| Patient 26 | 2 years                                    | No mutation                   | Insurance coverage              | 36 months                                | 7 weeks         | Yes                   | Stable (in remission) | 24          |
| Patient 27 | 9 months                                   | CFHR1, CFHR3 mutation         | Stable patient and complete remission | 4 months                                | 14 months       | Yes                   | Stable (in remission) | 60          |

Five patients showed HUS recurrence episodes once, and 2 patients were subjected to another trial of discontinuation after the relapse episodes were treated. Regarding the renal outcomes, 23 patients were in remission with normal renal function (including 12 (52%) patients who underwent a positive genetic study, and 10 (43.7%) patients with no detected gene mutation), 4 patients had chronic kidney disease (CKD) (three patients had pre-existing chronic kidney disease (CKD) before discontinuation, and one had a DKGE mutation and developed a new CKD after discontinuation), while one patient underwent transplantation

4. Discussion

Our study describes one of the largest pediatric aHUS populations. The majority of previously published aHUS studies included both adult and pediatric patients and focused mainly on eculizumab therapy rather than its discontinuation with the consideration of
its association with gene mutations at the same time. Eculizumab is a well-tolerated and effective treatment for patients with aHUS [12–14]. It has been reported that eculizumab discontinuation is possible and safe in children with aHUS [10]. Several factors reported in the literature should be considered, as they might be associated with the risk of disease progression after discontinuation, such as the age at first presentation, previous TMA manifestations, the prevalence of extrarenal manifestations, and the post-transplantation setting [13]. We did not find in our study an association between the previous factors and risks for disease progression after eculizumab discontinuation.

The reasons for treatment discontinuation include both medical and economical concerns as well as patient requests [13]. In our study, the most common reason for the discontinuation of eculizumab therapy was that patients experienced remission and their conditions were stable or they were negative for genetic mutations.

Sixteen (57.2%) patients in this study showed positive genetic testing results, possibly reflected in the high consanguinity percentage (71.4%). The risk of aHUS relapse following eculizumab discontinuation is mainly determined by the presence or absence of gene mutations [10]. Similarly, in our study, seven (87.5%) of the 8 relapsed patients had a detected gene mutation, while patients with no relapse were more likely to have no genetic mutation. Some reports suggest a potential relationship between the type of complement mutation identified and the risk of subsequent clinical manifestations of HUS [13]. Patients with CFH and MCP/CD46 variant mutations appear to be at higher risk of recurrence [1,13–17]. Patients with CFH or THBD/CD141 mutations had the earliest onset of aHUS and the highest mortality rates, whereas patients with MCP/CD46 mutations were associated with the least severe outcomes [16], but there was no significant relationship that could be identified between the type of mutation and the risk of HUS relapse in our study, which may be related to the differences in the background genetics of our population.

However, the time to recurrence was longer in this study (median of 3 months) than in the study by Ardissino et al. [1] (<6 weeks) and equal to that in the study by Macia et al. [13], but it was shorter than that in the study by Ariceta et al. [15] (median of 5 months). The current literature reports that the rates of HUS recurrence after eculizumab discontinuation are between 22% and 31% [1,10,13,15,18], while in our study, eight patients (28.5%) experienced HUS relapse after discontinuation, making the identification of at-risk patients essential. Contrary to other studies, where patients experiencing HUS recurrence had a shorter duration of treatment [10,13,18], the durations of eculizumab therapy in our study were similar between patients who did and did not suffer HUS recurrence.

When HUS occurs after discontinuation, restarting eculizumab can prevent further HUS manifestations. Vilalta et al. [17] reported HUS manifesting eight weeks after missing a single eculizumab dose. The rates of eculizumab discontinuation and re-initiation have also been reported for 296 patients receiving eculizumab in the global aHUS Registry. In patients aged <18 years, 28 (24%) discontinued treatment, of whom 7 (25%) restarted eculizumab treatment [19]. Similarly, in our study, of all eight patients who relapsed, eculizumab was restarted within 24–48 h in the majority of them. Most of the patients who relapsed achieved remission after resuming eculizumab. However, it has been reported that the restart of eculizumab could not prevent the deterioration of renal function, HUS recurrence, or subsequent ESKD [14]. We observed that 5 patients had a relapse within 1–3 months, with upper respiratory tract infection being the most common triggering factor for a new recurrence. It remains a debate between researchers about the safety and feasibility of the discontinuation of eculizumab therapy [17,20]. In a selected group of patients, a trial of gradual discontinuation by lengthening the dosing interval of eculizumab seems to be a reasonable and safe alternative to the indefinite continuation of the drug [15,18]. In this study, eleven of our patients underwent eculizumab dose spacing prior to complete discontinuation. We emphasize the importance of the previously suggested recommendation by other studies that in the case of discontinuation, patient education regarding early HUS symptoms should be ensured, along with regular urine dipsticks to monitor hematuria and proteinuria. Additionally, if possible, regular blood tests to monitor hemoglobin, platelet,
serum creatinine, and LDH levels are crucial. If HUS recurrence is suspected, eculizumab should be rapidly reinitiated [1,11,21,22].

