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Hemolytic Uremic Syndrome in Pregnancy and Post-Partum.

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Abstract:

Background: Pregnancy is associated with various forms of thrombotic microangiopathy, including the hemolytic uremic syndrome. A previous small French study suggested that pregnancy-associated hemolytic uremic syndrome was to be included in the spectrum of atypical HUS linked to complement alternative pathway dysregulation.

Design, setting, participants and measurements: We sought to retrospectively analyze the presentation, outcome, and frequency of complement alternative pathway genes variants in a larger international (France, United Kingdom, Italy) cohort of patients with pregnancy-associated hemolytic uremic syndrome.

Results: Eighty-seven patients with pregnancy-associated hemolytic uremic syndrome were included. Hemolytic uremic syndrome occurred mainly during the first pregnancy (58%) and in the post-partum period (76%). At diagnosis, 56 (71%) patients required dialysis. Fifty-six (78%) patients underwent plasma exchanges, 21 (41%) received plasma infusions and 4 (5%) received eculizumab. During follow-up (mean duration of 7.2 years), 41 (53%) patients reached end-stage renal disease, 15 (19%) had chronic kidney disease and 18 (28%) patients experienced hemolytic uremic syndrome relapse.. Twenty four patients (27%) received a renal transplant and a recurrence of hemolytic uremic syndrome occurred in 13 (54%) cases. Variants in complement genes were detected in 49 (56%) patients, mainly in the *CFH* (30%) and *CFI* genes (9%).

Conclusions: Pregnancy-associated hemolytic uremic syndrome and atypical hemolytic uremic syndrome non-related to pregnancy have the same severity at onset and during follow-up and the same frequency of complement gene variants.

Introduction

Hemolytic and uremic syndrome (HUS) is a rare and severe form of thrombotic microangiopathy associated with a poor renal prognosis. It is characterized by the association of mechanical hemolytic anemia, thrombocytopenia and renal failure[1]. HUS arises from an insult to endothelial cells that in turn may result from distinct pathogenic mechanisms: verotoxin-induced endothelial cells activation and apoptosis in Shiga toxin-induced HUS , acquired or constitutional complement alternative pathway dysregulation leading to complement-induced endothelial cells damage in atypical HUS or various, more or less well-defined, patterns of endothelial cells lesions in the heterogeneous group of secondary HUS associated with autoimmune diseases, drugs, infections and pregnancy. Pregnancy carries a high risk for various forms of thrombotic microangiopathy, including ADAMTS13-deficiency associated thrombotic thrombocytopenic purpura[2] but also HUS[3]. The diagnosis of pregnancy associated-HUS can be difficult with preeclampsia, HELLP syndrome and in some instances severe post-partum hemorrhage presenting with similar features[4,5]. Moreover, until few years ago, pregnancy associated-HUS was included in the group of secondary forms of thrombotic microangiopathy.

The identification of complement alternative pathway dysregulation as a major risk factor for atypical HUS[6,7] has led several authors to reconsider the pathogenesis of secondary forms of HUS and to assess whether in these settings complement alternative pathway dysregulation combined with specific precipitating events could lead to thrombotic microangiopathy. Previously, we tested this hypothesis in a small series of pregnancy-associated HUS and showed that 86% of patients with mainly post-partum HUS harbored complement genes variants and had an initial presentation and outcome similar to non-pregnancy related atypical HUS[3]. This first study was rather limited as it included only 21

patients from a single country. Thus, there remained some uncertainties and controversies regarding the pathogenesis and consequently the treatment of pregnancy-associated HUS, a crucial question since the clinical availability of the first complement inhibitor, eculizumab[4]. We sought to analyze the presentation, outcome, and frequency of complement alternative pathway genes variants in a large international cohort of patients presenting with pregnancy-associated HUS.

Methods and Methods

Patients

This was a retrospective multicentric study conducted in France, the United Kingdom and Italy, three countries in which a HUS registry has been established. We identified through computerized databases all women with pregnancy-related HUS included in these registries between 1983 and 2013 and for whom complement workup has been performed in the three national reference centers (Department of Biological Immunology at Hôpital Européen Georges Pompidou, Paris, France; Mario Negri Institute, Bergamo, Italy, and The UK National aHUS service in Newcastle).

