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

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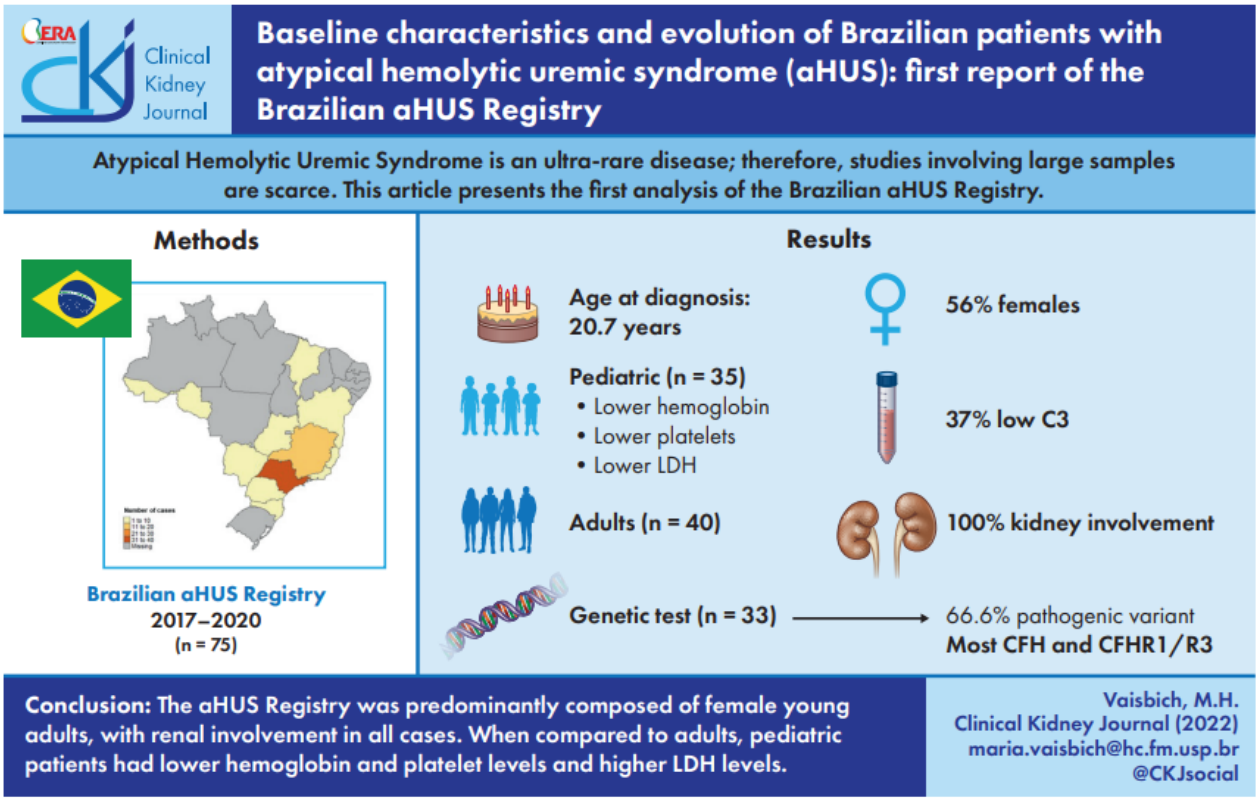
GRAPHICAL ABSTRACT



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. Cohort of 75 patients (40 adults and 35 pediatric). There was a predominance of females (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=0.01) and platelets (p=0.003), and higher levels of LDH (p=0.004) 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=0.005) and 97.3% of patients were treated with eculizumab and its earlier administration was associated with dialysis-free after 3 months (p=0.08).

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.

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 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, multicenter registry of patients with aHUS. The aims of the Registry are to assess 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 December 31st, 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 (# 09831719.7.0000.5411). 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 percent).
- 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 > 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 one 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, 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, lactate dehydrogenase (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 CKD-EPI 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 three months of first aHUS presentation.

The secondary outcomes were:

- Need for plasma exchange, blood, platelets, or plasma transfusions within the first three 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 ± 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 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 $p<0.05$.

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 with 49.3% of the total sample (Supplementary Figure 1). 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 < 18 years of age patients than adults (42.5% *vs* 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% *vs* 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%) 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 below 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 more than 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 level of hemoglobin (p=0.01) and platelets (p=0.003) were significantly lower and LDH levels were significantly higher (p=0.004) in children compared to

adult patients (Table 3, Supplementary Figure 2). The haptoglobin was reduced in the three groups and serum C3 complement was reduced in 26.7% of total cases (Table 3).

