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[QUERY TO AUTHOR: title and abstract rewritten by Editorial Office – not subject to change]

Atypical and secondary haemolytic uremic syndromes have distinct presentation and no common genetic risk factors.

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Running headline: Secondary HUS.

Word count:

Abstract: 247.

Text: 4240.

Number of tables: 5.

Number of figures: 1.

Number of references: 38.

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Sources of support: This study was supported by research grants from La Société Francophone de Néphrologie et de Dialyse (to FF) (unrestricted funding by Alexion Pharmaceuticals who was not involved at any stage in the study design, analysis of the data and writing of the manuscript), the Programme Hospitalier de Recherche Clinique (grant AOM08198) (to VFB), the EU FP7 program 2012-305608 (EURenOmics) (to VFB), the Fondation du rein (FRM, Prix 2012 FDR) (to VFB) and the Association pour l'Information et la Recherche dans les maladies Rénales Génétiques (AIRG France).

Abstract:

Secondary haemolytic uremic syndrome (HUS) is a heterogeneous group of thrombotic

microangiopathies associated with various underlying conditions. Whether it belongs to the spectrum of complement-mediated HUS remains controversial. We analysed the presentation, outcome, and frequency of complement gene rare variants in a cohort of 110 patients with secondary HUS attributed to drugs (29%), autoimmune diseases (24%), infections (17%), malignancies (10%), glomerulopathies (9%), extra-renal organ transplantation (8%), and pancreatitis (3%). The frequency of complement gene rare variants was similar in patients with secondary HUS (5%) and in healthy individuals (6% and 8% in French and European controls, respectively). At diagnosis, 40% of patients required dialysis and 18% had neurological manifestations. 50% of patients received plasmatherapy and 35% were treated with eculizumab. Haematological and complete renal remission was achieved in 80% and 24% of patients, respectively. Thirty-nine percent of patients progressed to chronic kidney disease (stages 3-4) and an additional 37% reached end-stage renal disease. Eleven percent of patients died, most often from complications of the underlying cause of HUS. Only one patient experienced an HUS relapse. Patients treated with eculizumab presented with more severe HUS and were more likely to require dialysis at the time of diagnosis as compared to patients not treated with eculizumab. Rates of haematological remission, chronic kidney disease (stages 3-4), and end-stage renal disease were similar in the two groups. Secondary HUS is an acute nonrelapsing form of HUS, not related to complement dysregulation. The efficacy of eculizumab in this setting is not yet established.

Key words: haemolytic uremic syndrome, thrombotic microangiopathy, complement, eculizumab.

Introduction:

The term hemolytic uremic syndrome (HUS) encompasses several forms of thrombotic microangiopathy affecting predominantly the kidney: shiga toxin-producing *Escherichia coli*. (STEC)-HUS, cobalamin C-defect HUS, Diacyl Glycerol Kinase epsilon-HUS, atypical HUS due to a dysregulation of the complement alternative pathway, and HUS associated with coexisting diseases and conditions (malignancy, drugs, transplantation, systemic diseases and infections) [1], usually termed "secondary HUS". These various forms of hemolytic uremic syndrome (HUS) have distinct mechanisms of initial endothelial cell injury but a common final phenotype of activated and prothrombotic endothelial cell [1]. Several classifications of HUS have been previously proposed, each carrying caveats [2-4], and the terminology of HUS is still evolving.

The group of secondary HUS is heterogeneous particularly in terms of mechanisms of endothelial cell injury [1]. It has also to date attracted a more limited number of studies as compared to atypical HUS or STEC-HUS and data regarding clinical presentation, response to treatments and outcome of secondary HUS, generated through large cohorts and registries, are lacking.

Moreover, the identification of the dysregulation of the complement alternative pathway as a major risk factor for atypical HUS [5, 6], and the clinical availability of the first complement inhibitor eculizumab [7-10] have transformed the approach to all types of HUS. Currently, one of the most debated questions is whether, beyond atypical HUS, complement dysregulation/overactivation is involved in the pathogenesis of other types of HUS. This is particularly true for the group of secondary HUS for which natural history and therapeutic options remain ill-defined. Thus, we analyzed the presentation and frequency of complement gene variants in a large cohort of secondary HUS included in the nationwide French HUS registry.

Results

Presentation at diagnosis

One hundred and ten patients with secondary HUS diagnosed between 1999 and 2017 were included in the study. Thirty (27%) patients were included before 2010 and 80 (73%) between 2011 and 2017. Mean age was 44 years (2-80). At diagnosis, mean serum creatinine was 3.9 mg/dL (0.5-25) and 45 (40%) of patients required dialysis. Mean platelet count was 94 G/L (10-450) and 11 (10%) patients had normal platelet count at presentation. Thirty-one (28%) patients presented with extra-renal manifestations, mainly (n=20, 65%) neurological involvement (confusion, seizure, paresthesia). A kidney biopsy was performed in 51 (46%) patients and disclosed signs of active TMA in all patients. Main clinical and biological characteristics of these patients are shown in Tables 1 and 2 and in Supplemental Figure S1 and Table S1.

Diseases/conditions associated to secondary HUS

Secondary HUS in our series was associated to drugs (n=32, 29%), autoimmune diseases (n=26, 24%), bacterial and viral infections (n=18, 17%), malignancies (n=11, 10%), glomerulopathies (n=10, 9%), extra-renal organ transplantation (n=9, 8%), and pancreatitis (n= 4, 3%) (Table 1 and Supplemental Table S1).

Complement work-up.

C3 serum level measured during HUS active phase was decreased in 17 (15%) patients, of whom nine had a systemic lupus erythematous-associated HUS. Eight patients had a low C3 and C4 plasma levels as a result of an activation of the classical pathway and 9 had only a low C3 level suggestive of an activation of the alternative pathway.

Six (5%) patients had rare variants with MAF<0.1% in one of the 6 tested complement genes. These variants are located in CFH (n=3), CFI (n=1) and THBD (n=2) genes (Tables 3 and 4). Out of the six

rare variants, two are classified as pathogenic, the remaining four variants being classified as of undetermined significance. Characteristics of the 6 patients with complement gene variants are shown in Table 4. One patient had anti-FH antibodies with moderate titre (< 1000 arbitrary unit) and did not carry the homozygous CFHR1-3 deletion. None had a family history suggestive of HUS.

The frequency of complement gene rare variants in secondary HUS patients did not differ from the one found in healthy donors (5 % (n=6/110) vs 6% (=5/80) and 8% (n=42/503) in French and European controls, respectively) (p=1/0.4) (Table 3 and Supplemental Tables S2, S3 and S4). Altogether, the frequency of rare variants per gene was not significantly different between secondary HUS patients and controls. A rare (MAF <0.1%) *pathogenic* complement gene variant was identified in 2/111 (2%) secondary HUS patients versus 0/80 (0%) French controls (p=0.5) and 3/503 (1%) European controls (p=0.2). In contrast, the frequency of complement gene rare variants significantly differed between aHUS and secondary HUS patients (Table 3).