Definitions

All patients with a diagnosis of pregnancy-associated HUS from the three registries were included in the study. Pregnancy-associated HUS was defined as a HUS occurring during pregnancy, or in the post-partum period (up to 12 weeks after delivery). HUS was defined by the association of at least three of the following criteria: mechanical hemolytic anemia (hemoglobin < 10g/dl, Lactate dehydrogenase > upper limit of normal, undetectable haptoglobin, presence of schistocytes), thrombocytopenia (platelets count < 150 x $10^3/\mu L$), acute kidney injury (serum creatinine > 1.1 mg/dLl or > 25% increase from baseline value) or typical features of thrombotic microangiopathy in a kidney biopsy (fibrin/platelet thrombi, endothelial cells swelling and detachment from the basement membrane, double contours). Patients with pre-eclampsia (defined by hypertension > 140/80 mmHg and proteinuria > 300 mg/day[8] after 20 weeks of gestation) prior to the development of thrombotic microangiopathy features, HELLP (Hemolysis, Elevated Liver enzymes and Low Platelet count) syndrome (defined by aminotransferase > 70 U/L, Lactate dehydrogenase > 600 U/L and platelet count < 100 x $10^3/\mu L$)[9], massive post-partum bleeding or other identified causes of secondary HUS

(lupus, antiphospholipid syndrome) were excluded. Chronic kidney disease was defined by a glomerular filtration rate estimated using the Modification of Diet in Renal Diseases formula < 60 ml/min/1.73m^2.

Medical records of included patients were reviewed and relevant clinical and biological features were collected.

Complement analysis

Complement evaluation and genetic analysis were performed in each reference center in the usual management of the patients. Plasma concentrations of C3, C4, factor B , factor F and I and membrane cofactor protein expression on granulocytes were measured according to local practice[6,7,10,11]. All coding sequences for complement factor H , complement factor I, membrane cofactor protein , *C3*, factor B and thrombomodulin genes were sequenced as described previously[6,7,11]. Variants were categorized as: a) Pathogenic: novel (not found in the general population) or rare (minor allele frequency in the general population <0.1%) variant reported to cause disease in literature; functional data indicating the variant affects protein function or expression. b) Likely pathogenic: novel or rare variants that change protein sequence or affect splicing and with highly deleterious effects by in silico predictions but without functional data; found in disease-related functional domains; c) Uncertain significance: novel or rare variants that change protein sequence or affect splicing with no available functional data; uncertain deleterious effects by in silico prediction[12].

All patients gave informed consent for genetic analysis according to the Declaration of Helsinki.

Statistical analysis

Data are presented as percentages or means (± standard deviation). 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.

Results

Eighty-seven patients who presented with pregnancy-associated HUS between 1983 and 2013 were included in the study. The distribution by country and decade is presented in supplementary table S1. Pregnancy-associated HUS represented 16% (87/547) of HUS cases occurring in females aged 18-45 years reported in the three national registries. The main characteristics of these patients are summarized in Table 1.

Patients' characteristics at presentation.

Mean age at the time of pregnancy-associated HUS was 29 ± 6 years (Table 1). Fourteen patients (16%) had a familial history (at least one affected family member) of atypical HUS. Seven (8%) patients had a personal history of atypical HUS and had experienced one (n=6) or several (4 in one patient) episodes of atypical HUS not related to pregnancy before presenting with pregnancy-associated HUS. One patient had a documented complement gene variant (complement factor H) prior to pregnancy. Five patients had previously underwent plasma exchanges for atypical HUS, and two had a stage 3 chronic kidney disease related to atypical HUS. The risk for HUS occurrence was similar for the first pregnancy (48/82, 58%) and for subsequent ($\geq 2^{nd}$ pregnancy) pregnancies (42%). HUS occurred mainly in the post-partum period (n=63, 76%; mean time of 14 ± 12 days after delivery) (Figure 1), regardless of the rank of pregnancy. Twenty patients (24%) presented with HUS during pregnancy, mostly (n=18, 77%) in the third trimester. Acute kidney injury was severe with 56 (71%) patients requiring dialysis at presentation. In contrast thrombocytopenia was mild (mean platelet count 97 $x10^3/\mu L$ ± 99) and even absent in 13 (15%) patients. All patients had aminotransferases levels < 70 U/L and ADAMTS13 activity > 10%. Extra-renal manifestations were noted in 11 patients (14%). Kidney biopsy performed in 8 patients (mostly with normal platelet count) disclosed typical features of thrombotic microangiopathy.

