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Screening for Fabry Disease among Dialysis Patients in Brazil: Findings from the First 18 months of a Nationwide Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author MPC managed the literature search, designed the study, collected, analyzed and interpreted the data, wrote and critically reviewed the study. Author OMVN did the literature search, collected and interpreted the data and critically reviewed the study. Author JCBA did the data analysis, data interpretation and figures. Author TMS wrote the study, managed the literature search, analyzed and interpreted the data and prepared figures and tables, critically reviewed the study and arranged the references. Author JEPL did the literature search, data collection, data interpretation and critical review. Author LRB did the literature search and data collection. Author MGR did the literature search, study design, data collection, data analysis, data interpretation, writing and critical review of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To estimate the frequency of Fabry disease (FD) among kidney failure patients on dialysis in Brazil using an algorithm designed to track FD-suspected patients.
Study Design: Cross-sectional study.
Place and Duration of Study: Dialysis Centers in Brazil, from July, 2013 to December, 2014.
Methodology: A total of 25,223 dialysis patients from 188 dialysis centers spread all over the country were analyzed. All collected data were entered in a database created and maintained by DataGenno Interactive Research®. An algorithm was created to sort dialysis patients into three main groups: FD-suspected patients, FD-non suspected patients, and patients for medical analysis. Further up, FD-suspected patients were submitted to *GLA* gene sequencing.
Results: Out of 25,223 patients, 2,956 (11.72%) were considered FD-suspected. From FD-suspected patients, 89 (3.0%; 2.0% female, 1.0% male) were diagnosed with FD. FD-positive patients represented 0.3% (0.2% female, 0.1% male) of all analyzed patients. Average age of FD-positive patients**:** 37.7 years (±16.6) and of FD-negative patients: 45.1 years (±11.5). Seventeen different mutations were found in FD-positive patients. Missense mutations c.352C>T(R118C), c.1102G>A(A368T) and c.870G>C(M290I) were the most frequent (60.7% of the patients). A368T and R118C were more frequent among 30 patients with depression. Six female patients had cerebrovascular disease and A368T mutation was more frequent. A368T, R118C and M290I were more frequent in patients with heart disease. Angiokeratoma frequency (14.6%) was higher than in previous findings in the Brazilian population.
Conclusion: The natural history and frequency of FD among Brazilian dialysis patients were found, in general, according to literature. Three missense mutations were highly frequent among FD-positive patients; none of them were directly related to end-stage renal disease caused by FD. The algorithm used could be a helpful tool to identify FD.

Keywords: Fabry disease; lysosomal storage disorders; end-stage renal disease; dialysis; screening; mutation.

ABBREVIATIONS

α-Gal A : Alpha-galactosidase A
CKD : chronic kidney disease
DM : diabetes mellitus
ESRD : end-stage renal disease
FD : Fabry disease
Gb3/GL3 : globotriaosylceramide
GLA : galactosidase, alpha gene
HBP : high blood pressure
HGMD : Human Gene Mutation Database
LOVD : Leiden Open-source Variation Database
MGR : Márcia Gonçalves Ribeiro
MPC : Marcelo Paula Coutinho

1. INTRODUCTION

Fabry disease (MIM: 301500), an X-linked lysosomal storage disorder, with an estimated incidence of 1:40,000–170,000, is caused by a deficiency of the alpha-galactosidase A (α-Gal A) enzyme, resulting in storage of globotriaosylceramide (Gb3/GL3) and related glycosphingolipids in the plasma and cellular lysosomes [1-3]. Currently, 845 variants in the galactosidase, alpha gene (*GLA,* MIM: 300644) have been described [4], most in single families [1]. Fabry disease (FD) is a chronic progressive condition, clinically heterogeneous with symptoms such as chronic neuropathic pain, acute pain crises, abdominal pain, heat and cold intolerance, and fatigue often beginning in childhood [2,3,5,6]. The average presentation age is: 6–8 years (y) (males) and 9y (females), although it may vary from individual to individual even within the same family [5,7,8]. The storage of Gb3/GL3 can result in: angiokeratoma, tinnitus, hearing loss, corneal whorls, vertigo, transient ischemic attacks, stroke, cardiomyopathy, left ventricular hypertrophy, cardiac arrhythmias and valve insufficiency, chronic alternating diarrhea and constipation, obstructive pulmonary disease, proteinuria, progressive renal disease, panic attacks, depression, and adaptive function disorders [2,3,5,7]. Heterozygous women may also be affected, with more variable phenotype [9-11]. Life expectancy is diminished, more apparent in men [12].