The frequency of at-risk polymorphisms tgtgt (CFH) and of CFHR1-3 deletion did not significantly differ between secondary HUS patients and healthy donors (Supplemental Tables S2 and S4). The frequency of at-risk polymorphisms ggaac (MCP) was increased in secondary HUS patients compared to French healthy donors (17% vs 6%; p=0.03)

Treatment

Fifty-five (50%) patients underwent plasma exchanges, ten (9%) received plasma infusions, thirty-one (27%) received corticosteroids and thirteen (27%) cyclophosphamide (used as a treatment of the underlying cause in all cases) (Table 1). Eculizumab was used in 38 (35%) cases. Treatment with eculizumab (regimen similar to the one used in atypical HUS [10]) was started as a second-line therapy in 28 (26%) patients after plasma exchanges and treatment of the underlying cause failed to induce HUS remission. All eculizumab-treated patients received anti-meningococcal vaccines and antibioprophylaxis with methylpenicillin.

Outcome

Eight (7%) patients were lost to follow-up. Mean follow-up was 21 months (0.3-107) (Tables 1 and 2). Haematological remission was achieved in 75/95 (80%) patients. At 3 months of diagnosis, complete renal remission was obtained in 20/102 (19%) patients, 45/102 (45%) patients had CKD stages 3-4 and 34/102 (33%) reached end-stage renal disease (ESRD). At last follow-up, complete renal remission was obtained in 24/102 (24%) patients, 40/102 (39%) patients had CKD stages 3-4 and 38/102 (37%) reached end-stage renal disease (ESRD). Over time, the incidence of ESRD did not significantly vary between patients diagnosed with secondary HUS between 2009 and 2010 (54%) and those diagnosed after 2011 (32%) (Supplemental Table S5). Six patients underwent renal transplantation (IgA nephropathy (n=2), systemic lupus erythematous (n=2), Still's disease (n=1), heart transplantation (n=1)) and none experienced a HUS recurrence.

Eleven (11%) patients died during follow-up and death rate did not vary over time (Table 1 and Supplemental tables S1 and S5). Death was related to complications of chemotherapy (n=2), malignancy (n=4), extra-renal transplantation (n=2), infection (n=1), auto-immune disease (n=1), and pancreatitis (n=1). Mean time between diagnosis and death was 254 days (12-1149). Death occurred within three months after HUS onset in three patients (pancreatitis, n=1; cancer, n=1; infection, n=1).

Among the 73 patients who were not lost to follow-up and who did not die or reach ESRD within 3 months of HUS onset, one patient (1%) experienced a relapse of HUS. This 32-year female patient with a metastatic melanoma (skin, liver, lung) treated with vemurafenib and cobemitinib presented with severe HUS (acute kidney injury requiring dialysis, liver and heart involvement) nine months after the diagnosis of melanoma and six months after the start of treatment. Chemotherapy was stopped, the patient received eleven PE and her renal function partially recovered (SCr 1.5-2 mg/dL). One month after PE discontinuation, HUS recurred (acute kidney injury, SCr 6 mg/dL, mechanical hemolysis), eculizumab was started and, subsequently, SCr decreased to 2 mg/dL. Eculizumab was stopped after six

months. One month later, SCr increased to 2.7 mg/dL, eculizumab was resumed and SCr decreased and stabilized at 1.1 mg/dL (eGFR 57 ml/min/1.73m^2). She is still treated with eculizumab and receives nivolumab for her metastatic melanoma. No complement gene variant was detected.

As compared to 125 atypical HUS patients (adult-onset of the disease) from the same registry, patients with secondary HUS required less frequently dialysis at presentation (41% vs 81%; $p < 0.001$), progressed less frequently to ESRD (37% vs 71%; $p < 0.001$) and experienced fewer relapses (1% vs 35%; $p < 0.001$) (Table 2). In contrast, patients with secondary HUS had a higher frequency neurological involvement (18% vs 8%; p=0.03) and death rates (11% vs 2%; p=0.007) compared to atypical HUS patients.

Impact of eculizumab on clinical outcome.

Characteristics and outcome of patients treated or not with eculiuzmab are shown in Table 5. The mean time between secondary HUS diagnosis and eculizumab initiation was 24 days (0.5-120), and in 11/38 (28%) patients eculizumab was started within 7 days of diagnosis. The mean duration of eculizumab treatment was 7 months (0.25-68) and the mean number of treatment doses was 20 (1-206).

The evolution of SCr, dialysis status, and platelet count at 3 months of eculizumab initiation is depicted in the Figure. No eculizumab-specific side effects (meningococcal infections) occurred. Eculizumab-treated patients had a more severe HUS at presentation with a more frequent need for dialysis (56% vs 32%; p=0.01) and more frequent neurological manifestations (28% vs 13%; p=0.04), as compared to patients not treated with eculizumab. In all, rates of haematological remission (69% vs 87%; p =0.06) of CKD stages 3-4 (51% vs 33%; p=0.09) of ESRD (36% vs 38%; p=1) and of death (10% vs 11%, p=1) rates were similar in in patients treated and those not treated with eculizumab. When the 38 patients treated with eculizumab were compared to 38 matched patients (based on age and SCr first, and platelet count and haemoglobin level second), renal outcome at 3 months of eculizumab start was not statistically different (Supplemental Table S6). When the comparison was restricted to the most severe

patients who required dialysis, the renal outcome was similar in patients treated (n=22) or not (n=23) with eculizumab (Supplement Table S7). Median time between secondary HUS diagnosis and eculizumab initiation tended to be shorter in patients who had complete renal remission, CKD stages 3-4 and ESRD, but the difference did not reach statistical significance (Supplemental Table S8). Response to eculizumab according to the underlying condition associated to secondary HUS is depicted in Supplemental Table S9, but the low number of patients in some groups precluded any relevant statistical analysis.

At last follow-up, only four (10%) patients remained on eculizumab. One patient (melanoma/chemotherapy) had a HUS relapse after eculizumab discontinuation and was restarted on treatment. Two patients, one with a pulmonary carcinoid tumour and a pathogenic CFH variant (c.3047A>G; p.Tyr1016Cys) and one with systemic lupus erythematous had severe cardiac involvement (heart failure) and ESRD and were maintained on eculizumab awaiting renal or renal and cardiac transplantation. In the remaining patient, the reason for eculizumab continuation was the physician preference.

Discussion

This large retrospective series provides new clinical and genetic insights into secondary HUS. The dissection of the genetic risk factors for atypical HUS and the availability of the first complement inhibitor, eculizumab, have undoubtedly fuelled the interest of clinicians for secondary HUS. Currently, one of the most debated question is whether secondary HUS belongs or not to the spectrum of complement-mediated atypical HUS.

The present series of 110 patients with secondary HUS and a complete complement work-up is the largest to date. It included a large variety of conditions associated to secondary HUS, with however a predominance of drug, auto-immune disease and infection-related HUS, in keeping with previously published series[11, 12].