The outcome after discontinuation in this study was fairly good, with the majority of children remaining in remission with normal kidney function, which is better than a previous report by Ariceta et al. [17], as 8.0% of their patients progressed to ESKD. This study had limitations that should be noted, including the retrospective nature of the study and the varying observation intervals. However, we have reported on the whole pediatric aHUS population in the Arabian Gulf region spanning over 9 years, and to the best of our knowledge this is one of the largest studies in a pediatric population.

5. Conclusions

Our study supports the possibility of discontinuing eculizumab treatment in children with aHUS, but this decision may depend on identified gene mutations. The majority of children in our cohort remained in remission after the discontinuation of eculizumab.

A full assessment of the risk factors before the decision to discontinue eculizumab is essential, with parents being involved in the decision. Resuming eculizumab immediately after relapse is crucial and effective in most patients. In this study, although relapses happened, resuming eculizumab therapy was effective in controlling the disease.

Author Contributions: S.A. (Saeed AlZabali), A.A., K.R., H.F., S.A. (Sawsan AlBatati), A.O., A.B. and K.A.A. designed the study. S.A. (Saeed AlZabali), A.O., K.A.A., A.I., H.N., E.A., L.E., M.A.R., O.A. and M.A. performed the data collection. S.A. (Saeed AlZabali), A.O. drafted the manuscript. A.A., K.A.A., J.A.K., S.S., C.L. and M.A.S., reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by the Institutional Review Board of King Fahad Medical City, Riyadh, Saudi Arabia (IRB number: 20-722, in 9 January 2021). The study design was also approved by the Ethical Committees of all other centers involved in the study, which waived the need to obtain informed consent, as retrospective data collection was performed.

Informed Consent Statement: Written informed consent from the participants’ legal guardian or next of kin was not required to participate in this study, in accordance with the national legislation and the institutional requirements.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank the Research Center at King Fahad Medical City for their valuable support in conducting this study.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Ardissino, G.; Testa, S.; Possenti, I.; Tel, F.; Paglialonga, F.; Salardi, S.; Tedeschi, S.; Belingheri, M.; Cugno, M. Discontinuation of Eculizumab Maintenance Treatment for Atypical Hemolytic Uremic Syndrome: A Report of 10 Cases. Am. J. Kidney Dis. 2014, 64, 633–637. [CrossRef] [PubMed]
2. Yoshida, Y.; Kato, H.; Ikeda, Y.; Nangaku, M. Pathogenesis of Atypical Hemolytic Uremic Syndrome. J. Atheroscler. Thromb. 2019, 26, 99–110. [CrossRef] [PubMed]
3. Wijnsma, K.L.; Duineveld, C.; Volokhina, E.B.; Heuvel, L.P.V.D.; Kar, N.C.A.J.V.D.; Wetzels, J.F.M. Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic uremic syndrome. Nephrol. Dial. Transplant. 2017, 33, 635–645. [CrossRef] [PubMed]
4. Lee, H.; Kang, E.; Kang, H.G.; Kim, Y.H.; Kim, J.S.; Kim, H.-J.; Moon, K.C.; Ban, T.H.; Oh, S.W.; Jo, S.K.; et al. Consensus regarding diagnosis and management of atypical hemolytic uremic syndrome. Korean J. Intern. Med. 2020, 35, 25–40. [CrossRef]
5. Raina, R.; Krishnappa, V.; Blaha, T.; Kann, T.; Hein, W.; Burke, L.; Bagga, A. Atypical Hemolytic-Uremic Syndrome: An Update on Pathophysiology, Diagnosis, and Treatment. Ther. Apher. Dial. 2018, 23, 4–21. [CrossRef]
6. Menne, J.; Delmas, Y.; Fakhouri, F.; Licht, C.; Lommelé, Á.; Minetti, E.E.; Provôt, F.; Rondeau, E.; Sheerin, N.S.; Wang, J.; et al. Outcomes in patients with atypical hemolytic uremic syndrome treated with eculizumab in a long-term observational study. BMC Nephrol. 2019, 20, 125. [CrossRef]
7. Siedlecki, A.M.; Isbel, N.; Walle, J.V.; Eggleston, J.J.; Cohen, D.J.; Licht, C.; Frémeaux-Bacchi, V.; Ariceta, G.; Ardissino, G.; Fakhouri, F.; et al. Eculizumab Use for Kidney Transplantation in Patients with a Diagnosis of Atypical Hemolytic Uremic Syndrome. *Kidney Int. Rep.* 2018, 4, 434–446. [CrossRef]

8. Merrill, S.A.; Brittingham, Z.D.; Yuan, X.; Moliterno, A.R.; Sperati, C.J.; Brodsky, R.A. Eculizumab cessation in atypical hemolytic uremic syndrome. *Blood* 2017, 130, 368–372. [CrossRef]