Treatment

Fifty-six patients (56/72, 78%) underwent plasma exchanges as first-line therapy, 21 (21/51, 41%) received plasma infusions and sixteen (16/60, 27%) corticosteroids (Table 1). Eculizumab was used in 4 cases starting 2011, as a second-line therapy after plasma exchanges. Three patients had HUS in the post-partum, one during pregnancy but eculizumab was started after delivery in all cases. The four patients had severe acute kidney injury requiring dialysis but no extra-renal manifestations and received eculizumab 4 days, 5 days, 1 month and 2 months after HUS diagnosis. Three of them had complement gene variants (an isolated *complement factor H* variant (n=2) and combined *complement factor H/complement factor I* variants (n=1)).

Outcome.

Short-term outcome

No maternal death was reported. Fetal or neonatal death occurred in 11 (14%) cases (Table 2). Twenty-five patients (32%) reached end-stage renal disease within three months of first manifestations of pregnancy-associated HUS and 15 (19%) developed chronic kidney disease. The risk of end-stage renal disease (29/56 (51%) vs (7/16) 47% p= 0.77) and chronic kidney disease (11/56 (20%) vs 3/16 (20%), p= 1) did not differ between patients treated with plasma exchanges and those who did not undergo plasma exchanges. Among the four patients treated with eculizumab, three (two with complement genes variants, one without) had a complete recovery of renal function. The remaining patient treated with eculizumab two months after diagnosis remained dialysis-dependent.

Long-term outcome

Eight patients out of the 62 (13%) who did not reach end-stage renal disease within 3 months of pregnancy-associated HUS had an HUS relapse: in the post-partum of a subsequent pregnancy (n=1), in the setting of malignant hypertension (n=1) or an infectious illness (n=1), or without any identified triggering event (n=5). In 6 (75%) relapsing patients, HUS relapse led to end-stage renal disease. At last follow-up (7.2 years ± 5.2), 22/78 (28%) patients had an estimated glomerular filtration rate > 60 ml/min/1.73 m^2, 15 (19%) had chronic kidney disease and 41 (53%) had progressed to end-stage renal disease. Twenty four patients (24/87, 27%) received a renal transplantation and a recurrence of HUS occurred in 10 patients (10/24, 42%). Overall, eighteen patients (18/87, 21%) experienced HUS relapse either in their native kidneys or in a renal graft.

Complement work up

Available results for complement component assays and complement genes sequencing are presented in tables 3 and 4. C3 serum level was low in 29/74 (39%) of patients. Twenty-three women among the 29 (79%) with low serum C3 level had complement genes variants. Novel or rare variants in complement genes (missense, nonsense variants, ins-del and exon-intron boundary variants affecting splicing) were detected in 49/87 (56%) patients, most frequently in the *complement factor H* (n=26, 30%) and *complement factor I* genes (n=8, 9%). Eight patients (9%) presented combined variants: *complement factor H* and *complement factor I* variants (n=2), *complement factor H* and *membrane cofactor protein* (n=2), *complement factor H* and *C3* (n=2), *complement factor I* and *membrane cofactor protein* (n=1) or *complement factor H* and *thrombomodulin* (n=1). One patient had two heterozygous variants in *complement factor I gene*. Thirty-eight patients (44%) had no detected variant in

complement genes. Detailed description of the 59 variants detected in complement genes are shown in table 4. Based on published functional data, low protein level and/or high pathogenicity predicted by in silico analysis, 40 variants were considered to be pathogenic, the remaining 19 variants being of uncertain significance.

Patients' characteristics and outcome depending on the presence or absence of complement genes variants.