Our results indicate that atypical HUS and secondary HUS have no common genetic risk factors distinct presentation and outcome. We showed that the frequency of rare (MAF < 0.1%) genetic variants in complement genes was similar in secondary HUS patients (5%) and healthy donors (6% and 8% of the French of the European controls, respectively). More interestingly, the frequency of pathogenic variants that impair complement regulatory activity was extremely low and did not differ between secondary HUS cases and healthy French and European donors (1% vs 0% and 1%, respectively). This is in sharp contrast to atypical HUS that has been linked in a significant number of large studies to a high incidence (40-70%) of rare and mostly pathogenic complement genes variants and is thus assumed to be mediated by uncontrolled complement activation[2, 5, 13]. However, homozygous MCP haplotype ggaac, a known risk factor for atypical HUS[5], was more frequently found in secondary HUS patients as compared to controls (Supplemental Table S2). This haplotype is probably associated to a decreased transcriptional activity of the MCP gene promotor[14] and its implication in the pathogenesis of secondary HUS warrants further assessment in larger cohorts. Moreover, one cannot exclude that transient complement activation may occur in some patients with secondary HUS and thus promotes TMA process, as suggested in pneumococcal or anti-VEGF drug-induced HUS [15, 16]. In the present

series, 15% of included patients presented at onset with a low C3 serum level secondary to classical or alternative pathway consumption. Thus, even if complement activation does not seem to be the initial trigger in secondary HUS cases, such activation may occur in a subset of patients as a "second-hit" and perpetuates thrombotic microangiopathy. This hypothesis warrants additional ex vivo assays at the level of the endothelial cell to document a potential inadequate complement regulation at the endothelium surface[17]. However, to date, in clinical practice, systematic screening for complement gene variants is probably not warranted in patients with secondary HUS.

Secondary HUS is an acute, non-relapsing disease. Only one patient (1%) included in the present series experienced secondary HUS relapse in the setting of metastatic melanoma and chemotherapy, whereas, even in the eculizumab era, atypical HUS relapses rate remains high (31-50%) in the absence of specific treatment or after treatment discontinuation in patients with pathogenic complement gene variants [1, 18-20]. The withdrawal or the treatment of the triggering factor or condition usually prevents secondary HUS relapse. Interestingly, patients included in the present study had been treated for a longer mean period of time (8 weeks versus 7 months) and had received a higher mean number of doses (6 versus 20) as compared to the previous study from Spain[12]. This may be due to the uncertainties about the potential overlap between atypical HUS and secondary HUS prior to the completion of the present study.

In the present series, the risk of ESRD in secondary HUS patients from the present series was high (37%) but lower than previously reported in atypical HUS in the pre-eculizumab era (64-67%) [5, 13]. Besides, a significant proportion (39%) of secondary HUS patients progressed to CKD stages 3-4.

However, progression of CKD in some patients may not be entirely related to HUS per se but to the underlying cause of HUS – autoimmune diseases, cancer, chemotherapy, glomerulopathy, transplantation - that may negatively impact renal function regardless of thrombotic microangiopathy. Another remarkable difference with atypical HUS is the significant mortality rate in secondary HUS

(11% vs 2%). Nevertheless, death was mainly related to the underlying condition rather than to HUS complications per se.

Even in the absence of a definite proven link between complement activation and secondary HUS, eculizumab is increasingly used in patients with this type of HUS. In the present series, more than one third of included patients who were diagnosed with secondary HUS after the approval of eculizumab for atypical HUS were treated with this C5 blocker. A growing number of case reports suggest potential benefit of eculizumab but carry the bias of preferential publication of positive results [21-26]. A recent retrospective study reviewed 29 cases of secondary HUS treated with eculizumab in eleven Spanish nephrology centers [12]. HUS was mainly drug-induced (n=15) or related to systemic diseases (n=8) and was severe as 14 (52%) patients required dialysis and 11 (38%) presented with extra-renal manifestations (mainly neurological). In 24 (83%) patients, plasmapheresis failed to control TMA leading to the use of eculizumab. C5 blockade was associated with a rapid (within 1 month of start of treatment) improvement of renal and haematological features of TMA in 20 (69%) patients. In six (21%) additional patients, hematological remission occurred without any renal improvement and in the three (10%) remaining patients no benefit of eculizumab was noted. However, a comparison with historical controls not treated with eculizumab was not performed in this retrospective study.

The analysis of the impact of eculizumab on the outcome of secondary HUS is not straightforward. Firstly, underlying conditions and pathogenic mechanisms are heterogeneous, several triggering factors may coexist particularly in patients with malignancies treated with chemotherapy and it is hard to ascertain the respective role of each condition in triggering HUS. Moreover, the exact mechanism of TMA is illusive in some subtypes of secondary HUS (pancreatitis-associated HUS, for example). Thus, response to treatments may vary accordingly. Secondly, management of patients with secondary HUS usually includes the withdrawal or treatment of the underlying disease, and thus the removal or control of the initial main trigger of TMA. Such approach may be sufficient in some instances to halt the TMA process. Finally, in contrast to patients with atypical HUS (excluding those

with anti-CFH antibodies), eculizumab is not frequently the sole treatment used in patients with secondary HUS.

In the present series, 13% of eculizumab-treated patients had a complete renal and haematological remission. An additional two-thirds had a haematological remission and an improvement or stabilization of their renal function. However, a similar haematological and renal outcome was noted in patients not treated with eculizumab (38% of whom received plasmatherapy). Nevertheless, compared with patients not treated with eculizumab, patients who received eculizumab had a more severe disease, as exemplified by a more frequent need for dialysis and more frequent neurological manifestations. The finding that eculizumab-treated patients shared with patients not treated with eculizumab a similar haematological and renal outcome, despite a more severe presentation, may suggest a potential benefit of complement blockade in this subset of patients. Nevertheless, when the eculizumab-treated patients were compared to matched patients or when the comparison (eculizumab versus no eculizumab) was restricted to the most severe cases requiring dialysis, the renal outcome was not significantly altered by the use of the complement inhibitor. Noteworthy, in contrast to secondary HUS, the beneficial impact of eculizumab on the renal outcome of patients with atypical HUS was suggested by a similar retrospective analysis of even a smaller series from the same French registry [27].

No firm conclusion can be drawn regarding the efficacy of eculizumab in secondary HUS. However, treatment was well tolerated and short-term complement blockade may represent a reasonable therapeutic option in patients with severe renal and/or extra-renal manifestations of HUS. Early use of eculizumab in secondary HUS may be associated to a better renal recovery as suggested, but not fully proven, by the present series (supplemental table S8) and by the previous one from Spain[12]. The rapid achievement of at least a haematological remission with eculizumab may also facilitate the use of chemotherapy and/or cytotoxic agents for the treatment of the underlying cause of HUS. Prospective controlled studies with eculizumab in distinct subtypes of secondary HUS are obviously needed but

several already outlined issues (wide heterogeneity of secondary HUS, effect of the treatment/withdrawal of the underlying cause/condition) may be an obstacle to the optimal design of such studies.

Our study has limitations. It was retrospective and included a heterogeneous cohort of secondary HUS. Patients with hematopoietic stem cell transplantation-TMA from the French HUS registry were excluded, as their cases have been previously reported[28]. However, hematopoietic stem cell transplantation-TMA is a peculiar type of secondary TMA that has attracted a substantial number of specific studies[29-32], and in which complement involvement has been assessed with discrepant results[28, 31]. Nevertheless, our study is the first large study to provide a global clinical and genetic picture of secondary HUS.