9. Loirat, C.; HUS International; Fakhouri, F.; Ariceta, G.; Besbas, N.; Bitzan, M.; Bjerre, A.; Coppo, R.; Emma, F.; Johnson, S.; et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. *Pediatr. Nephrol.* 2015, 31, 15–39. [CrossRef]

10. Fakhouri, F.; Fila, M.; Hummel, A.; Ribes, D.; Sellier-Leclerc, A.-L.; Ville, S.; Pouteil-Noble, C.; Coindre, J.-P.; Le Quintrec, M.; Rondeau, E.; et al. Eculizumab discontinuation in children and adults with atypical hemolytic-uremic syndrome: A prospective multicenter study. *Blood* 2021, 137, 2438–2449. [CrossRef]

11. Olson, S.R.; Lu, E.; Sulpizio, E.; Shatzel, J.J.; Rueda, J.F.; Deloughery, T.G. When to stop Eculizumab in Complement-Mediated Thrombotic Microangiopathies. *Am. J. Nephrol.* 2018, 48, 96–107. [CrossRef] [PubMed]

12. Bamhraz, A.A.; Rahim, K.A.; Faqeehi, H.Y.; Alanazi, A. Improved Renal Recovery with Eculizumab Therapy among Children with High Prevalence of Mutation-Associated Atypical Hemolytic Uremic Syndrome: A Retrospective Cohort Study. *Kidney Blood Press. Res.* 2020, 45, 939–954. [CrossRef]

13. Macia, M.; Moreno, F.D.A.; Dutt, T.; Fehrman, I.; Hadaya, K.; Gasteiger, C.; Heyne, N. Current evidence on the discontinuation of eculizumab in patients with atypical haemolytic uraemic syndrome. *Clin. Kidney J.* 2016, 10, 310–319. [CrossRef] [PubMed]

14. Alzabli, S.M.; Al Anazi, A.; Rahman, M.A.U.; Rahim, K.A. Complete Renal Recovery Following Delayed Therapy with Eculizumab in Atypical Hemolytic Uremic Syndrome. *Asian J. Pediatr. Nephrol.* 2019, 2, 25–30. Available online: www.ajpn-online.org (accessed on 15 July 2018).

15. Fakhouri, F.; Fila, M.; Provôt, F.; Delmas, Y.; Barbet, C.; Châtelet, V.; Rafat, C.; Cailliez, M.; Hogan, J.; Servais, A.; et al. Pathogenic Variants in Complement Genes and Risk of Atypical Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. *Clin. J. Am. Soc. Nephrol.* 2016, 12, 50–59. [CrossRef] [PubMed]

16. Noris, M.; Caprioli, J.; Bresin, E.; Mozzali, C.; Pianetti, G.; Gamba, S.; Daina, E.; Ferilli, C.; Casteletti, F.; Sorosina, A.; et al. Relative Role of Genetic Complement Abnormalities in Sporadic and Familial aHUS and Their Impact on Clinical Phenotype. *Clin. J. Am. Soc. Nephrol.* 2010, 5, 1844–1859. [CrossRef]

17. Ariceta, G.; Fakhouri, F.; Sartz, L.; Miller, B.; Nikolaou, V.; Cohen, D.; Siedlecki, A.M.; Ardissino, G. Eculizumab discontinuation in atypical haemolytic uraemic syndrome: TMA recurrence risk and renal outcomes. *Clin. Kidney J.* 2021, 14, 2075–2084. [CrossRef]

18. Khedraki, R.; Noor, Z.; Rick, J. The Most Expensive Drug in the World: To Continue or Discontinue, That Is the Question. *Fed. Pract.* 2016, 33, 22.

19. Rodrigues, E.; Barrios, C.; Soler, M.J. Should eculizumab be discontinued in patients with atypical hemolytic uraemic syndrome? *Clin. Kidney J.* 2017, 10, 320–322. [CrossRef]

20. Vilalta, R.; Lara, E.; Madrid, A.; Chocron, S.; Muñoz, M.; Casquero, A.; Nieto, J. Long-term eculizumab improves clinical outcomes in atypical hemolytic uremic syndrome. *Pediatr. Nephrol.* 2012, 27, 2323–2326. [CrossRef]

21. Licht, C.; Ardissino, G.; Ariceta, G. Characteristics of 521 adult and pediatric patients in the Global aHUS Registry. In Proceedings of the American Society of Nephrology (ASN) Kidney Week 2014 Annual Meeting, Philadelphia, PA, USA, 11–16 November 2014.

22. Sheerin, N.S.; Kavanagh, D.; Goodship, T.H.J.; Johnson, S. A national specialized service in England for atypical haemolytic uraemic syndrome—the first year’s experience. *QJM* 2016, 109, 27–33. [CrossRef] [PubMed]