Renal Biopsy

Kidney biopsy reports were described for 44 (58.7%) of patients and it was more frequently performed in adults when compared to pediatric patients (80% vs 58.8% and 42.9%, p=0,033) (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 Table 02).

Evolution of kidney function

Among the 75 patients enrolled in the registry, 45% were on dialysis three months after diagnosis, ranging from 42.5% (≥ 18 years of age) to 50% (between 2 and 18 years of age) (Table 5). Most patients were classified as CKD stage 5 at 3 months (46.2% of the total cases) (Table 5). Evolution of kidney function in patients grouped below 18 years and more than 18 ys are provided in supplementary Table01.

Treatment

Plasma exchange was more frequently performed in adults (42.1%) than children (20%), p=0.005. There was a high frequency of eculizumab treatment in all age groups, reaching 97.3% of the total population (Table 4). 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 to 34 (15 – 372) days in ongoing-dialysis cases at three months of follow-up, p=0.081 (Figure 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 4A). Other genetic variants were identified in other *CFHR* (*CFH*-Related proteins) (18% of patients), *CFI* (12%), *C3* (9%), *CFB* (3%) and *CD46* (3%) (Table 4A). Table 4B 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 4B). Negative genetic tests were found in 33.5% of the cohort. The genetic profile was similar between pediatric and adult patients (Table 4A).

Summary of worldwide registries or case series

Supplemental 2 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 [8,10,11,12,17,22,23]. 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.[8,17,18,20] aHUS is a disease that can affect adults and children. There was a slightly higher prevalence in adults aged more than 18 years (53.4%) as reported by others.[17,20,21,22]

In the Pediatric group (< 18 years of age), the frequency of aHUS in patients under 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)[17,18] yet, in the Turkish Pediatric Registry the percentage of cases under 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 [12,17,18, 20, 22,23] as well as the highest rate of males in the under-2 year of age group.[17] 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 reported the same proportion of male and female patients from the Pediatric Korean cohort[10].

In this Brazilian Registry, family history was reported in only 8% of cases, much lower when compared to 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[22,23], 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 to adult patients in some cohorts [17, 20, 21].

The diagnosis of aHUS demands tailored steps and, not infrequently, aHUS is considered a diagnosis of exclusion.[4,24] 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 Thrombocytopenic Purpura (TTP), Shiga Toxin Uremic Hemolytic Syndrome (STEC-HUS) and Atypical Hemolytic Uremic Syndrome (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.[25]

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, 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 one 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 lower than 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 one 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.[8,20,23,11, 22] In our registry fatigue was a frequent finding and it is a very important patient-reported symptom that have been studied by Greenbaum et al in patients from the Global Registry of aHUS. The recovering of fatigue remained over the time with continuous treatment with eculizumab[26].

Laboratory exams at diagnosis showed that pediatric patients had a different profile presenting with lower levels of hemoglobin and platelets compared to adults as well as higher levels of LDH. These could suggest that children have a more pronounced hemolytic effect compared to adults. To the best of our knowledge, these aHUS laboratory patterns were rarely described earlier.

Fremeaux-Bacchi et al have already observed lower hemoglobin and platelet levels in children compared to adults, but no mention was made to higher LDH levels in their paper.[17] In addition, a high proportion of patients evolved with dialysis dependence in the first three 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 to 40% in the Global aHUS Registry[20] and compared to 78.4% in the Canadian cohort of the Global aHUS Registry[22]. In the Pediatric Turkish Registry, 81% of patients had at least 1 mutation[8]; however, Çakar et al, studying the < 2 years of age-group from the same population, detected only 14/53 (36%) 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 as well as *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 it was observed a recurrent missense variant 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 frequently followed by C3 mutations [8].

We identified a higher proportion of variants in genes encoding Factor H-related proteins (*CFRH*) compared to the Italian and French cohorts.[27,28] 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 (Table 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.[29–31]

Eculizumab was administered to 97.3% of the patients compared to 68% of the Australian cohort Registry[23], and superior to aHUS Global Registry (59.1%).[18] 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.[32]

We also showed that patients with lower time between diagnosis and eculizumab infusion had a lower probability to be on dialysis at the three-month follow-up (Figure 1), which was similar to previous reports.[33] 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*[18] 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 this data was reported.[34]

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 to adult patients, especially those under 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 which can be influenced by precipitating factors, including some external ones, can vary among regions of the globe and populations.[1,3,4] Therefore, the knowledge in 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 this current Brazilian aHUS Registry.