In all, secondary HUS appears as an acute, non-relapsing form of HUS, not related to an autoimmune or constitutional dysregulation of the CAP. It is nevertheless a severe form of HUS with a high morbidity and mortality. Complement blockade may represent an empirical therapeutic option in severe forms of HUS with life-threatening manifestations. However, its efficacy in this setting is, so far, not established.

Methods

Study population

In this academic non industry-sponsored study, we retrospectively identified through a computerized database all patients with secondary HUS diagnosed between 1999 and 2017 and included in the French national registry of HUS patients referred for complement analysis. This registry is based in the Laboratory of Immunology at Hôpital Européen Georges Pompidou, a national reference centre for complement analysis in France. Blood samples were collected from 80 French controls (healthy adult blood donors at Hôpital Européen Georges Pompidou where the French HUS registry is based), to establish normal values of complement factors and the frequency of complement variants in the French

population. Previously published clinical, biological and genetic data of 125 patients with atypical HUS from the same registry were also included in the analysis[5].

Definitions

All patients with a diagnosis of secondary HUS and available clinical data were included in the study. Their medical records were reviewed and relevant clinical and biological features were collected. HUS was defined by the association of at least three of the following criteria: mechanical hemolytic anemia (hemoglobin < 10g/dl, lactate dehydrogenase level > upper limit of normal, undetectable haptoglobin, presence of schistocytes on blood smear), thrombocytopenia (platelets count < 150 G/L), acute kidney injury or typical features of thrombotic microangiopathy in a kidney biopsy (fibrin/platelet thrombi, endothelial cells swelling and detachment from the basement membrane, double contours). Secondary HUS was defined as a HUS associated with an active disease or condition or to an ongoing treatment including: an uncontrolled autoimmune disease, an ongoing bacterial or viral infection (excluding post-diarrheal STEC-HUS), a progressing (or not in full remission) malignancy in the last 6 months preceding HUS, the use of medications previously reported to be associated with HUS, a glomerulopathy documented by a kidney biopsy, an extra-renal transplantation or a pancreatitis. In patients with malignancies, HUS was considered to be "malignancy-associated" if it had occurred *before* the start of chemotherapy and "drug-induced" if it had occurred *after* the start of chemotherapy. HUS relapse was defined by the association of at least two of the biological criteria of HUS, occurring after at least 6 weeks of haematological remission. All included patients diagnosed with secondary HUS after 2000 had detectable ADAMTS 13 activity (> 10%). Cases of HUS occurring de novo after renal transplantation [33], following hematopoietic stem cell transplantation [28] or overlapping cases of HUS and C3 glomerulopathy [34] have been reported previously and are not included in the present series. A previously published series [5] of hundred and twenty-five patients with adult-onset atypical HUS (without coexisting conditions/diseases) from the same French HUS registry was included in the analysis as historical controls.

Haematological remission of HUS was defined by a normalization of platelet count and a LDH level < 1.5 upper limit of normal for at least 8 consecutive weeks. Complete renal remission was defined by a glomerular filtration rate estimated using the Modification of Diet in Renal Diseases formula > 60 ml/min/1.73m^2 and proteinuria/creatininuria ratio < 0.05 g/mmol. Chronic kidney disease (CKD) was defined by an estimated glomerular filtration rate < 60 ml/min/1.73m^2 and end-stage renal disease (CKD stage 5) was defined by an estimated glomerular filtration rate < 15 ml/min/1.73m^2 or the need to start chronic dialysis. In patients who required dialysis, the initial SCr refers to the highest value measured prior to the start of dialysis. Normalization of proteinuria was defined by a proteinuria/creatininuria ratio < 0.05 g/mmol. Stable SCr was defined by a SCr value unchanged (±10%) on at least two measurements three months apart. Unless specified, outcomes were assessed at the last visit for living patients who had not reached CKD stage 5 or ESRD.

Complement analysis

Complement work-up and genetic analysis were performed in the usual clinical management of the patients. All patients gave informed consent for genetic analysis according to the Declaration of Helsinki. Measurement of plasma concentrations of C3, C4, complement Factor H (FH) and complement Factor I (FI), membrane cofactor protein (MCP) expression on granulocytes, and test for anti-FH antibodies were performed as previously described[35].

All coding sequences for CFH, CFI, MCP, *C3*, Factor B (FB) and thrombomodulin (THBD) genes were sequenced using next-generation sequencing (NGS). Multiplex ligation-dependent probe amplification was performed to detect CFH hybrid genes and CFH-related protein 1-3 (CFHR1-CFHR3) genes deletion. The Minor allele frequency (MAF) of the genetic changes was obtained from the Exome aggregation consortium database (http://exac.broadinstitute.org)[36].

We collected the genotypes in 503 European individuals from the 1000 Genomes project[37] for 6 genes of interest: CFH, CFI, CFB, MCP, C3 and THBD. The 1000 Genomes project was an

international study that applied whole-genome sequencing to a large cohort of individuals from multiple populations (2,504 individuals from 26 populations), including 503 individuals with European ancestry (Northern and Western European, Finnish, British, Spanish and Tuscan). The variant call format files located on the 1000 Genomes server (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/) were parsed using the Ferret tool[38] to extract the genetic information of rare coding variants (minor allele frequency (MAF) <1%). From the genotypes, we computed the occurrence of rare coding variants in each individual.

In this study, a variant is defined as rare when its MAF in the general population is < 0.1%. A variant was classified as pathogenic when the genetic change affects the protein function (well-established in vitro functional studies supportive of a damaging effect on the gene product), and/or the genetic change is found in a disease-related functional domain or affects the protein expression (nonsense, frameshift, canonical ±1 or 2 splice sites variants, or well demonstrated lack of in vitro synthesis, or quantitative deficiency in the patient's plasma). The others variants were classified as Variants of Uncertain Significance (VUS) when no available functional data are available (definitions adapted from).

Statistical analysis

Data are presented as percentages or means (ranges). The Wilcoxon test was performed for quantitative variables, and Fischer's exact test for qualitative data. All analyses with p value <0.05 were considered statistically significant.

Disclosures: François Provôt, Moglie Le Quintrec, Yahsou Delmas, Jean-Michel Halimi, Steven Grangé, Eric Rondeau, Véronique Frémeaux-Bacchi and Fadi Fakhouri have received consultancy and/or lecture fees and/or travel support from Alexion Pharmaceuticals. Yahsou Delmas, Jean-Michel Halimi and Steven Grangé have received consultancy fees from Ablynx. Noémie Joude-Chiche has received a research grant from Genzyme.

Acknowledgments:

We would like to thank Pr Christine Piétrement (CHU de Reims), Dr Sophie Taque (CHU de Rennes) and Dr Laurent Guillaume (CH de Perpignan) for their help.

Authorship contributions:

ALC, VF-B and FF designed the study, and collected and analyzed the data. VF-B and P V-M performed the complement work-up and genetic screening. All authors were involved in the clinical management of the patients. FF and VFB drafted the manuscript and all authors reviewed, amended and approved the manuscript. FF had full access to the data in the study and final responsibility for the decision to submit for publication.