Frequency of complement genes variants in patients presenting with HUS during their first pregnancy and in those presenting with HUS during subsequent (≥ 2nd) pregnancies did not significantly differ (23/48 (47%) vs 23/35 (65%), p = 0.11) (Figure 1). Patients with complement genes variants required dialysis at presentation more frequently than those with no detected variant (35/89 (81%) vs 21/38 (58%), p=0.02). The frequency of neurological involvement was similar in patients with or without complement genes variants (5/49 (12%) vs 2/38 (6%), p=0.38) (table 5). The long-term outcome of HUS was more severe in patients with documented complement gene variants compared to patients with no identified variant (table 5). Patients with variants progressed to end-stage renal disease more frequently than patients with no variant detected (29/49 (64%) vs 12/38 (36%), p = 0.01). The risk of relapse was also significantly greater in patients with variants compared to those without (13/49 (38%) vs 5/38 (16%), p =0.04). Among patients with mutations, there was no difference according to the type of genetic abnormality (supplementary table S2).

Patients' characteristics and outcome depending on the timing of HUS (during pregnancy or in the post-partum).

Compared to patients with HUS in the post-partum, patients presenting with HUS during pregnancy had more frequently a personal history of chronic kidney disease or HUS (25% vs 5%, p= 0.02) and tended to require less frequently dialysis in the acute phase (56% vs 76%, p =0.08) even though this difference was not statistically significant (table 6). However, the frequency of complement genes variants and the risk of HUS relapse, chronic kidney disease and end-stage renal disease did not differ between the two groups.

Discussion

This is the largest to date cohort study assessing the presentation, the outcome and the frequency of complement genes variants in pregnancy-associated HUS. It was performed using three distinct registries in three national reference centers for HUS. Cases were diagnosed over a rather long period of time spanning over four decades, but until the very recent availability of eculizumab the care of HUS did not significantly vary.

The results indicate that pregnancy-associated HUS has a severity at onset (2/3 of patients requiring dialysis) and in the long-term (more than half of the patients reaching end-stage renal disease), and a frequency (56%) and distribution of complement genes variants similar to those of atypical HUS cases from the same three national registries[6,7,13]. Pregnancy-associated HUS is thus an atypical HUS triggered by pregnancy, in keeping with the findings of a previous small study performed in one country[3]. It remains unknown why a significant proportion of women presented with HUS in the second or subsequent pregnancies and not in earlier pregnancies and overall the precise mechanism by which pregnancy precipitates HUS remains ill-defined. During normal pregnancy, complement activation occurs in the placenta at the interface between the mother and the fetus[14] and one would expect pregnancy-associated HUS to occur mainly during pregnancy. Nevertheless, complement alternative pathway regulation in the placenta depends predominantly on CD59 and decay accelerating factor [15,16], two membrane-bound proteins that negatively control complement alternative pathway. Neither CD59 nor Decay Accelerating Factor has been to date implicated in the pathogenesis of atypical HUS and their overexpression in the placenta may compensate for the deficiency in other regulatory proteins implicated in the pathogenesis of atypical HUS. In contrast, during the post-partum, the protection conferred by the overexpression of Decay Accelerating Factor and CD59 is lost with placental delivery. Post-partum bleeding and

infection may also trigger excessive complement activation in predisposed women and ultimately lead to HUS.

Three-quarters of the cases included in the present study occurred in the post-partum of uneventful pregnancies and in this setting the diagnosis of HUS is rather straightforward. For the remaining one-quarter of cases occurring during pregnancy (mainly during the third trimester) the differential diagnosis is more complex as several disorders may mimic HUS, including preeclampsia, HELLP syndrome or severe bleeding[4]. We have carefully excluded patients with these complications of pregnancy from the study. Besides, one-quarter of patients with HUS during pregnancy had a history of chronic kidney disease/HUS and the existence of renal vascular damage prior to gestation may explain the occurrence of HUS early during pregnancy and before delivery. Furthermore, patients with HUS during pregnancy and those with HUS in the post-partum had a similar high frequency (50.0%-59%) of complement genes mutations. In contrast, only a minority (8-10%) of patients with preeclampsia and HELLP syndrome harbor complement genes variants, mostly of unknown significance or non-pathogenic[17]. Finally, patients with HUS during pregnancy and those with HUS in the post-partum shared the same severe renal outcome (risk of end-stage renal disease of 44%-55%), in a sharp contrast to the usual complete renal recovery in preeclampsia and HELLP syndrome patients. Thus, the present cohort reflects the presentation, genetic risk factors and outcome of HUS occurring during pregnancy or in the post-partum.