FD manifestations tend to be non-specific and often unrecognized. Patients are therefore

frequently misdiagnosed or delayed diagnosed [2,3,5,7]. Screening of FD patients in high-risk populations allows the diagnostic investigation and confirmation, the identification of asymptomatic/oligosymptomatic affected relatives and genetic counseling for couples at risk. This corroborates the importance of an early diagnosis of FD in these populations [1,13]. An increasing number of screening studies in high-risk populations and newborn screening studies have been performed since enzyme replacement therapy became available [1,13,14]. The interest of nephrologists in FD increased after the description of the "renal variant" phenotype – patients without classic symptoms of FD who develop end-stage renal disease (ESRD) [15]. Large-scale screening efforts of ESRD populations in dialysis treatment have been carried out [1,13,16], as ESRD is an important outcome in FD. Currently, Brazil has around 90,000 patients with ESRD being treated in 692 dialysis centers (Ministry of Health; Fig. 1) [17]. Considering FD prevalence from 0.12-0.94% in dialysis centers [16], the estimative of FD patients in Brazil would be from 108–846. This number can increase as FD may be characterized as a family trait disorder [2,18].

The main objective of this study was to estimate the frequency of FD among Brazilian dialysis patients using a screening tool designed for screening FD-suspects.

2. METHODOLOGY

2.1 Study Population

A cross-sectional study was undertaken from July 2013 to December 2014, with kidney failure patients from dialysis centers in Brazil. It's part of a large ongoing study that aims to assess around 90,000 kidney failure patients in order to track FD-suspected patients and may be considered a pilot study. Inclusion criteria: kidney failure patients on hemodialysis or peritoneal dialysis from dialysis centers throughout Brazil (Fig. 1), both sexes, having or not underlying causes of chronic kidney disease – CKD: high blood pressure (HBP), diabetes mellitus (DM), obesity, rheumatoid arthritis, polycystic kidney disease, and Berger's disease [16,17]. Exclusion criteria: patients with confirmed laboratory and/or clinical diagnosis of underlying causes of CKD ruling out the possibility of FD. Underlying causes of CKD which might be present in patients enrolled in the study were different from those considered as exclusion criteria.

2.2 Ethics, Consent and Permissions

The study protocol was approved by the Research Ethics Committee of Campos Medical School (legal opinion #305·988; 06/28/2013). Patients invited to participate were informed about the study purposes and each subject freely signed an individual consent form agreeing to participate. Patients and the health care team involved had their anonymity fully preserved according to Resolution no. 196/96 of the National Board of Health, 1988 Medical Ethics Code and 1964 Declaration of Helsinki.

2.3 Screening Strategy

The screening strategy started with an invitation letter sent to all Brazilian dialysis centers. After the Ethics Committee's approval and formal acceptance by the head of the dialysis center, clinical questionnaires were sent to be answered by dialysis patients. Dialysis centers' healthcare staffs, mainly nurses, were trained to apply the questionnaire. Questionnaires with filling inconsistencies were sent back to be reapplied. The results were analyzed by a team of medical specialists (MPC - Marcelo Paula Coutinho and MGR – Márcia Gonçalves Ribeiro). It should be clear that this study did not actively request the participant dialysis centers to run FD tests or send FD test results they have decided to run by themselves. However, the dialysis centers kindly sent us FD test results of the FD-suspected patients to the study investigators. The flowchart is in Fig. 2.