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Brazilian Society of Nephrology

AUTHORS' CONTRIBUTIONS

MHV, CABS, LCS, GB, VSPV, PMF, VSC, JGG, AFPL, LCS, PGMM, OMV-N designed the Registry. MHV, LGMA, LMPP, MCRC, MINH, MGGP, OAFN, RMLS, SMCM, HMT, CR, RMS, CAAC, DJBM, AMSTS, ARS, ERR, FHSB, JCLN, LSSO, LCS, RW, SON provided patient data. MHV, LGMA, PDMMN, LMPP, MCRC, CABS, MINHB, MGMGP, OAFN, RMLS e SMCM provided intellectual content to the manuscript. MHV, LGMA e PDMMN designed the study and were responsible for data analysis. MHV, LGMA, PDMMN, LMPP and MCRC drafted and revised the article. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST STRATEMENT

MHV reports lecture fees from Alexion Pharmaceuticals and grants from Roche. LGMA reports lecture fees from Alexion Pharmaceuticals, Takeda and Sanofi. LMPP reports lecture fees from Alexion Pharmaceuticals. MCRC reports lecture fees from Alexion Pharmaceuticals. MINHB 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.

DATA AVAILABILITY STATEMENT

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

REFERENCES

1. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009; 361(17):1676-87.

2. Dragon-Durey MA, Loirat C, Cloarec S, et al. Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2005; 16(2):555-63.

3. Campistol JM, Arias M, Ariceta G, et al. An update for atypical haemolytic uraemic syndrome: diagnosis and treatment. A consensus document. Nefrologia. 2015; 35(5):421-47.

4. Goodship TH, Cook HT, Fakhouri F, et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. Kidney Int. 201; 91(3):539-551.

5. Zuber J, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V. French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol. 2012; 8(11):643-57.

6. Nga HS, Palma LMP, Ernandes Neto M, et al. Thrombotic microangiopathy after kidney transplantation: Analysis of the Brazilian Atypical Hemolytic Uremic Syndrome cohort. PloS One. 2021; 16(11):e0258319.

7. de Andrade LGM, Contti MM, Nga HS, et al. Long-term outcomes of the Atypical Hemolytic Uremic Syndrome after kidney transplantation treated with eculizumab as first choice. PloS One. 2017; 12(11):e0188155.

8. Besbas N, Gulhan B, Soylemezoglu O, et al. Turkish pediatric atypical hemolytic uremic syndrome registry: initial analysis of 146 patients. BMC Nephrol. 2017; 18(1):6.

9. Palma LMP, Eick RG, Dantas GC, Tino MKDS, de Holanda MI; Brazilian Thrombotic Microangiopathy and Atypical Hemolytic Uremic Syndrome Study Group (aHUS Brazil). Atypical hemolytic uremic syndrome in Brazil: clinical presentation, genetic findings and outcomes of a case series in adults and children treated with eculizumab. Clin Kidney J. 2020; 14(4):1126-1135.

10. Lee JM, Park YS, Lee JH, et al. Atypical hemolytic uremic syndrome: Korean pediatric series. Pediatr Int. 2015; 57(3):431-8.

11. Çakar N, Ozcakar ZB, Ozaltin F, et al. Atypical Hemolytic Uremic Syndrome in Children Aged <2 Years. Nephron. 2018; 139(3):211-218.

12. Yun JW, Oh J, Lee KO, et al. Distinct genetic profile with recurrent population-specific missense variants in Korean adult atypical hemolytic uremic syndrome. Thromb Res. 2020; 194:45-53

13. Kodra Y, Weinbach J, Posada-de-la-Paz M, et al. Recommendations for Improving the Quality of Rare Disease Registries. Int J Environ Res Public Health. 2018; 15(8):1644.

14. Levey AS, Stevens LA, Schmid CH, et al. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150(9):604-12.

15. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. Clin J Am Soc Nephrol. 2009; 4(11):1832-43.

16. 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. 2010; 5(10):1844-59.

17. Fremeaux-Bacchi V, Fakhouri F, Garnier A, et al. Clin J Am Soc Nephrol. 2013; 8(4):554-62.

18. Licht C, Ardissino G, Ariceta G, et al. The global aHUS registry: methodology and initial patient characteristics. BMC Nephrol. 2015; 16:207.

19. Walle JV, Delmas Y, Ardissino G, Wang J, Kincaid JF, Haller H. Improved renal recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of eculizumab treatment. J Nephrol. 2017; 30(1):127-134.

20. Schaefer F, Ardissino G, Ariceta G, et al. Global aHUS Registry. Clinical and genetic predictors of atypical hemolytic uremic syndrome phenotype and outcome. Kidney Int. 2018; 94(2):408-418.

