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Original Article

ORIGINAL ARTICLE

Baseline characteristics and evolution of Brazilian patients with atypical hemolytic uremic syndrome: first report of the Brazilian aHUS Registry

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ABSTRACT

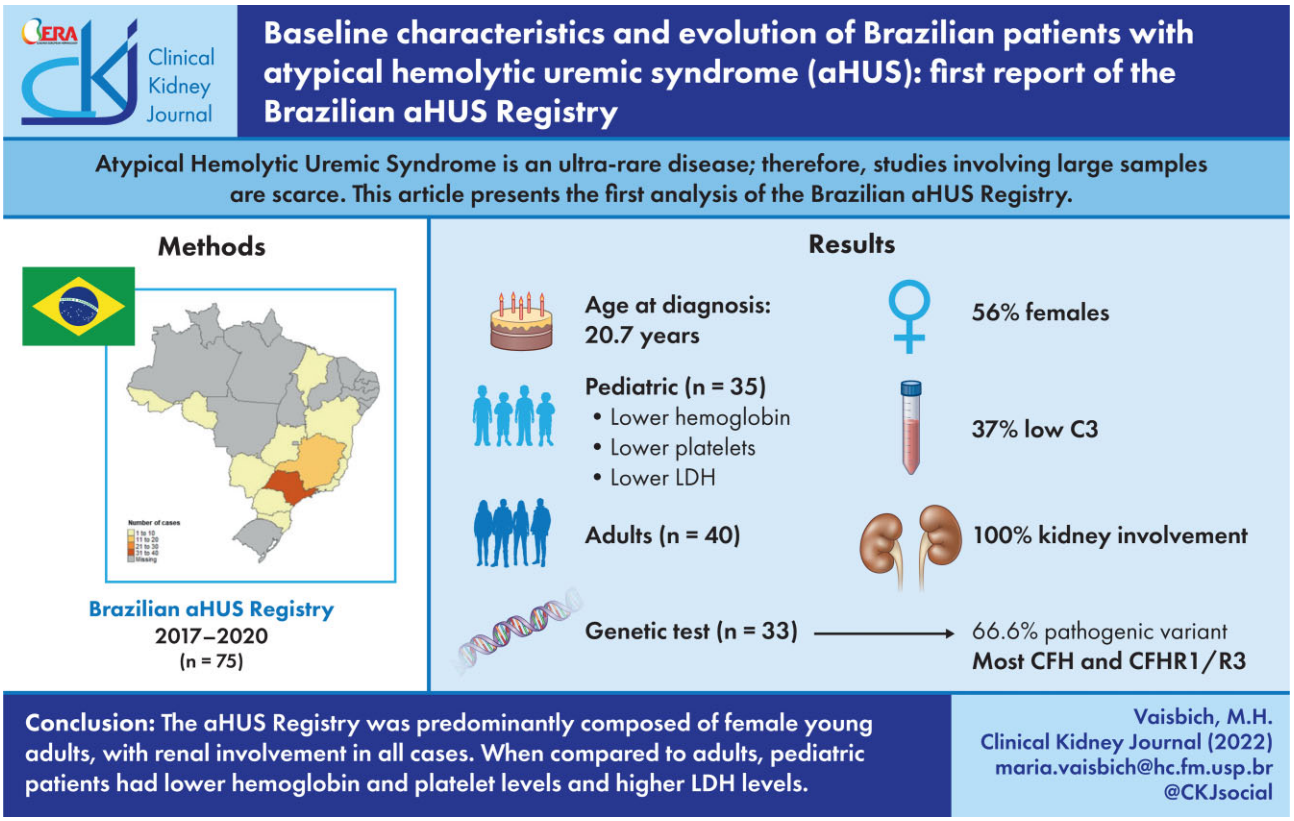
Background. Atypical hemolytic uremic syndrome (aHUS) is an ultra-rare disease. Therefore, studies involving large samples are scarce, making registries powerful tools to evaluate cases. We present herein the first analysis of the Brazilian aHUS Registry (BRaHUS).

Methods. Analysis of clinical, laboratory, genetic and treatment data from patients inserted in the BRaHUS, from 2017 to 2020, as an initiative of the Rare Diseases Committee of the Brazilian Society of Nephrology.

Results. The cohort consisted of 75 patients (40 adults and 35 pediatric). There was a predominance of women (56%), median age at diagnosis of 20.7 years and a positive family history in 8% of cases. Renal involvement was observed in all cases and 37% had low C3 levels. In the <2 years of age group, males were predominant. Children presented lower levels of hemoglobin ($P = .01$) and platelets ($P = .003$), and higher levels of lactate dehydrogenase (LDH) ($P = .004$) than adults. Genetic analysis performed in 44% of patients revealed pathogenic variants in 66.6% of them, mainly in *CFH* and the *CFHR1-3* deletion. Plasmapheresis was performed more often in adults ($P = .005$) and 97.3% of patients were treated with eculizumab and its earlier administration was associated with dialysis-free after 3 months ($P = .08$).

Conclusions. The cohort of BRaHUS was predominantly composed of female young adults, with renal involvement in all cases. Pediatric patients had lower hemoglobin and platelet levels and higher LDH levels than adults, and the most common genetic variants were identified in *CFH* and the *CFHR1-3* deletion with no preference of age, a peculiar pattern of Brazilian patients.