Supplementary Material:

Supplementary Figure S1: Flow chart of 110 patients with secondary hemolytic uremic syndrome (HUS) included in the study.

* Among 73 patients who were not lost to follow-up and who did not die or reach end-stage renal disease within 3 months of HUS onset.

Supplementary Table S1: Characteristics of 110 secondary HUS patients according to the associated conditions/diseases.
Abbreviations: VEGF, vascular endothelial growth factor. CMV, cytomegalovirus. HIV, human immunodeficiency virus. HSV, herpes simplex virus. GBM, glomerular basement membrane. ANCA, anti-neutrophil cytoplasm antibodies.

*, Interferon-β (n=9), interferon-α (n=1), ** including lupus with APS (n=4). *** sacroidosis (n=1), undetermined connective disease (n=1).

a, the two patients had chronic kidney disease (stages 3-4). b, the patient had reached end-stage renal disease. c, the patient had chronic kidney disease (stages 3-4). d, one patient had a complete remission, two had chronic kidney disease (stages 3-4) and one had reached end-stage renal disease. e, both patients had chronic kidney disease (stages 3-4). f, the patient had reached end-stage renal disease.

Supplementary Table S2: Number and frequency (%) of patients who carried at least one rare variant in one of the 6 tested complement genes among 110 patients with secondary HUS, 80 French healthy controls and 503 controls from the 1000 Genomes project database.

Abbreviations: HUS, hemolytic uremic syndrome; MAF, minor allele frequency. CFH, complement factor H; MCP, membrane-cofactor protein. CFHR, complement factor H-related protein.

Supplementary Table S3: Rare complement gene variants identified in 3 out of 503 (1 %) European individuals from the 1000 Genomes project.

a) Atypical HUS mutation database, http://www.fh-hus.org/. b) Author VFB, personal communication: CFI variants p.Cys 54 Phe, p.Gly162Asp variants and C3 p.Lys155Gln variant found in aHUS patients (French cohort) .

Supplementary Table S4: Frequency of complement gene variants and of at-risk tgtgt and ggaac haplotypes in distinct subtypes of secondary HUS and in 80 French healthy donors.

Abbreviations: CFH, complement factor H. MCP, membrane cofactor protein. * vs healthy donors.

Supplementary Table S5: Trend over time of death, complete renal remission, chronic kidney disease and end-stage renal disease rates in patients with secondary HUS. * six patients were lost to follow-up ** two patients were lost to follow-up.

Supplementary Table S6: Outcome of 38 secondary HUS patients treated with eculizumab and of 38 matched patients not treated with eculizumab. Patients were matched first on age and serum creatinine level (requirement for dialysis) and second on hemoglobin and platelet count.

Supplementary Table S7: Outcome of 45 secondary HUS patients who required dialysis and who were treated (n=22) or not (n=23) with eculizumab.

Supplementary Table S8: Mean time between secondary HUS diagnosis and eculizumab initiation and renal outcome in 38 patients. *, two patients died within 3 months of secondary HUS onset.

Supplementary Table S9: Renal outcome according to the underlying associated condition in 39 patients with secondary HUS treated with eculizumab.

Supplementary information is available at Kidney International's website.

References:

1. Fakhouri F, Zuber J, Fremeaux-Bacchi V*, et al.*: Haemolytic uraemic syndrome. *Lancet* 217:681-696, 2017
2. George JN, Nester CM: Syndromes of thrombotic microangiopathy. *N Engl J Med* 371:1847-1848, 2014
3. Scully M, Goodship T: How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. *Br J Haematol* 164:759-766, 2014
4. Cataland SR, Wu HM: How I treat: the clinical differentiation and initial treatment of adult patients with atypical hemolytic uremic syndrome. *Blood* 123:2478-2484, 2014
5. Fremeaux-Bacchi V, Fakhouri F, Garnier A*, et al.*: Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin J Am Soc Nephrol* 8:554-562, 2013
6. Noris M, Remuzzi G: Atypical hemolytic-uremic syndrome. *N Engl J Med* 361:1676-1687, 2009
7. Greenbaum LA, Fila M, Ardissino G*, et al.*: Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. *Kidney Int* 89:701-711, 2016
8. Legendre CM, Licht C, Muus P*, et al.*: Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 368:2169-2181, 2013
9. Licht C, Greenbaum LA, Muus P*, et al.*: Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int* 87:1061-1073, 2015
10. Fakhouri F, Hourmant M, Campistol JM*, et al.*: Terminal Complement Inhibitor Eculizumab in Adult Patients With Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. *Am J Kidney Dis* 2016:84-93, 2016
11. Terrell DR, Williams LA, Vesely SK*, et al.*: The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. *J Thromb Haemost* 3:1432-1436, 2005
12. Cavero T, Rabasco C, Lopez A*, et al.*: Eculizumab in secondary atypical haemolytic uraemic syndrome. *Nephrol Dial Transplant* 32:466-474, 2017
13. Noris M, Caprioli J, Bresin E*, et al.*: Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 5:1844-1859, 2010
14. Esparza-Gordillo J, Goicoechea de Jorge E, Buil A*, et al.*: Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. *Hum Mol Genet* 14:703-712, 2005
15. Gilbert RD, Nagra A, Haq MR: Does dysregulated complement activation contribute to haemolytic uraemic syndrome secondary to Streptococcus pneumoniae? *Med Hypotheses* 81:400-403, 2013
16. Keir LS, Firth R, Aponik L*, et al.*: VEGF regulates local inhibitory complement proteins in the eye and kidney. *J Clin Invest* 127:199-214, 2017
17. Noris M, Galbusera M, Gastoldi S*, et al.*: Dynamics of complement activation in aHUS and how to monitor eculizumab therapy. *Blood* 124:1715-1726, 2014
18. Ardissino G, Possenti I, Tel F*, et al.*: Discontinuation of eculizumab treatment in atypical hemolytic uremic syndrome: an update. *Am J Kidney Dis* 66:172-173, 2015
19. Fakhouri F, Fila M, Provot F*, et al.*: Pathogenic Variants in Complement Genes and Risk of Atypical Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. *Clin J Am Soc Nephrol* 12:50-59, 2017
20. Wetzels JF, van de Kar NC: Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome. *Am J Kidney Dis* 65:342, 2015
21. Al Ustwani O, Lohr J, Dy G*, et al.*: Eculizumab therapy for gemcitabine induced hemolytic uremic syndrome: case series and concise review. *J Gastrointest Oncol* 5:E30-33, 2014
22. Chandran S, Baxter-Lowe L, Olson JL*, et al.*: Eculizumab for the treatment of de novo thrombotic microangiopathy post simultaneous pancreas-kidney transplantation--a case report. *Transplant Proc* 43:2097-2101, 2011
23. El-Husseini A, Hannan S, Awad A*, et al.*: Thrombotic microangiopathy in systemic lupus erythematosus: efficacy of eculizumab. *Am J Kidney Dis* 65:127-130, 2015