2.3.1 Clinical questionnaire

The clinical questionnaire (Fig. 3) has a list of questions about FD signs and symptoms [2,3,5,7] that were divided into seven groups: 1, nephrological; 2, cardiological; 3, rheumatological; 4, neurological; 5, gastrointestinal/ otorhinolaryngological; 6, dermatological; 7, ophthalmological. The questionnaire also has questions about underlying causes of CKD [19,20]. Content validity was done by clinical geneticists and nephrologists. The questionnaire was previously applied to 88 dialysis patients: five with FD (positive molecular test) and 83 without FD (negative molecular test); all five FD patients were considered suspected for FD, and the remaining were considered non-suspected by the algorithm (unpublished data).

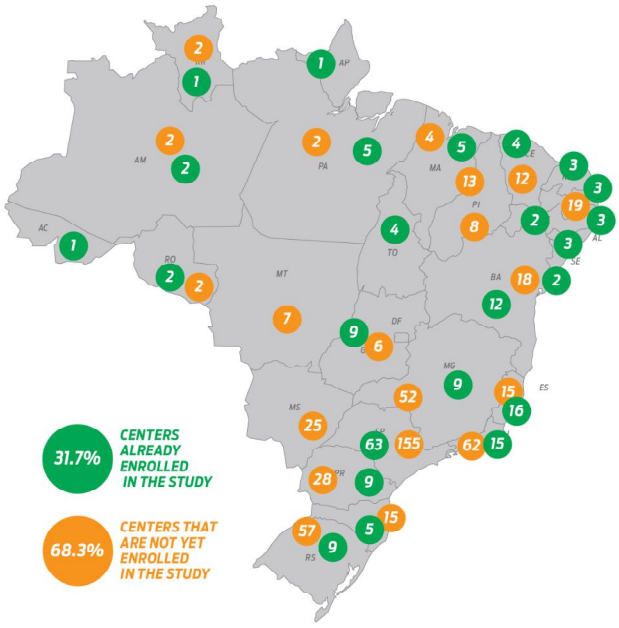


Fig. 1. Map of the dialysis centers in Brazil

Centers already enrolled in the study = 188; Centers that are not yet enrolled or refused to participate in the study = 504. Total of 692 centers in Brazil [17]

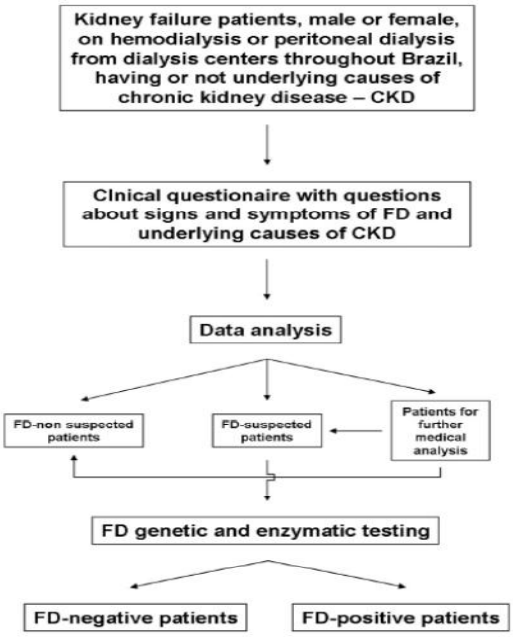


Fig. 2. Flowchart depicting a method of screening for FD detection in Brazilian dialysis patients

2.3.2 Data analysis

All collected data were entered in a database created and maintained by DataGenno Interactive Research® [21]. The algorithm proposed (Fig. 4) was created by DataGenno to sort dialysis patients into three main groups: FD-suspected patients, FD-non suspected patients, and patients for further medical analysis.