GRAPHICAL ABSTRACT



Keywords: atypical hemolytic uremic syndrome, Brazil, eculizumab, genetic, rare diseases

INTRODUCTION

Atypical hemolytic uremic syndrome (aHUS) is a thrombotic microangiopathy caused by the inability to self-regulate the alternative complement pathway. As consequence of this pathway imbalance, a massive membrane attack complex (MAC, C5b-9) production occurs causing severe damage to endothelial cells throughout the body [1].

There is a well-known genetic basis for nearly two-thirds of cases of aHUS, most related to inactivating mutations in genes codifying inhibiting proteins of the alternative pathway: Factor H (*CFH*), Factor I (*CFI*), membrane cofactor protein (*MCP* or *CD46*), thrombomodulin (*THBD*), large deletions or insertions in Factor H-related proteins 1 to 5 (*CFHR1* to 5) or gain-of-function mutations in genes codifying activating factors of this complement pathway (C3 or Factor B) [2–4].

aHUS is a rare genetic disease and the knowledge of its epidemiological data, natural history, genetic profile and pathophysiology have been increasing over recent years [5]. However, reports from low-middle income countries' populations of aHUS are restricted to a few cohort series [6–12]. The availability of data from these countries can broaden the spectrum of genotype according to the region.

In rare diseases, studies enrolling a large population are difficult to achieve and registries are powerful tools to overcome this obstacle. Registry data of rare diseases are important in understanding and providing clinical insights and are essential for strategic planning in structuring support and allocation of healthcare resources. In addition, rare diseases registries can provide research opportunities and solve issues related to scientific studies. They can facilitate patient's recruitment for clinical trials as well as providing historical controls data [13].

The Brazilian aHUS Registry (http://comdora-sbn.org.br/registros) is an observational, non-interventional, industry-independent and multicenter registry of patients with aHUS. The aims of the Registry are to assess the clinical and epidemiological characteristics, genetic profile as well as long-term outcomes of aHUS patients in Brazil.

The Brazilian aHUS Registry was an initiative of the Rare Diseases Committee of the Brazilian Society of Nephrology, named COMDORA, which is in charge of scientific oversight, governance and coordination of all COMDORA's registries. COMDORA is formed by expert physicians in the diagnosis and management of aHUS patients (e.g. adult and pediatric nephrologists). These members are responsible for validating the aHUS diagnosis of each registered case, and for contacting the physician who registered the patient, in case of doubts. The registry recommends a clinical update at 6 months and then annually.

The aim of this study was to describe the epidemiological and clinical characteristics, genetic profile and evolution of Brazilian aHUS patients.

MATERIALS AND METHODS

Study population

Eligible patients included individuals of all ages with a clinical diagnosis of aHUS as determined by the treating clinicians at each site in Brazil, with or without an identified complement regulatory factor genetic abnormality. This first report is related to data from July 2017 (first data inclusion) to 31 December 2020.

Patient data were collected following a research protocol based mainly on the choice of alternatives related to clinical data but with space for remarks that the attending physician reported spontaneously.

All procedures were performed in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki. The study was approved by the Research Ethics Committee of the Faculty of Medicine of Botucatu-UNESP (# 09 831 719.7.0000.5411). Informed consent was available on the platform and was presented to the patient/parent/guardian by the attending clinician. Patients were identified by encrypted codes in the datasheets, hosted on the Brazilian Society of Nephrology website and in full compliance with Brazilian data protection law.

Inclusion criteria

- Male or female patients of any age who have been diagnosed with aHUS.
- Patients with or without an identified complement pathogenic variant or anti-complement factor antibody.

Exclusion criteria

- Secondary causes of TMA, in the setting of drug use, infections, cobalamin metabolism defects, neoplasia, scleroderma, antiphospholipid antibody syndrome and others.
- Thrombotic thrombocytopenic purpura (TTP): TMA resulting from severe ADAMTS13 deficiency. TTP was defined by a severe deficiency of ADAMTS13 (activity $<10\%$).
- Shiga toxin-mediated hemolytic uremic syndrome (ST-HUS): related to Shiga toxin. Shiga toxins are produced by *Shigella dysenteriae* and some serotypes of *Escherichia coli*, such as O157:H7 and O104:H4.

Diagnosis of aHUS

The diagnosis of TMA was performed using the clinical history and laboratory exams compatible with TMA [microangiopathic hemolytic anemia, increased lactate dehydrogenase (LDH) >1.5 upper normal limit, thrombocytopenia and kidney injury] after exclusion of other causes of TMA [4].

The authors checked the accuracy of aHUS diagnosis of all included patients based on history and baseline exams. The presence of genetic analysis was not necessary to diagnose aHUS. All patients should have an ADAMTS13 activity measurement performed with a result higher than 10% before receiving plasma therapy, if applicable. All patients with the presence of diarrhea should have a negative Shiga Toxin PCR and/or negative stool culture. In case of concomitant infection, it should be resolved before the establishment of aHUS diagnosis. In patients using known TMA-inducing medications, the diagnosis of aHUS was established if TMA persisted for 1 week after discontinuation of the putative drug. The TMA-inducing medications list included cyclosporine, tacrolimus, rifampicin, cisplatin, bleomycin, mitomycin, bevacizumab, clopidogrel and ticlopidine.