24. Faguer S, Huart A, Fremeaux-Bacchi V*, et al.*: Eculizumab and drug-induced haemolytic-uraemic syndrome. *Clin Kidney J* 6:484-485, 2013
25. Favre GA, Touzot M, Fremeaux-Bacchi V*, et al.*: Malignancy and thrombotic microangiopathy or atypical haemolytic and uraemic syndrome? *Br J Haematol* 166:802-805, 2014
26. Merrill SA, Brittingham ZD, Yuan X*, et al.*: Eculizumab cessation in atypical hemolytic uremic syndrome. *Blood* 130:368-372, 2017
27. Fakhouri F, Delmas Y, Provot F*, et al.*: Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am J Kidney Dis* 63:40-48, 2014
28. de Fontbrune FS, Galambrun C, Sirvent A*, et al.*: Use of Eculizumab in Patients With Allogeneic Stem Cell Transplant-Associated Thrombotic Microangiopathy: A Study From the SFGM-TC. *Transplantation* 99:1953-1959, 2015
29. Jodele S, Fukuda T, Vinks A*, et al.*: Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Biol Blood Marrow Transplant* 20:518-525, 2014
30. Jodele S, Laskin BL, Dandoy CE*, et al.*: A new paradigm: Diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. *Blood Rev* 29:191-204, 2015
31. Jodele S, Licht C, Goebel J*, et al.*: Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. *Blood* 122:2003-2007, 2013
32. Jodele S, Zhang K, Zou F*, et al.*: The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. *Blood* 127:989-996, 2016
33. Le Quintrec M, Lionet A, Kamar N*, et al.*: Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. *Am J Transplant* 8:1694-1701, 2008
34. Servais A, Noel LH, Roumenina LT*, et al.*: Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int* 82:454-464, 2012
35. Roumenina LT, Loirat C, Dragon-Durey MA*, et al.*: Alternative complement pathway assessment in patients with atypical HUS. *J Immunol Methods* 365:8-26, 2011
36. Lek M, Karczewski KJ, Minikel EV*, et al.*: Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536:285-291, 2016
37. Auton A, Brooks LD, Durbin RM*, et al.*: A global reference for human genetic variation. *Nature* 526:68-74, 2015
38. Limou S, Taverner AM, Winkler CA: Ferret: a user-friendly Java tool to extract data from the 1000 Genomes Project. *Bioinformatics* 32:2224-2226, 2016

	n= 110
Female / Male	65 (59%) / 45 (41%)
Age	44 [2-80]
< 18 years	8 (7%)
≥ 18 years	102 (93%)
Associated conditions/treatments	
Drugs	32 (29%)
Auto-immune diseases	26 (24%)
Infections	18 (17%)
Malignancies	11 (10%)
Glomerulopathy	10 (9%)
Extra-renal transplantation	9 (8%)
Pancreatitis	4 (3%)
Features at diagnosis	
Serum creatinine (mg/dL)	3.9 [0.5-25]
Hemoglobin level (d/dL)	8.7 [4.6 -15]
Platelet count (G/L)	94 [10 – 450]
Normal platelet count (n)	11 (10%)
Requirement for dialysis	45 (40%)
Kidney biopsy	51 (46%)
Extra-renal manifestations	31 (28%)
Neurological involvement	20 (18%)
Other*	11 (10%)
Treatment	
Plasma exchanges	55 (50%)
Eculizumab	39 (35%)
Corticosteroids	31 (27%)
Plasma infusions	10 (9%)
Cyclophosphamide	13 (27%)**
Outcome	
Follow-up (months)	21 (0.3-107)
Hematological remission at 3 months (n=96)	76 (80%)
Renal outcome (n=103)***	
> 50% decrease in serum creatinine	27/102 (27%)
< 50% decrease in serum creatinine	24/102 (24%)
Increase in serum creatinine	25/102 (24%)
Stable serum creatinine	26/102 (25%)
Weaning from dialysis at 3 months	16/45 (36%)
Weaning from dialysis at last follow-up	19/45 (42%)
Complete renal remission at 3 months	20/102 (19%)
Complete remission at last follow-up	24/102 (24%)
Chronic kidney disease (stages 3-4) at 3 months	45/102 (45%)
Chronic kidney disease (stages 3-4) at last follow-up	40/102 (39%)
End-stage renal disease at 3 months	34/102 (33%)
End-stage renal disease at last follow-up	38/102 (37%)
Relapse	1/73 (1%)€
Death	11/102 (11%)#
Death within 3 months	3/102 (3%)

Table 1: Main characteristics of 110 patients with secondary HUS included in the study.

*Heart failure (n=8), digestive system (colitis, ischemic cholangitis and jejunitis) (n=3). ** Used for the treatment of the underlying cause of HUS (lupus (n=9), myositis (n=1), ANCA-associated vasculitis (n=1), heart transplantation (n=1), drug (bevacizumab, n=1)). *** Eight patients were lost to follow-up.

€Among 73 patients who were not lost to follow-up and who did die or reach end-stage renal disease within 3 months of HUS onset. # Among the 11 patients who died, one had a complete remission, six had chronic kidney disease (stages 3-4) and four had reached end-stage renal disease.

	Secondary HUS (n=110)	aHUS (n=125)	p-values
At presentation			
Requirement for dialysis	45 (41%)	93/115 (81%)	**$< 10^{-4}$**
Neurological involvement	20 (18%)	10 (8%)	0.03
Renal outcome			
Chronic kidney disease (stages 3-4)	40/102 (40%)	NA	
End-stage renal disease	38/102 (37%)	89 (71%)	**$< 10^{-4}$**
Relapse	1/73 (1%)	23/66 (35%)	**$< 10^{-4}$**
Death	11 (11%)	2 (2%)	0.007

Table 2: Presentation and outcome of 110 patients with secondary HUS and 125 patients with adult-onset atypical HUS in the pre-eculizumab era (from the French HUS registry). Abbreviations: HUS, haemolytic and uremic syndrome.

Complement gene rare variants	Secondary HUS (n=110)	aHUS (n=125)	French controls (n=80)	1000 Genomes controls (n=503)	Secondary vs French controls	Secondary HUS vs 1000 Genome Controls	aHUS vs French controls	aHUS vs 1000 Genome Controls	Secondary HUS vs aHUS
CFH	3 (2.7%)	40 (32%)	1 (1%)	8 (2%)	0.6	0.4	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$
MCP	0 (0%)	8 (6%)	0 (0%)	2 (<1%)	1	1	0.02	$< 10^{-4}$	0.007
CFI	1 (0.9%)	12 (10%)	0 (0%)	8 (2%)	1	1	0.003	$< 10^{-4}$	$< 10^{-4}$
FB	0 (0%)	2 (1%)	0 (0%)	8 (2%)	1	0.4	0.5	0.1	0.5
C3	0 (0%)	11 (9%)	4 (5%)	12 (2%)	0.2	0.1	0.4	0.002	0.0009
THBD	2 (2%)	0 (0%)	0 (0%)	2 (<1%)	0.5	0.2	1	$< 10^{-4}$	0.2
Combined	0 (0 %)	6 (5%)	0 (0%)	2 (<1%)	1	1	0.08	0.001	0.02
Total	6 (5%)	79 (63%)	5 (6%)	42 (8%)	1	0.4	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$
Anti-CFH antibodies	1 (2%)	4 (3%)	0 (0%)	-	1		0.2	-	0.2

Table 3: Frequency of complement gene rare variants in 110 secondary HUS cases, 125 adult-onset atypical HUS cases included in the French HUS Registry, 80 French healthy donors and 503 European healthy individuals from the 1000 Genomes project. All donors were screened for variants in the 6 tested complement genes.