The choice of combinations created to distinguish patients in FD-suspected, FD-non suspected and for further medical analysis was based on combinations of the frequency of signs and symptoms of FD described in the literature, patient's gender and age and the natural history of FD (Tables 1, 2). Patients for further medical analysis were clinically evaluated by a team of medical specialists (MPC, MGR) in order to decide if they could be included either in FD-suspected or FD-non suspected patient groups (Fig. 2). Statistical analysis was done by frequency distribution, measures of central tendency, dispersion, and the chi-square test. The results were sent back to the dialysis centers which were entirely responsible for moving forward and order or not FD diagnostic tests for FD-suspected patients.

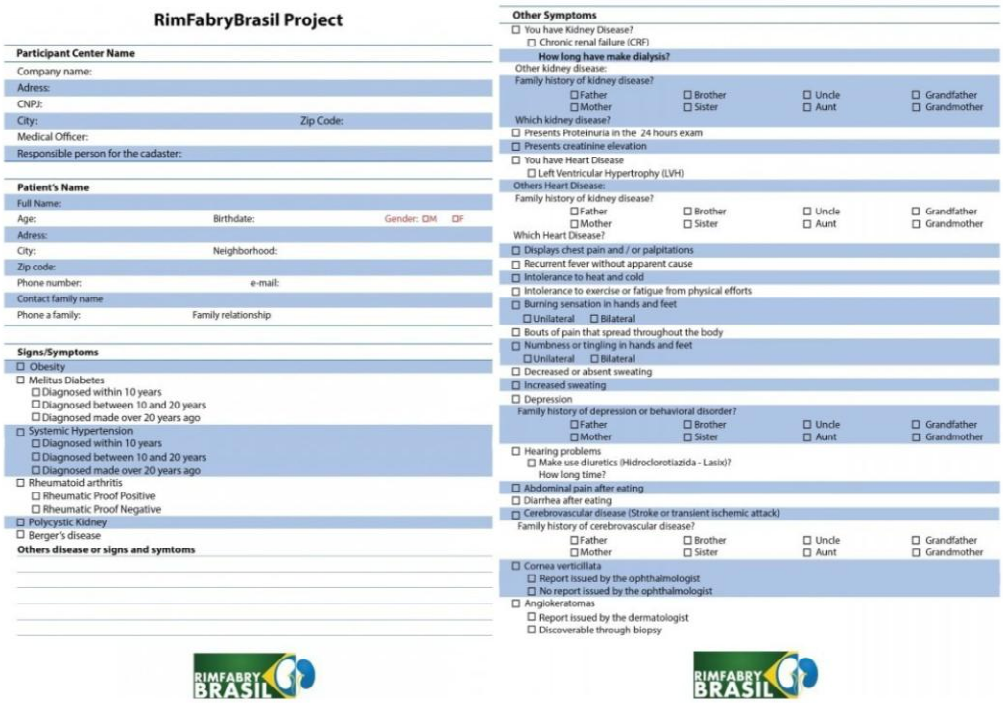
2.3.3 FD laboratory testing

Male FD-positive patients: positive enzymatic test (low/undetectable α-Gal A activity) and/or

presence of pathogenic mutation in the *GLA* gene. Female FD-positive patients: pathogenic mutation in the *GLA* gene. FD genetic and enzymatic tests were run independently by the participant dialysis centers.

2.3.4 Searches in human mutation databases

Searches for *GLA* gene mutations were performed at the Human Gene Mutation Database – HGMD [4], Leiden Open-source


RimFabryBrasil Project

Participant Center Name
Company name:
Adress:
CNPJ:
City: Zip Code:
Medical Officer:
Responsible person for the cadaster:

Patient's Name
Full Name:
Age: Birthdate: Gender: ☐M ☐F
Adress:
City: Neighborhood:
Zip code:
Phone number: e-mail:
Contact family name
Phone a family: Family relationship