Genetic analysis

Genetic analysis was performed according to the indication of each center. The most common test employed was an aHUS panel, which comprised the PCR amplification and target sequencing (next-generation sequencing) of complete regions of genes encoding at least the following genes according to KDIGO recommendations [4]: *CFH*, *CD46*, *CFI*, *C3*, *CFB*, *THBD*, *CFHR1*, *CFHR5* and *DGKE* and including 10 base pairs next to exons. However, in some cases, more extended panels were performed.

Data collection

Demographic data included gender, age at presentation and diagnosis, family history of kidney diseases, comorbidities and clinical presentation (kidney, cardiovascular, neurological, gastrointestinal and pulmonary involvements). We evaluated all investigational diagnostic tests and exams at diagnosis. The reported exams were the most recent prior to aHUS diagnosis and included hemoglobin, platelets, LDH, haptoglobin, direct Coombs Test, presence of schistocytes in peripheral blood smears, serum creatinine, urinary protein/creatinine ratio, serum complement fractions C3 and C4, SHIGA-toxin PCR, stool culture, serum ADAMTS-13 activity, antinuclear factor test, anti-DNA test and serum homocysteine. The glomerular filtration rate (eGFR) was estimated by Chronic Kidney Disease Epidemiology Collaboration equation [14] for patients older than 18 years and the Schwartz Modified equation for patients younger than 18 years [15], using serum creatinine at presentation. Renal biopsy results were also analyzed when available.

Groups

Patients were divided into three groups according to the age at diagnosis: under 2, between 2 and 18, and older than 18 years of age. Demographic data, baseline exams, outcome and genetic tests were analyzed.

Outcomes

The primary outcome was change in eGFR and need for dialysis within 3 months of first aHUS presentation.

The secondary outcomes were:

- Need for plasma exchange, blood, platelets or plasma transfusions within the first 3 months.
- Time between aHUS diagnosis and eculizumab administration, if applicable.
- Correlation between time from aHUS diagnosis to first eculizumab dose with long-term dialysis need.

Statistical analysis

The distribution of variables was assessed with the Shapiro–Wilk test. Qualitative variables were expressed as proportions and compared among each other via the chi-squared test or Fisher's exact test. Variables following a parametric distribution were expressed as mean $\pm$ standard error and compared among each other with ANOVA test. Variables with non-parametric distributions were expressed as median (percentiles 25 and 75) and compared among each other with the Kruskal–Wallis test. We

Table 1. Baseline characteristics on Brazilian aHUS Registry patients divided by age: <2 years old, between 2 and 18 years old and >18 years old

	<2 years old (*n* = 17)	2–18 years old (*n* = 18)	>18 years old (*n* = 40)	Total (*n* = 75)	*P*-value
Female (*n*/%)	3 (17.6%)	13 (72.2%)	26 (65%)	42 (56%)	.001
Age (years)	0.81 (0.7–1.2)	8.84 (6.5–14.8)	29.7 (25.95–34.5)	20.7 (2.4–30.3)	<.001
Family history (*n*/%)	1 (6.2%)	1 (5.6%)	4 (10%)	6 (8.1%)	.809
Previous hypertension (*n*/%)	3 (30%)	7 (50%)	31 (93.7%)	41 (71.9%)	<.001
Kidney transplant (*n*/%)	1 (5.8%)	3 (16.6%)	18 (45%)	22 (29%)	<.001
Clinical presentation (*n*/%)					
Hypertension	14 (87.5%)	11 (64.7%)	31 (77.5%)	56 (76.7%)	.516
Diarrhea	3 (17.6%)	2 (11.8%)	5 (12.5%)	10 (19.6%)	.831
Dyspnea	3 (30%)	1 (12.5%)	8 (32%)	12 (27.9%)	.556
Fatigue	3 (27.3%)	8 (72.7%)	30 (85.7%)	41 (71.9%)	<.001
Elevated creatinine	16 (94.1%)	16 (94.1%)	37 (94.9%)	69 (94.5%)	.990

Continuous variables were expressed as median and percentiles 25 and 75.

Table 2. Concomitant conditions on Brazilian aHUS Registry patients divided by age: <2 years old, between 2 and 18 years old and >18 years old

	<2 years old (*n* = 17)	2–18 years old (*n* = 18)	>18 years old (*n* = 40)	Total (*N* = 75)	*P*-value
Cobalamin defect	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0
Malignant HTN	1 (5.9%)	1 (5.9%)	3 (8.6%)	5 (7.2%)	.911
Pregnancy	0 (0.0%)	0 (0.0%)	5 (13.5%)	5 (7.0%)	.084
SLE	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (1.4%)	.619
Scleroderma	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0
APS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0
Infection	0 (0.0%)	5 (33.3%)	2 (12.5%)	4 (10.8%)	.122
Neoplasia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0
Medications[a]	0 (0.0%)	0 (0.0%)	15 (39.5%)	15 (20.8%)	<.001

Abbreviations: APS, antiphospholipid syndrome; HTN, hypertension; SLE, systemic lupus erythematous. [a]TMA-inducing medications.

provided the number of missing values in the tables. For statistical analysis, the R program was used (https://www.r-project.org/). Statistical significance was assigned to $P < .05$.