Abbreviations: aHUS, atypical haemolytic uremic syndrome. CFH, complement factor H. CFI, complement factor I. MCP, membrane cofactor-protein. FB, factor B. THBD, thrombomodulin.

Pt	Gender /Age	Associated disease/ condition	Plt (G/L)	SCr (mg/dL)	Hb (g/dL)	Treatment	Outcome	Complement gene	Variant	MAF[c] (%)	Polyphen 2 prediction	Functional consequences	Variant categorization
1	F/26	SLE	40	4.3	8.5	PE/Ecu/Cs/CYP	ESRD	THBD	c.707C>G p.Ala236Gly	Novel	Benign	NA	VUS
2	F/80	Breast cancer	129	1.3	10.6	PI	CKD / Death[#]	THBD	c.91G>A p.Val31Ile	Novel	Benign	NA	VUS
3[a]	F/39	Drug (IFN)	54	4	8.1	Drug withdrawal/ PE/Cs	CKD	CFI	c.11T>A p.L4H	0.0033	Benign	NA	VUS
4	M/59	Infection (E.Coli)	27	ND/HD	ND	Antibiotics	CKD	CFH	c.643G>A p.Val215Ile	Novel	Benign	NA	VUS
5	F/43	Pulmonary carcinoid tumor	80	6.3/HD	7.2	PE/Ecu	ESRD*	CFH	c.3047A>G p.Tyr1016Cys	Novel	Probably damaging	Located in disease-related functional domain	Pathogenic
6[b]	F/32	Drug (Vemurafenib)	27	5.7	7.8	Drug withdrawal/ PE/Ecu	CKD / Relapse after Ecu discontinuation	CFH	c.3596T>C p.Phe1199Ser	Novel	Probably damaging	Located in disease-related functional domain	Pathogenic

Table 4: Main characteristics of the 6 patients with secondary HUS and complement gene rare variants.

Abbreviations: Pt, patient. F, female. M, male. SLE, systemic lupus erythematous. VEGF, vascular endothelial growth factor. APS, antiphospholipid syndrome. IFN, interferon. Plt, platelet count. SCr, serum creatinine. HD, hemodialysis. NA, not available. PE, plasma exchanges. Ecu, eculizumab. Cs, corticosteroids. CYP, cyclophosphamide. ESRD, end-stage renal disease. CR, complete remission. CKD, chronic kidney disease. Hb, haemoglobin. MAF, minor allele frequency. CFH, complement factor H. CFI, complement factor I. . THBD, thrombomodulin.. VUS, variant of unknown significance.

NA: not available

[a)] This patient also carried a VUS in CFI gene with a minor allele frequency of 0.8 (c.1642G>C p.E548Q). [b)] This patient carried a C3 p.Lys155Gln (c.463A>C) pathogenic variant with a minor allele frequency of 0.33 in control populations.

[c)] Allele frequency given by Exome aggregation consortium

* This patient had a partial renal recovery under eculizumab (SCr, 1.2 mg/dL) but developed severe hypertension, uncontrolled despite the use of six antihypertensive drugs. She also had severe heart failure (left ventricular ejection fraction 15-20%) due to HUS but also to the intractable hypertension and she underwent binephrectomy. Subsequently, hypertension improved and ejection fraction increased to 55%.

This patient died 2 weeks after the onset of HUS in the setting of sepsis metastatic cancer.

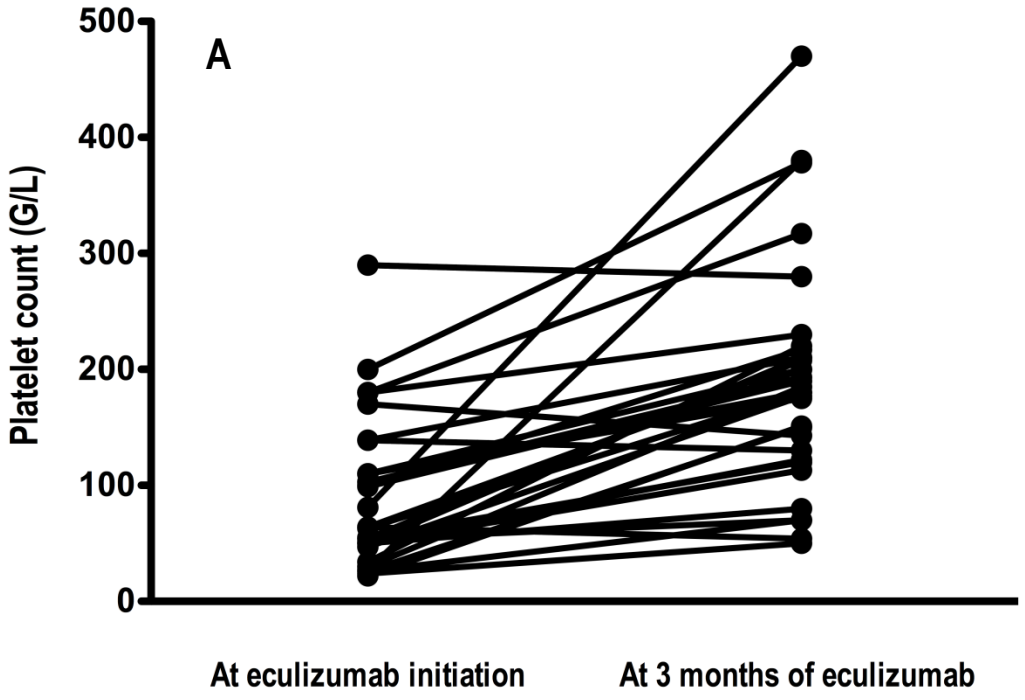
n	Patients treated with eculizumab (n= 38)	Patients not treated with eculizumab (n= 72)	p
Age (years)	51 [17-74]	43 [2-80]	0.6
< 18 years	1 (5%)	7 (10%)	0.5
Associated conditions/treatments			
Drugs	13 (33%)	19 (26%)	0.6
Malignancies	8 (20%)	3 (4%)	**0.01**
Auto-immune disorders	9 (26%)	17 (24%)	0.8
Infection	2 (5%)	16 (22%)	**0.02**
Pancreatitis	1(3%)	3 (4%)	1
Glomerulopathies	0 (0%)	10 (14%)	**0.01**
Extra-renal transplantation	5 (13%)	4 (6%)	0.3
Features at eculizumab initiation			
Serum creatinine (mg/dL)	4.4 (1.3-12)	3,9 (0.5-25)	0.05
Hemoglobin (g/dL)	7.8 (6 – 11)	9.0 (5-15)	**0.02**
Platelet count (G/L)	86 (22 – 290)	112 (10 – 450)	0.29
Requirement for dialysis	22 (56%)	23 (32%)	**0.01**
Kidney Biopsy	19 (51%)	32 (44%)	0.7
Neurological involvement	11 (29%)	9 (13%)	**0.04**
Treatments other than eculizumab			
Plasma exchange	28 (74%)	27 (38%)	**<0.001**
Plasma infusion	6 (15%)	4 (6%)	0.2
Steroids	11 (28%)	20 (28%)	1
Cyclophosphamide	7 (18%)	6 (8%)	0.2
Eculizumab treatment			
Time between diagnosis and eculizumab initiation (days)	24 (0.5-120)		
Initiation of eculizumab whithin 7 days of diagnosis	11/38 (28%)		
Duration (months)	7 (0.25-68)	-	
Number of doses	20 (1-206)	-	
Discontinuation	34 (90%)	-	
Outcome			
Duration of follow-up (months)	18 (0.5-79)	22 (0.5-105)	0.4
Hematological remission	24/35 (69%)	52/60 (87%)	0.06
Weaning from dialysis at 3 months	10/22 (45%)	6/23 (26%)	0.22
Weaning from dialysis at last follow-up	11/22 (50%)	8/23 (35%)	0.4
Complete renal remission at 3 months	3/38 (8%)	17/64 (27%)	**0.02**
Complete renal remission at last follow-up	5/39 (13%)	19/64 (29%)	0.1
> 50% decrease in serum creatinine at 3 months	11/38 (28%)	12/64 (20%)	0.3
> 50% decrease in serum creatinine at last follow-up	14/39 (36%)	13/64 (20%)	0.1
< 50% decrease in serum creatinine at 3 months	8/38 (23%)	10/64 (16%)	0.6
< 50% decrease in serum creatinine at last follow-up	9/38 (26%)	15/64 (23%)	0.8
Increase in serum creatinine at 3 months	11/38 (28%)	16/64 (23%)	0.9
Increase in serum creatinine at last follow-up	12/38 (31%)	13/64 (20%)	0.2
Stable serum creatinine at 3 months	8/38 (21%)	26/64 (40%)	0.05
Stable serum creatinine at last follow-up	3/38 (8%)	23/64 (36%)	**0.001**
Chronic kidney disease (stages 3-4) at 3 months	21/38 (56%)	25/64 (39%)	0.1
Chronic kidney disease (stages 3-4) at last follow-up	19/38 (51%)	21/64 (33%)	0.09
End-stage renal disease at 3 months	14/38 (36%)	20/64 (31%)	0.7
End-stage renal disease at last follow-up	14/38 (36%)	24/64 (38%)	1
Death within 3 months of onset	2/38 (5%)	2/64 (3%)	0.61
Death*	4/38 (10%)	7/64 (11%)	0.4
Relapse	1 (2%)	0 (0%)	