Signs/Symptoms
☐ Obesity
☐ Melitus Diabetes
☐ Diagnosed within 10 years
☐ Diagnosed between 10 and 20 years
☐ Diagnosed made over 20 years ago
☐ Systemic Hypertension
☐ Diagnosed within 10 years
☐ Diagnosed between 10 and 20 years
☐ Diagnosed made over 20 years ago
☐ Rheumatoid arthritis
☐ Rheumatic Proof Positive
☐ Rheumatic Proof Negative
☐ Polycystic Kidney
☐ Berger's disease
Others disease or signs and symtoms

Other Symptoms
☐ You have Kidney Disease?
☐ Chronic renal failure (CRF)
How long have make dialysis?
Other kidney disease:
Family history of kidney disease?
☐ Father ☐ Brother ☐ Uncle ☐ Grandfather
☐ Mother ☐ Sister ☐ Aunt ☐ Grandmother
Which kidney disease?
☐ Presents Proteinuria in the 24 hours exam
☐ Presents creatinine elevation
☐ You have Heart Disease
☐ Left Ventricular Hypertrophy (LVH)
Others Heart Disease:
Family history of kidney disease?
☐ Father ☐ Brother ☐ Uncle ☐ Grandfather
☐ Mother ☐ Sister ☐ Aunt ☐ Grandmother
Which Heart Disease?
☐ Displays chest pain and / or palpitations
☐ Recurrent fever without apparent cause
☐ Intolerance to heat and cold
☐ Intolerance to exercise or fatigue from physical efforts
☐ Burning sensation in hands and feet
☐ Unilateral ☐ Bilateral
☐ Bouts of pain that spread throughout the body
☐ Numbness or tingling in hands and feet
☐ Unilateral ☐ Bilateral
☐ Decreased or absent sweating
☐ Increased sweating
☐ Depression
Family history of depression or behavioral disorder?
☐ Father ☐ Brother ☐ Uncle ☐ Grandfather
☐ Mother ☐ Sister ☐ Aunt ☐ Grandmother
☐ Hearing problems
☐ Make use diuretics (Hidroclorotiazida - Lasix)?
How long time?
☐ Abdominal pain after eating
☐ Diarrhea after eating
☐ Cerebrovascular disease (Stroke or transient ischemic attack)
Family history of cerebrovascular disease?
☐ Father ☐ Brother ☐ Uncle ☐ Grandfather
☐ Mother ☐ Sister ☐ Aunt ☐ Grandmother
☐ Cornea verticillata
☐ Report issued by the ophthalmologist
☐ No report issued by the ophthalmologist
☐ Angiokeratomas
☐ Report issued by the dermatologist
☐ Discoverable through biopsy

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Fig. 3. The clinical questionnaire used for interviewing the patients

Table 1. Groups of FD signs and symptoms

Group	FD signs and symptoms	
1 Nephrological	1.1 Family history of kidney disease	1.2 Kidney disease
	1.3 Proteinuria in the 24 hours exam	1.4 Creatinine elevation
2 Cardiological	2.1 Family history of heart disease	2.2 Heart Disease
	2.3 Chest pain and/or palpitations	
3 Rheumatological	3.1 Recurrent fever without apparent cause	
	3.2 Heat and cold intolerance	
	3.3 Exercise intolerance or fatigue from physical efforts	
	3.4 Burning sensation in hands and feet	
	3.5 Bouts of pain that spread throughout the body	
	3.6 Numbness or tingling in hands and feet	
	3.7 Sweating decrease or absence 3.8 Sweating increase	
4 Neurological	4.1 Family history of cerebrovascular disease	
	4.2 Cerebrovascular disease (stroke/transient ischemic attack)	
	4.3 Family history of depression/behavioral disorder	
	4.4 Depression	
Gastrointestinal / Otorhinolaryngological	5.1 Hearing problems	5.2 Abdominal pain (after eating)
	5.3 Diarrhea (after eating)	
6 Dermatological	6.1 Angiokeratomas	
7 Ophthalmological	7.1 Cornea verticillata	