RESULTS

In the first report of Brazilian aHUS registry, most cases were from the Southeast region of Brazil (74.6%), with the state of São Paulo contributing 49.3% of the total sample (Supplementary data, Fig. S1). During the selected period of this report, 75 cases were registered, 35 of which (46.6%) were pediatric patients (17 cases <2 years of age) and 40 (53.4%) were adults. The median age at diagnosis was 20.7 years (percentiles 2.4–30.3, range 3 months to 54 years of age) and there was a predominance of females (56%). However, in patients under 2 years of age, male gender was predominant, approximately 82% of cases (14/17 patients) (Table 1). For the majority of the patients (76%), the diagnosis of aHUS was made in the first episode of TMA. Family history was reported in only 8% of cases (6/75).

The most frequent clinical characteristic was hypertension (76.8% of all cases), regardless of the age at diagnosis, followed by fatigue in patients older than 2 years of age (Table 1). Neurological manifestations were more frequent in patients <18 years of age than in adults (42.5% versus 32.5%). Drowsiness and seizures were the most frequent neurological findings in both groups. Gastrointestinal manifestations were also more often observed in children than adults (45.5% versus 30%), and nausea and vomiting were most frequently reported. Among the 75 cases, 22 cases (29%) were kidney-transplanted recipients, 18 of whom (81.8%) were older than 18 years of age (Table 1).

Among the 26 adult female patients, five (19.2%) were diagnosed at pregnancy. A history of concomitant infectious disease was detected in 16.2% of the total population. History of drug use was present in 20% of the cases—all of them kidney-transplanted patients—tacrolimus (11 cases), everolimus (2 patients), cyclosporine (1 case) and sirolimus (1 case) (Table 2). There was no patient with cobalamin metabolism defect.

The most common aHUS-associated condition in the age group <2 years was malignant hypertension, present in 5.9% of total cases; the infection was most associated with aHUS in the age group between 2 and 18 years (33.3%), and in age >18 years the principal conditions associated with aHUS were medications (39.5%) followed by infections (10.8%).

Hematological exams at baseline

Anemia, negative direct Coombs Test, platelet consumption, presence of schistocytes and high levels of LDH were reported in all age groups. The levels of hemoglobin ($P = .01$) and platelets ($P = .003$) were significantly lower and LDH levels were significantly higher ($P = .004$) in children compared with adult patients (Table 3; Supplementary data, Fig. S2). The haptoglobin was reduced in the three groups and serum C3 complement was reduced in 26.7% of total cases (Table 3).

Table 3. Baseline laboratory exams at diagnosis onset in Brazilian aHUS registry patients divided by age: <2 years old, between 2 and 18 years old and >18 years old

	<2 years old (*n* = 17)	2–18 years old (*n* = 18)	>18 years old (*n* = 40)	Total (*n* = 75)	*P*-value
Hemoglobin (g/dl)	6.0 (5.3–6.9)	6.5 (6.0–7.4)	7.7 (6.2–9.4)	7.0 (6.0–8.6)	.012
Coombs Test positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Platelets ($\times 10^3$/mm^3)	53 (34–55)	55 (35.7–88.7)	89.5 (53–126.7)	65 (40–107)	.003
LDH (U/dl)	1855 (1484–3408)	2097 (1186–2625)	1000 (677–1567)	1400 (850–2344)	.004
Schistocyte	14 (100.0%)	13 (86.7%)	25 (71.4%)	52 (81.2%)	.150
Not performed	0 (0.0%)	2 (13.3%)	6 (17.1%)	8 (12.5%)	
Haptoglobin (mg/dL)	12.5 (10–26)	12 (6–16)	20 (6–37)	13 (7–30)	.388
Proteinuria					.493
Absent	1 (7.7%)	1 (6.7%)	3 (11.1%)	5 (9.1%)	
nephrotic	5 (38.5%)	2 (13.3%)	6 (22.2%)	13 (23.6%)	
Not nephrotic	6 (46.2%)	11 (73.3%)	15 (55.6%)	32 (58.2%)	
albuminuria	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (1.8%)	
Not performed	1 (7.7%)	0 (0.0%)	3 (11.1%)	4 (7.3%)	
Creatinine (mg/dL)	1.9 (1.5–2.1)	4.8 (2.8–9.4)	4.6 (2.7–7.6)	3.9 (1.9–7)	.003
eGFR (mL/min)	14.2 (7–15.6)	14.6 (9.4–37)	12.6 (7.7–26)	14.2 (8.1–23.3)	.715
ADAMTS-13 activity (%)	93 (40–100)	87 (85–100)	79 (70–98)	85 (68–100)	.424
Shiga Toxin PCR					.007
Negative	5 (33.3%)	5 (33.3%)	2 (5.4%)	12 (17.9%)	
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Not performed	10 (66.7%)	10 (66.7%)	35 (94.6%)	55 (82.1%)	
Stool culture					.199
Negative	5 (38.5%)	7 (43.8%)	7 (20.6%)	19 (30.2%)	
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Not performed	8 (61.5%)	9 (56.2%)	27 (79.4%)	44 (69.8%)	
Antinuclear factor test					.016
Negative	10 (71.4%)	16 (100.0%)	28 (75.7%)	54 (80.6%)	
Positive	0 (0.0%)	0 (0.0%)	6 (16.2%)	6 (9.0%)	
Not performed	4 (28.6%)	0 (0.0%)	3 (8.1%)	7 (10.4%)	
Complement C3 serum					.927
Normal	8 (72.7%)	9 (69.2%)	21 (75.0%)	38 (73.1%)	
Reduced	3 (27.3%)	4 (30.8%)	7 (25.0%)	14 (26.9%)	
Complement C4 serum					.314
Normal	9 (81.8%)	13 (92.9%)	26 (96.3%)	48 (92.3%)	
Reduced	2 (18.2%)	1 (7.1%)	1 (3.7%)	4 (7.7%)	
Kidney biopsy	6 (42.9%)	10 (58.8%)	28 (80.0%)	44 (58.6%)	.033