Two-thirds of complement genes variants detected in patients with pregnancy-associated HUS were considered to be pathogenic, based on quantitative defects and proven or predicted functional abnormalities of the encoded protein. These findings are similar to those of complement genes variants detected in atypical HUS[12]. The presence of an identified complement gene variant was associated with a more severe outcome of pregnancy-associated

HUS with an increased risk of HUS relapse and of progression to end-stage renal disease. However, there was no difference in terms of presentation and outcome between patients with different types of complement genes mutations.

The present study raises several issues regarding the management of pregnancy-associated HUS. Firstly, plasma exchanges did not improve the renal outcome of pregnancy-associated HUS, and the risk of end-stage renal disease remained similarly high (around 50%) in patients who underwent plasma exchanges and in those who did not. Secondly, the availability of the anti-C5 antibody eculizumab has dramatically changed the treatment and outcome of atypical HUS[18,19]. However, pregnant women were excluded from prospective trials with eculizumab and no specific data were reported for the few patients with post-partum HUS. In the present series only four patients with pregnancy-associated HUS received eculizumab and a limited number of cases reports of the use of the drug in this setting are available in the literature[20-23]. In all these cases except one, eculizumab proved efficacious in controlling HUS and improving renal function. Based on the experience with the eculizumab in patients with paroxysmal nocturnal hemoglobinuria[24], the use of eculizumab during gestation seems safe, even though the drug was detected in one-third of cord blood samples but was not present in maternal breast-milk. Careful monitoring of complement blockade is mandatory as pregnancy may require an increase in the dosage and/or the frequency of eculizumab infusions due to the increase in the distribution volume or C5 synthesis[25]. Based on all these available data, treatment of P-HUS should be similar to the treatment of non-pregnancy-related atypical HUS, and relies on eculizumab use, as first-line treatment if the clinical presentation (post-partum of an uneventful pregnancy) and/or personal or familial history is highly suggestive of HUS or as second-line treatment after plasma exchange if a

diagnostic work-up is necessary. Nevertheless, a further specific assessment of eculizumab use in pregnant women with HUS is warranted.

Secondly, the present data will help clinicians counsel patients with a history of atypical HUS or healthy carriers of complement genes variants who wish to start a pregnancy. In our study the risk of HUS was the highest during the first pregnancy, but remained relatively high during subsequent pregnancies and may even increase as eculizumab rescues patients from end-stage renal disease and thus preserves their ability to conduct a pregnancy. However, the availability of eculizumab has dramatically improved the outcome of atypical HUS and nowadays most clinicians do not advise against pregnancy in a patient with a history of atypical HUS or a healthy carrier of complement gene variant. Patients should make their decision about pregnancy after being informed of the risk of atypical HUS relapse (estimated however based mainly on studies in atypical HUS non-related to pregnancies) but also of the risks inherent to potential clinical or subclinical chronic renal damage due to previous episodes of atypical HUS, including hypertensive complications of pregnancy that may occur despite treatment of eculizumab[25]. Pregnancy in these patients requires close collaborative monitoring by nephrologists and obstetricians in level III maternity hospitals, starting in the first weeks of pregnancy and up to three months after delivery, in order to rapidly detect and thus treat the early manifestations of HUS.

In conclusion, pregnancy-related atypical HUS is a very severe disease, with a poor prognosis for maternal renal function if left without specific efficacious treatment. It is associated with variants in complement genes in 56% of patients, and pregnancy seems an important trigger for the disease in these at-risk patients. Prospective studies are needed in order to specifically assess the efficacy and safety of eculizumab in pregnancy-associated HUS.

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The other authors declare no conflict of interest.