Table 2. Combinations of FD signs and symptoms and patient's gender and age used to sort dialysis patients into three groups

Combinations of FD signs and symptoms, gender or age		Group of patients
A	1+ 2+3 (with four or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
B	Male patient >60y with 1+2+3 (with four or more FD signs and symptoms of Group 3, without sweating increase)	Analysis
C	1+1.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
D	Male patient >60y with 1+1.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	Analysis
E	1+2+ 2.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	FD-suspected
F	Male patient >60y with 1+2+2.1+3 (with three or more FD signs and symptoms of Group 3, without sweating increase)	Analysis
G	Combinations A, B or C where Group 3 of FD signs and symptoms includes sweating increase	Analysis
H	Male patient >60y with combinations of signs and symptoms A,C or E where Group 3 of FD signs and symptoms includes sweating increase	FD-non suspected
I	Male patient with 1.1 and/or 2.1 whose father has 1.1 and/or 2.1 (healthy mother)	Analysis
J	1+2+7 (one or more FD signs and symptoms of Group 7)	FD-suspected
K	Patient with polycystic kidney disease, excluding FD signs and symptoms of Groups 5 and/or 6	Analysis
L	1+5	FD-suspected
M	1+6	FD-suspected
N	1	Analysis
O	1+5	FD-suspected
P	1+6	FD-suspected
Q	1+2+3 (four or more FD signs and symptoms of Group 3)	Analysis
R	1+1.1+3 (three or more FD signs and symptoms of Group 3)	Analysis
S	1+2+2.1+3 (three or more FD signs and symptoms of Group 3)	Analysis
T	1+3 (three or more FD signs and symptoms of Group 3) +4 or 5	Analysis
U	Male patient >60y with combinations of signs and symptoms R,S or T where the FD signs and symptoms of Group 3 includes sweating increase	FD-non suspected
V	Patients with confirmed laboratory and/or clinical diagnosis of underlying causes of CKD ruling out the possibility of FD	FD-non suspected

A to V – combinations of FD signs/symptoms, gender or age used to define three main groups of patients: FD-suspected, FD-non suspected, and analysis.

1 to 7 – groups of FD signs/symptoms: 1, nephrological; 2, cardiological; 3, rheumatological; 4, neurological; 5, gastrointestinal/otorhinolaryngological; 6, dermatological; 7, ophthalmological

Variation Database – LOVD platform [22], available at Zhejiang University Center for Genetic and Genomic Medicine website [23], and at NCBI's ClinVar [24].

3. RESULTS

3.1 Patient Demographics

A total of 25,223 dialysis patients from 188 dialysis centers were analyzed. Nine of the invited dialysis centers did not join the study. Male patients were 59.3% (14,957) and female, 40.7% (10,266) (Fig. 5A). FD-suspected patients were 2,956 (11.7%). The total of FD-negative patients was 2,867, among FD-suspected patients and the total of FD-positive patients was 89, most female (58 [65.2%] female; 31 [34.8%] male) (Fig. 5C). Female predominance in FD-positives was significant (χ^2 = 7.39; P = 0.0065). FD-positive patients represented 3.0% (2.0% female, 1.0% male) of all FD-suspected patients and 0.3% (0.2% female, 0.1% male) of all participant dialysis patients. FD-negatives were

2,867 (Fig. 5B), both sexes in similar proportions (50.5% female; 49.5% male).

The average age of FD-positive patients was 37.7y (±16.6); for FD-negative patients it was 45.1y (±11.5). FD-positive patients showed lower average age (male: 33.8±16.0; female: 37.8±17.6) when compared to FD-negatives (male: 44.9±11.6; female: 45.2±11.5). It is possible to see a sharp decline in the number of FD-positive male patients from 40 y (Fig. 5C) while the decline in the number of FD-positive female patients becomes more significant from 60y. The distribution of FD-negative patients by gender and age was also different from the total of analyzed patients (Fig. 5B, 5A). In FD-negative patients, both genders, a sharp decline in the number of patients is only seen from 60y (Fig. 5B) and in the total of analyzed patients that happens from 70 y (Fig. 5A).