Continuous variables were expressed as median and percentiles 25 and 75. LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate.

Renal biopsy

Kidney biopsy reports were described for 44 (58.7%) of patients and it was more frequently performed in adults when compared with pediatric patients (80% versus 58.8% and 42.9%, $P = .033$) (Table 3). Although there are specific blanks for filling with the description of light microscopy, immunofluorescence microscopy and electron microscopy reports, most physicians reported only the diagnosis of TMA (Supplementary data, Table S02).

Evolution of kidney function

Among the 75 patients enrolled in the registry, 45% were on dialysis 3 months after diagnosis, ranging from 42.5% (≥18 years of age) to 50% (between 2 and 18 years of age) (Table 4). Most patients were classified as CKD stage 5 at 3 months (46.2% of the total cases) (Table 4). Evolution of kidney function in patients grouped <18 years and >18 years are provided in Supplementary data, Table S01.

Treatment

Plasma exchange was more frequently performed in adults (42.1%) than children (20%), $P = .005$. There was a high frequency of eculizumab treatment in all age groups, reaching 97.3% of the total population (Tables 5 and 6). The median time to eculizumab administration after aHUS diagnosis was 25 (7–188) days with no significant difference among the three groups. In dialysis-free patients, the median time to eculizumab administration was 16 days (6–87) compared with 34 (15–372) days in ongoing-dialysis cases at 3 months of follow-up, $P = .081$ (Fig. 1).

Genetics

Genetic analysis was performed in 33/75 cases (44%). Overall, the most frequent variants identified were in *CFH* (7 patients) and the *CFHR1-3* deletion (7 patients) (Table 5). Other genetic variants were identified in other *CFHR* (*CFH*-Related proteins) (18% of patients), *CFI* (12%), *C3* (9%), *CFB* (3%) and *CD46* (3%) (Table 5).

Table 4. Clinical evolution of Brazilian aHUS Registry patients divided by age: <2 years old, between 2 and 18 years old and >18 years old

	<2 years old ($n = 17$)	2–18 years old ($n = 18$)	>18 years old ($n = 40$)	Total ($n = 75$)	P-value
Renal injury within 3 months (*n*/%)					
No kidney damage	2 (14.4%)	1 (7.7%)	4 (16%)	7 (13.5%)	.334
Chronic kidney disease stage 1	1 (7.1%)	2 (23.1%)	1 (4%)	5 (9.6%)	
Chronic kidney disease stage 2	1 (7.1%)	2 (15.4%)	2 (8%)	5 (9.6%)	
Chronic kidney disease stage 3	2 (14.3%)	1 (7.7%)	6 (24%)	9 (17.3%)	
Chronic kidney disease stage 4	2 (14.3%)	0	0	2 (3.8%)	
Chronic kidney disease stage 5	6 (42.9%)	6 (46.2%)	12 (48%)	24 (46.2%)	
Dialysis need	8 (47%)	9 (50%)	17 (42.5%)	34 (45%)	.956
Treatment within 3 months (*n*/%)					
Red blood cells transfusion	15 (93.8%)	14 (82.4%)	22 (59.5%)	51 (72.9%)	.095
Platelet transfusion	10 (66.7%)	4 (23.5%)	8 (21.1%)	22 (31.4%)	.019
Plasma transfusion	6 (42.9%)	7 (43.8%)	12 (32.4%)	25 (37.3%)	.891
Plasma exchange	0	3 (20%)	16 (42.1%)	19 (27.5%)	.005
Treatment ≥3 months (*n*/%)					
Eculizumab use	16 (94.1%)	16 (94.1%)	39 (100%)	71 (97.3%)	.307
Time eculizumab infusion (days)	15 (14–25)	30 (14–44)	45 (6–260)	25 (7–118)	.600

Table 5. Genetic variants in aHUS Brazilian Registry divided by age ≤18 years old and >18 years old

	≤18 years ($n = 35$)	>18 years ($n = 40$)	Total ($n = 75$)
Genetic test performed	14 (40%)	19 (47.5%)	33 (44%)
Patients where genetic test were performed			
Negative genetics (*n*/%)	6/43	5/26	11/33,5
CFH (*n*/%)	2/14	5/26	7/21
CFHR1/R3 deletion	2/14	5/26	7/21
Other *CFHR* (*n*/%)	3/21	3/16	6/18
CFI (*n*/%)	1 (VUS)/7	3/16	4/12
C3 (*n*/%)	0/0	3/16	3/9
CFB (*n*/%)	0/0	1/5	1/3
CD46 (*n*/%)	0/0	1/5	1/3
Not specified (*n*/%)	1/7	1/5	0/0

CFHR, *CFH*-Related protein; VUS, variant of unknown significance.