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	Number (%) / mean ± SD
Number of patients	87
Age at HUS onset (years)	29 ± 6.0
Number of previous pregnancies	0.7 ± 1.2
Rank of pregnancy HUS was diagnosed in (n=83)	
1st	48 (58%)
2nd	23 (28%)
3rd	5 (6%)
≥ 4th	7 (8%)
Pre-eclampsia during previous pregnancies (n=53)	5 (9%)
Fetal loss during previous pregnancies (n=49)	10 (20%)
Familial history of atypical HUS	14 (16%)
Personal history of atypical HUS	7 (8%)
Timing of HUS*	
Post-partum	63 (76%)
During pregnancy	20 (24%)
Features at hemolytic and uremic syndrome onset	
Serum creatinine (mg/dL)	6.1 ± 5.2
Dialysis	56 (71%)
Platelet count x10^3(/μL)	97 ± 99
Hemoglobin (g/dL) (n=66)	7.8 ± 1.9
Lactate dehydrogenase (U/L) (n=56)	2225 ± 1617
Neurological involvement	7 (9%)
*Other extra-renal manifestations***	4 (6%)
Treatment	
Number of patients who underwent plasma exchange (n=72)	56 (78%)
Number of plasma exchange sessions performed per patient (n=41)	13 ± 10
Number of patients who received plasma infusion (n=51)	21 (41%)
Number of patients who received eculizumab	4 (5%)

Steroids (n=60)	16 (27%)
Other ***	3 (5%)

Table 1: Characteristics of 87 patients with pregnancy-associated hemolytic uremic syndrome .

* Timing of HUS is unknown for four patients.

**pulmonary edema (n=2), pulmonary embolism (n=1).

*** Intravenous immunoglobulins (n=2), rituximab (n=1).

The numbers of patients for whom data are available are reported in brackets

SD, standard deviation.

	Number (%) / mean ± SD
Duration of follow-up (years) (n=78)	**7.2 ± 5.2**
Patients who reached end-stage renal disease*	41 (53%)
End-stage renal disease within 3 months of pregnancy-HUS (n=78)	25 (32%)
Patients with an estimated GFR <60 mL/min/1.73m2 without end-stage renal disease	15 (19%)
Patients with an hemolytic and uremic syndrome relapse	18 (28%)
Relapse in the native kidneys	8/62* (13%)
Number of relapses	1.6 ± 1.4
Patients reaching end-stage renal disease after a relapse	6/8 (75%)
Relapse in the renal graft	10/24 (42%)

Table 2: Outcome of 87 patients with pregnancy-associated hemolytic uremic syndrome.

Abbreviations: SD, standarddeviation. eGFR, glomerular filtration rate estimated using the MDRD formula..

* Nine patients progressed to end-stage renal disease during follow-up without overt hemolytic and uremic syndrome relapse. The timing of end-stage renal disease is unknown in 2 patients.**, number of patients who did not reach end-stage renal disease within 3 months of pregnancy-associated HUS onset.

	Number (%)
Complement components assays	
Low serum C3	29/74 (39%)*
Low serum CFH	8/54 (15%)**
Low serum FI	5/43 (12%)***
Low serum FB	0/45 (0%)
Low MCP expression on granulocytes	6/39 (15%)****
Complement and THBD genes sequencing (n=87)	
Number of patients with a variant detected	**49 (56%)**
Isolated CFH variant	26 (31%)
Isolated CFI variant	8 (9%)
Isolated MCP variant	3 (3%)
Isolated C3 variant	3 (3%)
Isolated FB variant	0 (0%)
Isolated THBD variant	1 (1%)
Combined mutations	8 (9%)
No variant detected	**38 (44%)**

Table 3: Results of complement components assays and complement genes sequencing in patients with pregnancy-associated hemolytic uremic syndrome.
Abbreviations: CFH, complement factor H. CFI, complement factor I. MCP, membrane cofactor protein. FB, factor B. THBD, thrombomodulin.

*60% of patients with CFH or C3 variants had low serum C3 level.
**32% of patients with CFH variant had low serum CFH level.
***44% of patients with CFI variants had low serum CFI level.
****100% of patients with MCP variants had low MCP expression on granulocytes.