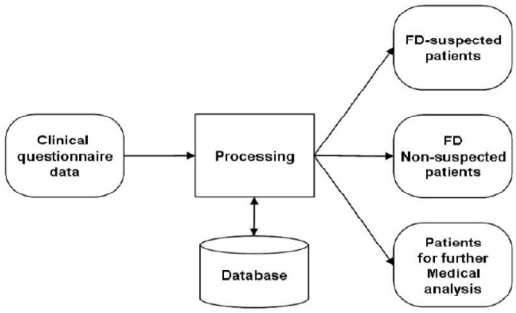

Fig. 4. Algorithm for detection of FD-suspected in Brazilian dialysis patients

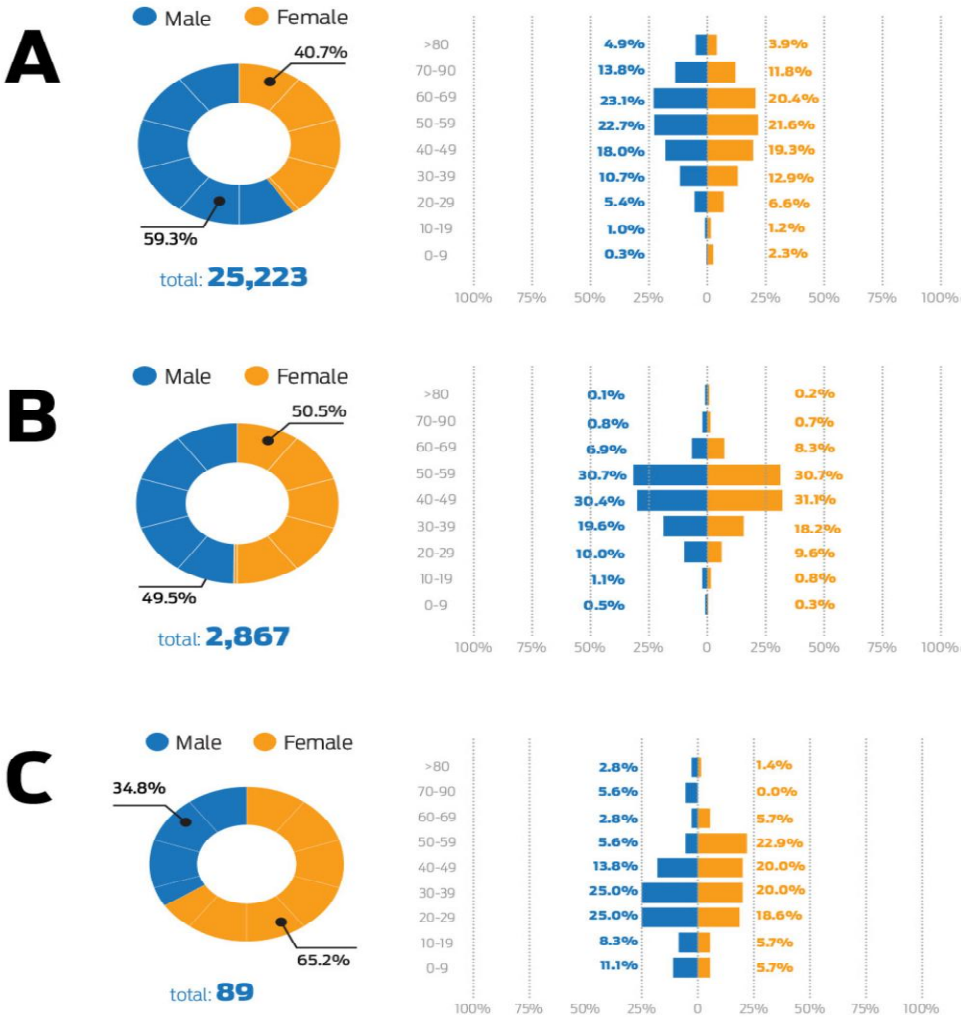

Fig. 5. Distribution of the Brazilian dialysis patients by gender, age group, and FD diagnosis. A) Total; B) FD-negatives; C) FD-positives