Table 6 shows detailed genetic results by patient and age of manifestation, including 5 patients with combined genetic abnormalities identified: *CFH* + *CFHR1/R3* del ($n = 1$), *CFH* + *CFI* (VUS) ($n = 1$), *CFI* + *CFB* ($n = 1$), *CFI* + *C3* ($n = 1$), *CFI* + *CFHR1/R3* del ($n = 1$) (Table 6). Negative genetic tests were found in 33.5% of the cohort. The genetic profile was similar between pediatric and adult patients (Table 5).

Summary of worldwide registries or case series

Supplementary data S2 highlights a summary of cohort data from other registries or significant case series around the world that we selected to be compared with this current Brazilian Registry. In this table, we performed a review of the clinical, laboratory and genetic data, response to treatment and mortality of aHUS patients, from those pediatric and adult cohorts.

DISCUSSION

aHUS is a rare disease and registries are useful to evaluate the natural history and progression of the disease as well as to address some questions related to diagnosis and treatment. Although investigators are aware about the influence of ethnic background on genetic abnormalities and characteristics of the disease, there are a few registries of patients with different ethnic backgrounds [8, 10, 11, 12, 16–18]. In this first Brazilian aHUS Registry report, demographic, clinical, laboratory and genetic data were analyzed. Patients were divided in three age groups based on observations from previous studies regarding differences among different ages such as gender, genetic findings, triggers and outcomes [8, 16, 19, 20]. aHUS is a disease that can affect adults and children. There was a slightly higher prevalence in adults aged >18 years (53.4%) as reported by others [16, 17, 20, 21].

In the pediatric group (<18 years of age), the frequency of aHUS in patients <2 years of age was 48.5%. This finding was similar to what was observed in the global and French registries (43.9% and 56%, respectively) [16, 19] yet, in the Turkish Pediatric Registry the percentage of cases <2 years of age was lower, around 36% [8].

The female gender was predominant in all ages except in the very young group. The female predominance in adults was previously described [12, 16–20] as was the highest rate of males in the <2 years of age group [16]. However, the Pediatric Turkish Registry revealed a female prevalence in children (57.6%) [8] even in those <2 years of age (57%) [11]. In addition, Lee *et al.* have

Table 6. Detailed genetic variants in aHUS Brazilian Registry

Variant	Number of cases	Female/male	Age at diagnosis
CFH	5	3/2	4 mo; 1y; 23y; 29y; 32y
del *CFHR1/R3*	5	4/1	1.5y; 2.2y; 8y; 20y; 29y
CFHR1	1	1/0	28y
C3	2	1/1	29y; 32y
CD46	1	1/0	31y
CFHR2	1	0/1	20y
CFHR3	2	1/1	17y; 44y
CFHR5	2	2/0	3 mo; 17y
CFH + del *CFHR1/R3*	1	0/1	20y
CFH + *CFI* (VUS)	1	1/0	16y
CFI + *CFB*	1	0/1	34y
CFI + *C3*	1	1/0	22y
CFI + del *CFHR1/R3*	1	0/1	38y
Heterozygous variant in *ADAMTS13*	1	0/1	49y
PLAT	1	0/1	26y

y, years; mo, months of age; VUS, variant of unknown significance.