21. Rondeau E, Cataland SR, Al-Dakkak I, Miller B, Webb NJA, Landau D. Eculizumab Safety: Five-Year Experience From the Global Atypical Hemolytic Uremic Syndrome Registry. Kidney Int Rep. 2019; 4(11):1568-1576.

22. Lapeyraque AL, Bitzan M, Al-Dakkak I, et al. Clinical Characteristics and Outcome of Canadian Patients Diagnosed With Atypical Hemolytic Uremic Syndrome. Can J Kidney Health Dis. 2020; 7:2054358119897229.

23. Soraru J, Isbel N, Wong G, et al. Baseline characteristics of patients with atypical haemolytic uraemic syndrome (aHUS): The Australian cohort in a global aHUS registry. Nephrology (Carlton). 2020; 25(9):683-690.

24. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011; 6:60.

25. Fox LC, Cohney SJ, Kausman JY, et al. Consensus opinion on diagnosis and management of thrombotic microangiopathy in Australia and New Zealand. Nephrology (Carlton). 2018; 23(6):507-517.

26. Greenbaum LA, Licht C, Nikolaou V, et al. Functional Assessment of Fatigue and Other Patient-Reported Outcomes in Patients Enrolled in the Global aHUS Registry. Kidney Int Rep. 2020; 5(8):1161-1171.

27. Ardissino G, Testa S, Possenti I, et al. Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome: a report of 10 cases. Am J Kidney Dis. 2014; 64(4):633-7.

28. Fakhouri F, Fila M, Provôt F, et al. Pathogenic Variants in Complement Genes and Risk of Atypical Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. Clin J Am Soc Nephrol. 2017; 12(1):50-59.

29. Moura RR, Coelho AV, Balbino Vde Q, Crovella S, Brandão LA. Meta-analysis of Brazilian genetic admixture and comparison with other Latin America countries. Am J Hum Biol. 2015; 27(5):674-80.

30. Pena SDJ, Santos FR, Tarazona-Santos E. Genetic admixture in Brazil. Am J Med Genet C Semin Med Genet. 2020; 184(4):928-938.

31. Kehdy FS, Gouveia MH, Machado M, et al. Brazilian EPIGEN Project Consortium. Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. Proc Natl Acad Sci U S A. 2015; 112(28):8696-701.

32. Neto ME, de Moraes Soler L, Vasconcelos HVG, et al. Eculizumab interruption in atypical hemolytic uremic syndrome due to shortage: analysis of a Brazilian cohort. J Nephrol. 2021; 34(4):1373-1380.

33. Siedlecki AM, Isbel N, Vande Walle J, James Eggleston J, Cohen DJ. Global aHUS Registry. Eculizumab Use for Kidney Transplantation in Patients With a Diagnosis of Atypical Hemolytic Uremic Syndrome. Kidney Int Rep. 2018; 4(3):434-446.

34. Lazem M, Sheikhtaheri A, Hooman N. Lessons learned from hemolytic uremic syndrome registries: recommendations for implementation. Orphanet J Rare Dis. 2021; 16(1):240.

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

	<2 years-old (n=17)	2-18 years-old (n=18)	>18years-old (n=40)	Total (n=75)	p value
Female (n / %)	3 (17.6%)	13 (72.2%)	26 (65%)	42 (56%)	0.001
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)	<0.001
Family history (n / %)	1 (6.2%)	1 (5.6%)	4 (10%)	6 (8.1%)	0.809
Previous hypertension (n / %)	3 (30%)	7 (50%)	31 (93.7%)	41 (71.9%)	<0.001
Kidney Transplant (n / %)	1 (5.8%)	3 (16.6%)	18 (45%)	22 (29%)	<0.001
Clinical presentation (n / %)					
Hypertension	14 (87.5%)	11 (64.7%)	31 (77.5%)	56 (76.7%)	0.516
Diarrhea	3 (17.6%)	2 (11.8%)	5 (12.5%)	10 (19.6%)	0.831
Dyspnea	3 (30%)	1 (12.5%)	8 (32%)	12 (27.9%)	0.556
Fatigue	3 (27.3%)	8 (72.7%)	30 (85.7%)	41 (71.9%)	<0.001
Elevated Creatinine	16 (94.1%)	16 (94.1%)	37 (94.9%)	69 (94.5%)	0.990

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

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

	<2 years-old (n=17)	2-18 years-old (n=18)	>18years-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%)	0.911
Pregnancy	0 (0.0%)	0 (0.0%)	5 (13.5%)	5 (7.0%)	0.084
SLE	0 (0.0%)	0 (0.0%)	1 (2.8%)	1 (1.4%)	0.619
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%)	0.122
Neoplasia	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0
Medications*	0 (0.0%)	0 (0.0%)	15 (39.5%)	15 (20.8%)	< 0.001