Patient	Gene	Variation Classification	Animo acid change*	Population frequency**	Protein Level	Protein Function	References
1	*CFH*	Pathogenic	p. Arg1215Gly	novel	↔	↓	26,27
2	*C3*	Pathogenic	p.Arg592Gln	0.000008241	↔	↓	28
3	*MCP*	Pathogenic	p.Met44Leu	0.0004454	↓	N/A	
4	*CFH*	Pathogenic	p.Arg1210C	0.0001730	↔	↓	26,27,29
5	*CFH*	Pathogenic	p.Thr645ArgfsX20	novel	↓	N/A	
6	*CFH*	Pathogenic	p.Trp71*	novel	↓	N/A	
7	*CFH*	Pathogenic	c.3468dupA	novel	↓	N/A	30
	CFI	VUS	Pro553Ser	0.000008243	↔	N/A	10,31
8	*CFH*	Pathogenic	p.Ser1191Leu	novel	↔	↓	26,32
9	*CFH*	Pathogenic	p.Val1197Ala	novel	↔	↓	29,32,33
10	*CFH*	Pathogenic	p.Arg1215X		↔	N/A	
11	*CFH*	Pathogenic	CFH/CFHR3 hybrid	novel	↔	↓	34
12	*CFH*	Pathogenic	c.3486delA		↓	N/A	
13	*CFI*	Pathogenic	p.W145X	novel	↓	N/A	35
14	*CFI*	Pathogenic	p.Ile416Leu	0.001113	↓	N/A	6,10,36,37
15	*CFI*	VUS	p.Ile578Thr	0.00002477	↔	N/A	10
16	*CFH*	Pathogenic	p.G1194D	0.00003295	N/A	N/A	38
	MCP	Pathogenic	p.F242C	novel	↓	N/A	39,40
17	*CFI*	Pathogenic	p.A240G	0.0002720	↓	N/A	7,31,36
18	*CFH*	VUS	p.P279L	novel	N/A	N/A	
19	*THBD*	VUS	p.D163N	novel	N/A	N/A	
20	*CFH*	Pathogenic	K474Nfs6X				
21	*MCP*	Pathogenic	IVS2+2T>G	0.00003311	↓	N/A	41
	MCP	Pathogenic	p.Y189D	novel	↓	N/A	41
	CFI	VUS	p.D44N	0.00001648			
22	*CFI*	VUS	p.D519N	0.00001658	↔	↓	42
23	*CFH*	VUS	p.N516K	0.0004046			
24	*CFH*	Pathogenic	p.R78G	novel	↔	↓	38,43
25	*CFH*	VUS	p.R303Q	0.00001650			
26	*C3*	Pathogenic	p.Arg161Trp	0.000008240	↔	↓	6,44
27	*C3*	VUS	p.Ile1095Ser	novel	NA	NA	6
28	*CFH*	Pathogenic	p.Arg53Cys	0.00001652	↔	↓	45
29	*CFH*	Pathogenic	p.Ala161Ser	0.00004124	N/A	N/A	
30	*CFH*	Pathogenic	p.Gly397Arg	novel	N/A	N/A	
31	*CFH*	Pathogenic	p.Cys431Tyr	0.000008262	N/A	N/A	
32	*CFH*	Pathogenic	p.His893Arg	novel	N/A	N/A	
33	*CFH*	Pathogenic	p.Val1197Ala (Hom)	novel	↔	↓	29,32,46
34	*CFH*	Pathogenic	p.Val1197Ala	novel	↔	↓	13-15,18,19