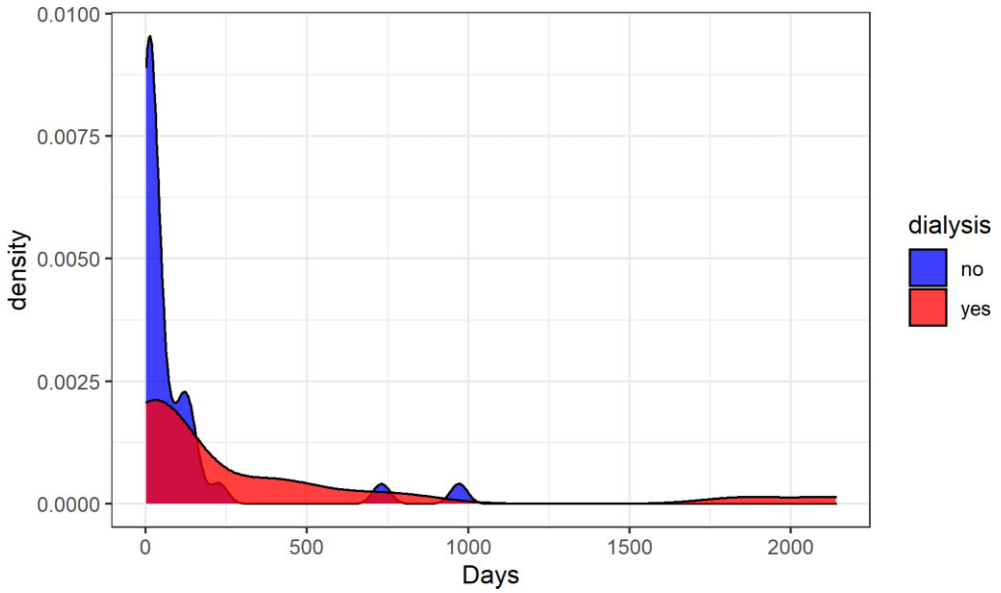


FIGURE 1: Density distribution of the time between aHUS diagnosis and eculizumab infusion in days. Patients are divided in two groups by dialysis need: the blue color refers to dialysis independent and red color to dialysis dependent.

reported the same proportion of male and female patients from a pediatric Korean cohort [10].

In this Brazilian Registry, family history was reported in only 8% of cases, much lower when compared with the aHUS Global Registry (20.4%) [20], but in accordance with the Canadian and Australian cohorts of the Global Registry, 5.4% and 10%, respectively [17, 18], and with the Turkish Pediatric Registry, in which only 4.8% of cases had a positive family history (despite a high consanguinity rate) [8]. A higher rate of positive family history has been found in children compared with adult patients in some cohorts [16, 20, 21].

The diagnosis of aHUS demands tailored steps and, not infrequently, aHUS is considered a diagnosis of exclusion [4, 22]. Traditionally, the causes of TMA are divided into primary and secondary. Primary TMA is designated when the endothelial injury mechanism is known. Classically, this group encompasses thrombotic TTP, Shiga Toxin uremic hemolytic syndrome (STEC-HUS) and aHUS. Secondary TMA usually occurs in the context of other diseases, frequently systemic and TMA tends to resolve with treatment or removal of the underlying cause [23].

In the Global aHUS Registry, diagnosis accuracy is not checked for each new entry case [20]. In this Brazilian Registry, for every patient TTP and STEC-HUS was excluded. Also, physicians were cautious with secondary causes, excluding cobalamin metabolism defects, neoplasia, scleroderma, antiphospholipid syndrome and other causes (infection and drugs) before designating a patient with aHUS. The diagnosis of aHUS must be established after the resolution of the infection and withdrawal of TMA-inducing medications by a minimum of 1 week. These steps were also part of the Brazilian Registry database, which directly instructed physicians during data entry through alerts and notes. For instance, if a value of

ADAMTS-13 activity <10% was inputted, the system showed a red alert informing that the aHUS diagnosis must be revised. In cases of drug-induced TMA, there was an extensive checkbox list with all possible medications. Additionally, there was a note that guided the physician to suspend the medication for a minimum of 1 week to validate the aHUS diagnosis if TMA persisted.

A kidney manifestation was almost universally present in all age groups (elevated serum creatinine, low creatinine clearance and/or proteinuria). Importantly, hypertension was the most frequent manifestation and occurred in 86.2% of the total cohort with no difference according to the age group. Yun *et al.* have also reported a high percentage of hypertension (64%) in aHUS adults (Korean TTP and TMA Registry) [12]; however, Lee *et al.* found only 47% of hypertension in the Pediatric Korean cohort [10].

In this Brazilian aHUS Registry, neurological and gastrointestinal manifestations were more frequently in pediatric patients than adults. Those manifestations have been evaluated in other registries and case series with great variability [8, 11, 20, 22, 23]. In our registry, fatigue was a frequent finding and it is a very important patient-reported symptom that has been studied by Greenbaum *et al.* in patients from the Global Registry of aHUS. The recovering of fatigue remained over time with continuous treatment with eculizumab [24].

Laboratory exams at diagnosis showed that pediatric patients had a different profile, presenting with lower levels of hemoglobin and platelets compared with adults as well as higher levels of LDH. These could suggest that children have a more pronounced hemolytic effect compared with adults. To the best of our knowledge, these aHUS laboratory patterns were rarely described earlier. Frémeaux-Bacchi *et al.* have already observed lower hemoglobin and platelet levels in children compared with adults, but no mention was made of higher LDH levels in their paper [16]. In addition, a high proportion of patients evolved with dialysis dependence in the first 3 months (45%), regardless of age, and a very high percentage of the cohort was treated with eculizumab (97.3%).

Among patients with genetic analysis, we found 33.5% negative compared with 40% in the Global aHUS Registry [20] and compared with 78.4% in the Canadian cohort of the Global aHUS Registry [17]. In the Pediatric Turkish Registry, 81% of patients had at least one mutation [8]; however, Çakar *et al.* studying the <2 years of age group from the same population, detected only 14/53 (26%) of positive mutation rate [11]. In addition, Yun *et al.* found a higher rate of positivity when the number of genes analyzed was increased [12].

The genetic findings in the Brazilian Registry are in agreement with those from the Global Registry in which *CFH* mutations were prevalent regardless of age group, in addition to which *CFI* variants were not identified in pediatric patients [20]. In 66 adults diagnosed with aHUS from the Korean TTP and TMA Registry, *CFH* mutations were prevalent (20%) followed by *THBD* mutations (14%), but a recurrent missense variant was observed in *THBD*, Asp486Tyr [12]. Yet, in a Pediatric Korean cohort, there was a predominance of AntiCFH antibodies (29%) [10]. In the Pediatric Turkish Registry, *MCP* variants were the most frequent, followed by C3 mutations [8].