APS: Antiphospholipid syndrome. HTN: Hypertension. SLE: Systemic Lupus Erythematous. *TMA-inducing medications

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

	<2 years-old (n=17)	2-18 years-old (n=18)	>18years-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)	0.012
Coombs Test Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Platelets ($x10^3$ / mm^3)	53 (34 - 55)	55 (35,7 - 88,7)	89,5 (53 - 126,7)	65 (40- 107)	0.003
LDH (U/dl)	1855 (1484- 3408)	2097 (1186- 2625)	1000 (677- 1567)	1400 (850- 2344)	0.004
Schistocyte	14 (100.0%)	13 (86.7%)	25 (71.4%)	52 (81.2%)	0.150
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)	0.388
Proteinuria					0.493
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)	0.003
eGFR (ml/min)	14.2 (7 - 15.6)	14.6 (9.4 - 37)	12.6 (7.7 - 26)	14.2 (8.1 - 23.3)	0.715
ADAMTS-13 activity (%)	93 (40- 100)	87 (85- 100)	79 (70- 98)	85 (68-100)	0.424
Shiga Toxin PCR					0.007
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					0.199
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					0.016
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					0.927
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					0.314
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%)	0.033

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

Table 4a. 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

Table 04b. Detailed Genetic Variants in aHUS Brazilian Registry

Variant	Number of cases	Female/Male	Age at diagnosis year=y; mo= months
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; 17 y
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

Table 5. Clinical evolution of Brazilian aHUS Registry patients divided by age: below 2 years-old, between 2 and 18 years-old and >18 years-old

	<2 years-old (n=17)	2-18 years-old (n=18)	>18years-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%)	0.334
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%)	0.956
Treatment within 3 months (n/%)					
Red blood cells transfusion	15 (93.8%)	14 (82.4%)	22 (59.5%)	51 (72.9%)	0.095
Platelet's transfusion	10 (66.7%)	4 (23.5%)	8 (21.1%)	22 (31.4%)	0.019
Plasma transfusion	6 (42.9%)	7 (43.8%)	12 (32.4%)	25 (37.3%)	0.891
Plasma exchange	0	3 (20%)	16 (42.1%)	19 (27.5%)	0.005
Treatment ≥3 months (n/%)					
Eculizumab Use	16 (94.1%)	16 (94.1%)	39 (100%)	71 (97.3%)	0.307
Time eculizumab infusion (days)	15 (14 – 25)	30 (14 -44)	45 (6 – 260)	25 (7 – 118)	0.600

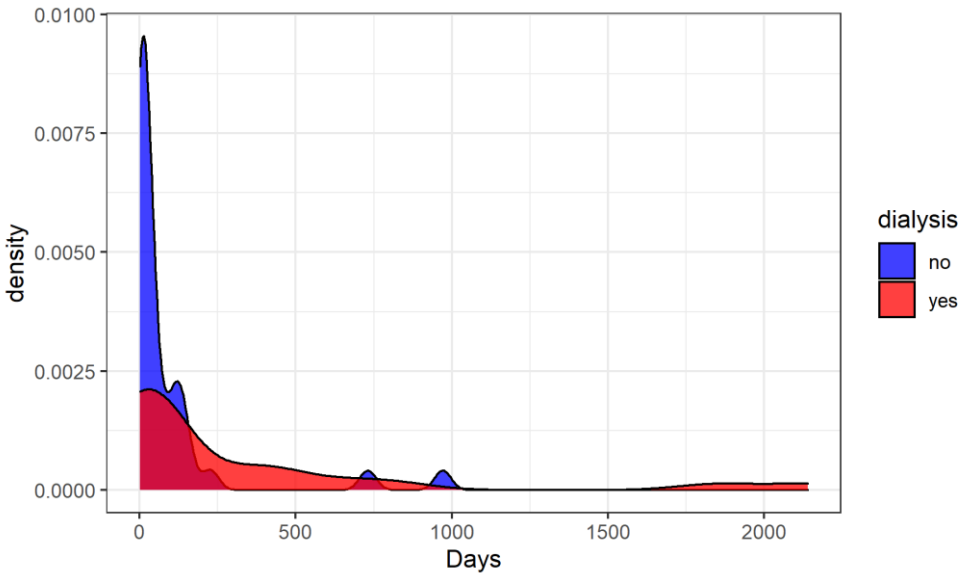

Figure 1. Density distribution of the time between aHUS diagnosis and eculizumab infusion in days. Patients are divided in two groups by dialysis need: the blue color refers to dialysis independent and red color to dialysis dependent.