35	*CFH*	Pathogenic	p.Arg1210Cys	0.0001730	↔	↓	26,27,29,47,48
	MCP	Pathogenic	p.Tyr29Stop	novel	↓	N/A	
					N/A	N/A	
36	*CFH*	Pathogenic	p.Gln81Pro	novel			
37	*CFH*	VUS	p.Lys1186Thr	novel	N/A	N/A	
	CFI	VUS	p.Ile340Thr	0.00004120	↔	↓	42
38	*C3*	Pathogenic	p.Lys155Gln	0.003362	N/A	N/A	
	CFH	Pathogenic	p.Lys584Stop	novel	N/A	N/A	
39	*C3*	Pathogenic	p.Arg161Trp	0.000008240	↔	↓	6,44
	CFH	VUS	p.Arg341His	0.00001653	N/A	N/A	
40	*CFH*	Pathogenic	p.Cys864Ser	novel	N/A	N/A	
	THBD	Pathogenic	p.Ala43Thr	0.003430	N/A	N/A	
41	*CFI*	Pathogenic	p.Arg474Stop	0.00004956	↓	N/A	6,36,49
42	*CFI*	Pathogenic	p.Gly119Arg	0.0005290	↓	N/A	6,31,36
43	*CFH*	Pathogenic	p.Gly218Glu	novel	↓	N/A	6,7
44	*CFI*	Pathogenic	p.Gly119Arg	0.0005290	↓	N/A	6,31,36
	CFI	VUS	p.Gly424Asp	novel	↔	N/A	36
45	*MCP*	Pathogenic	IVS3+2T>G	0.00003121	N/A	N/A	
46	*MCP*	Pathogenic	p.Tyr248Stop	novel	↓	N/A	
47	*CFH*	VUS	p.Lys82Arg;	0.00001649	N/A	N/A	
48	*CFH*	Pathogenic	p.Tyr1016* (Hom)	0.000008239	↓	N/A	
49	*CFH*	Pathogenic	p.Arg161Trp	0.000008240	↔	↓	6,44,50

Table 4: Characteristics of complement genes variants identified in patients with pregnancy-associated hemolytic uremic syndrome.
Abbreviations: CFH, complement factor H. CFI, complement factor I. MCP, membrane cofactor protein. FB, factor B. THBD, thrombomodulin. VUS, variant of undetermined significance. ↔, unchanged. ↓, decreased. * all heterozygous unless specified. **, from the Exome Aggregation Consortium (ExAc) database (http://exac.broadinstitute.org/).

	Complement gene variant detected (n=49)	No complement gene variant detected (n=38)	p
At presentation			
Age (years)	27.8 ± 6.0	30.4 ± 5.8	0.06
Personal history of HUS	4 (8%)	3 (9%)	1
Onset in the post-partum	39 (79%)	28 (72%)	0.49
Need for dialysis	35 (81%)	21 (58%)	0.02
Neurological involvement	5 (12%)	2 (6%)	0.38
Plasma exchange	30 (79%)	26 (77%)	0.80
During follow-up (n= 74)			
Duration of follow-up (years)	6.2 ± 3.6	6.7 ± 4.1	0.75
Relapse	13 (38%)	5 (16%)	0.04
Chronic kidney disease	9 (21%)	6 (18%)	0.81
End stage renal disease.	29 (64%)	12 (36%)	0.01

Table 5: Main characteristics of 87 patients with pregnancy-associated hemolytic uremic syndrome patients with (n=49) or without complement gene variants (n=38).

	HUS during pregnancy (n=20)	HUS in the post-partum (n=63)	p
Medical history			
Personal history of HUS	4 (20%)	3 (5%)	0.03
At onset			
Age (years)	28.5 ± 5	29.2 ± 6	0.68
Need for dialysis	10 (56%)	45 (76%)	0.08
Neurological involvement	0 (0%)	7 (12%)	0.12
Plasma exchange	10 (59%)	44 (83%)	0.04
Complement genes variants detected	10 (56%)	28 (78%)	0.09
Outcome			
Duration of follow-up (years)	6.9 ± 3.2	7.1 ± 5.1	0.80
HUS relapse	4 (20%)	14 (30%)	0.33
Chronic kidney disease	4 (22%)	11 (19%)	0.79
End stage renal disease	8 (44%)	32 (55%)	0.59

Table 6: Main characteristics of 83 patients who presented with hemolytic uremic syndrome during pregnancy (n=20) or in the post-partum (n=63).

Legend to Figures:

Figure 1: Rank of pregnancy and timing of pregnancy-associated hemolytic uremic syndrome in 72 patients for whom the precise data are available.