We identified a higher proportion of variants in genes encoding Factor H-related proteins (*CFRH*) compared with the Italian and French cohorts [25, 26]. We detected the *CFHR1-3* del in a high proportion of patients and it is important to emphasize that the presence of this deletion is related to presence of Anti-CFH antibodies [8], which were not evaluated in this current Brazilian Registry report.

All these findings taken together show that the rate of positivity as well as the spectrum of mutations can vary with the region and the genes analyzed (Tables 5 and 6). The Brazilian population has particularities such as the high rate of miscegenation and several ethnic origins. These factors can also determine different genetic and clinical characteristics of this disease in this population. More studies are needed to explore the potential differences [27–29].

Eculizumab was administered to 97.3% of the patients compared with 68% of the Australian cohort Registry [18] and superior to aHUS Global Registry (59.1%) [19]. This could be explained because the Brazilian aHUS Registry is relatively recent (it was created in 2017) combined with strictly aHUS criteria to enter data in the study. In Brazil, eculizumab has been available since 2011 with a progressive rise in aHUS therapy since then [30].

We also showed that patients with lower time between diagnosis and eculizumab infusion had a lower probability of being on dialysis at the 3-month follow-up (Fig. 1), which was similar to previous reports [31]. A more recent publication from the Global Registry compared Eculizumab-treated and untreated patients and showed that treated patients presented more severe clinical picture, but with low mortality rate [21]. Data on kidney or transplant loss or actual graft function are under analysis.

Among the strengths of this registry, we highlight the verification of the accuracy of aHUS diagnosis by the Committee members, as well as the fact that data were imputed by physicians. These actions have been recommended by Licht *et al.* [19] to improve the quality of the aHUS Global Registry.

Additionally, we provide details regarding clinical, laboratory and treatment data for these patients which have been rarely reported. We also provide data about laboratory diagnosis with missing data lower than 30%, except for haptoglobin, complement C3 and C4 values. Missing data report is a quality control tool and in this Brazilian aHUS Registry these data were reported [32].

The study has several limitations. Information regarding discontinuation of eculizumab and long-term renal outcomes in patients as well as allograft loss in kidney transplant recipients were not available. Additionally, we could not retrieve mortality data. Also, we were not able to check the pathogenicity of the variants and we had a lack of uniformity in the aHUS panel among centers.

In conclusion, we reported a cohort of aHUS Brazilian patients who were predominantly female young adults. aHUS patients had a high rate of renal involvement (100%) and the laboratory profile showed that pediatric patients had lower hemoglobin and platelet levels compared with adult patients, especially those <2 years of age. To the best of our knowledge, significant higher serum LDH levels in children is described for the first time in the current registry. The most common genetic variants were identified in *CFH* and the *CFHR1-3* deletion. We showed a high rate of eculizumab use, and the probability of dialysis-free evolution was correlated with shorter time between diagnosis and first infusion.

aHUS, as a genetic disease that can be influenced by precipitating factors, including some external ones, can vary among regions of the globe and populations [1, 3, 4]. Therefore, knowledge from different parts of the world is needed to complete the spectrum of genetic and clinical characteristics of this disease. This is an important contribution of the current Brazilian aHUS Registry.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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AUTHORS' CONTRIBUTIONS

M.H.V., C.A.B.S., L.C.S., G.B., V.S.P.V., P.M.F., V.S.C., J.G.G., A.F.P.L., L.C.S., P.G.M.M., and O.M.V.-N. designed the Registry. M.H.V., L.G.M.A., L.M.P.P., M.C.R.C., M.I.N.H.B., M.G.G.P., O.A.F.N., R.M.L.S., S.M.C.M., H.M.T., C.R., R.M.S., C.A.A.C., D.J.B.M., A.M.S.T.S., A.R.S., E.R.R., F.H.S.B., J.C.L.N., L.S.S.O., L.C.S., R.W., and S.O.N. provided patient data. M.H.V., L.G.M.A., P.D.M.M.N., L.M.P.P., M.C.R.C., C.A.B.S., M.I.N.H.B., M.G.M.G.P., O.A.F.N., R.M.L.S. and S.M.C.M. provided intellectual content to the manuscript. M.H.V., L.G.M.A. and P.D.M.M.N. designed the study and were responsible for data analysis. M.H.V., L.G.M.A., P.D.M.M.N., L.M.P.P. and M.C.R.C. drafted and revised the article. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST STRATEMENT

M.H.V. reports lecture fees from Alexion Pharmaceuticals and grants from Roche. L.G.M.A. reports lecture fees from Alexion Pharmaceuticals, Takeda and Sanofi. L.M.P.P. reports lecture fees from Alexion Pharmaceuticals. M.C.R.C. reports lecture fees from Alexion Pharmaceuticals. M.I.N.H.B. reports lecture fees from Alexion Pharmaceuticals. The other authors declare that they have no conflict of interest. The results presented in this article have not been published previously in whole or part.

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