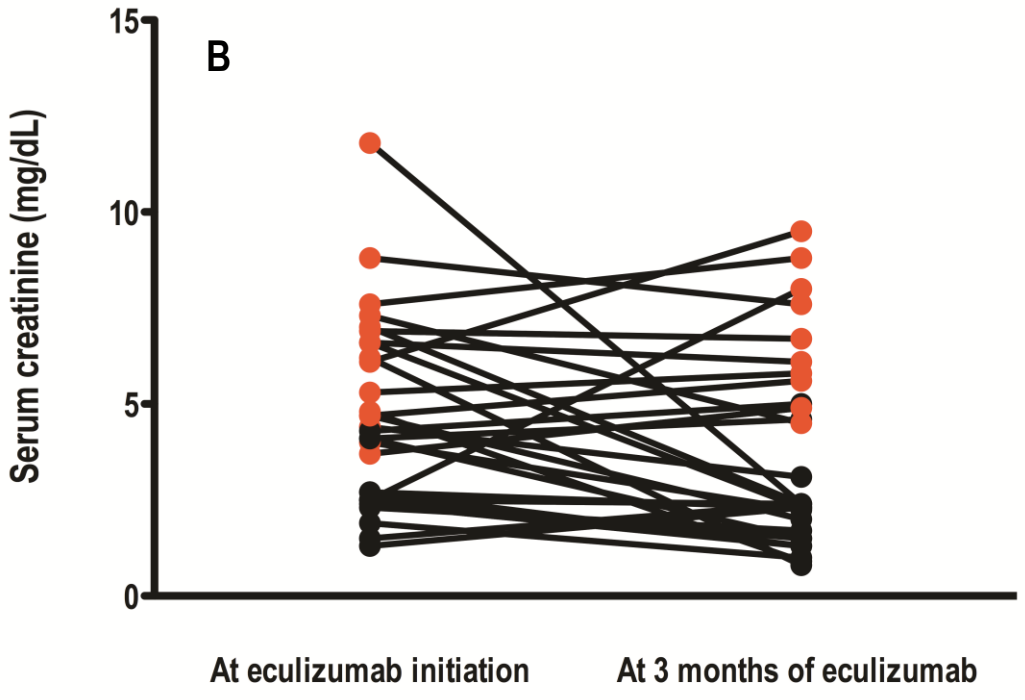
Table 5: Characteristics of patients with secondary HUS treated or not with eculizumab.

* Among the 4 eculizumab-treated patients who died, two had chronic kidney disease and 2 had reached end-stage renal disease. Among the 7 patients not treated with eculizumab who died, one had a complete remission, four had chronic kidney disease, and two had reached end-stage renal disease.

Figure legend:

Evolution of platelet count (panel A) and of serum creatinine (panel B) 3 months after eculizumab initiation in 32 patients with secondary HUS form whom detailed data are available. Coloured circles indicate requirement for dialysis.

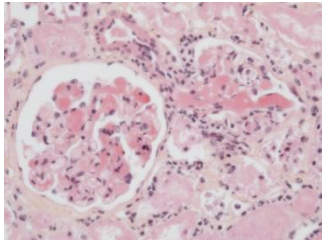

A
Platelet count (G/L)
500
400
300
200
100
0
At eculizumab initiation
At 3 months of eculizumab


B
Serum creatinine (mg/dL)
15
10
5
0
At eculizumab initiation
At 3 months of eculizumab

Atypical and secondary haemolytic uremic syndromes have distinct presentation and no common genetic risk factors.

Patient Selection

. 110 patients
(French HUS registry)
. Retrospective cohort
. **Secondary HUS**



Diseases/Conditions associated to HUS
Drugs (29%); Auto-immune diseases (24%); Infections (17%); Malignancies (10%), Glomerulopathies (9%); Extra-renal transplantation (8%); Pancreatitis (3%).

Presentation of secondary HUS

40% of patients required dialysis
18% had neurological manifestations

Genetics

Frequency of complement gene rare variants similar in secondary HUS patients (5%) and 2 cohorts of healthy individuals (6-8%).

Treatment

Plasmatherapy (50%)
Eculizumab (35%)

Outcome

Haematological remission: 80%
Complete renal remission: 24%
CKD (stages 3-4): 39%
ESRD: 37%
HUS relapse (<1%).

Benefit of eculizumab unclear

CONCLUSION:

Secondary HUS is an acute non-relapsing form of HUS, not related to a constitutional or autoimmune complement dysregulation.

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Le Clech, 2019