Table 3. Mutations found in FD-positive patients' *GLA* gene

Mutation no.	*GLA* gene nucleotide change	Location	Mutation	Number of patients with the mutation
M1	c.194+1G>A	intron	unknown	3
M2	c.370-1G>T	intron	splice acceptor variant	1
M3	c.801+36G>A	intron	unknown	1
M4	c.337T>C	exon 2	p.Phe113Leu (F113L)	3
M5	c.352C>T	exon 2	p.Arg118Cys (R118C)	18
M6	c.413delG	exon 3	p.Gly138Glufs (G138E)	1
M7	c.427G>A	exon 3	p.Ala143Thr (A143T)	1
M8	c.679C>T	exon 5	p.Arg227X (R227X)	4
M9	c.803T>C	exon 6	p.Leu268Ser (L268S)	1
M10	c.870G>A	exon 6	p. Met290Ile (M290I)	5
M11	c.870G>C	exon 6	p.Met290Ile (M290I)	17
M12	c.877C>T	exon 6	p.Pro293Ser (P293S)	3
M13	c.937G>T	exon 6	p.Asp313Tyr (D313Y)	4
M14	c.1025G>A	exon 7	p.Arg342Gln (R342Q)	4
M15	c.1067G>A	exon 7	p. Arg356Gln (R356Q)	1
M16	c.1102G>A	exon 7	p.Ala368Thr (A368T)	19
M17	c.1117G>A	exon 7	p.Gly373Ser (G373S)	1
**	c.870G>C/	exon 6	p.Met290Ile (M290I)/	2
	c.376A>G	exon 3	p.Ser126Gly (S126G)	

*** compound heterozygous (M11/variant of uncertain significance)*

3.2 *GLA* gene Mutation Analysis

A total of 17 different mutations were found in FD-positive patients' *GLA* gene (Table 3). Three mutations were highly frequent: c.1102G>A (A368T), c.352C>T (R118C) and c.870G>C (M290I). Mutations 194+1G>A, 370-1G>T and 801+36G>A were located in intronic regions of the *GLA* gene.

After renal involvement, heart disease was the most prevalent symptom; it was present in 56 patients (62.9%). Three mutations were more frequent: c.1102G>A (A368T), c.325C>T (R118C) and c.870G>C (M290I); 30.3%, 19.6% and 14.3% respectively. Thirty patients had depression and the most prevalent mutations were c.1102G>A (A368T; 40.0%) and c.352C>T (R118C; 13.4%). Six female had cerebrovascular disease and the mutation c.1102G>A (A368T) was frequent (66.7%). Thirteen patients had angiokeratoma and only three were male (23.1%); the mutations c.870G>A (M290I), c.870G>C (M290I) and c.870G>C/c.376A>G (M290I/S126G) were equally frequent (15.4% each). A total of 10 FD-positive had cornea verticillata; the mutations c.352C>T (R118C), c.870G>C (M290I) and c.937G>T (D313Y) were equally frequent (20.0% each).

3.3 Frequency of FD Symptoms

Heart and rheumatologic symptoms were more frequent below 59y and neurological and gastrointestinal/otorhinolaryngologic symptoms were more frequent below 39 y in FD-positive patients (Fig. 6B), unlike what was observed in FD-negative patients among FD suspected patients; it reminds the gaussian distribution (Fig. 6A). Symptoms in general occurred earlier in male then female FD-positive patients (Fig. 6C,6D).

3.4 Frequency of Underlying Causes of CKD

HBP (80.1%) and DM (33.3%) were highly frequent underlying causes of CKD in FD-negative patients (Fig. 7), followed by obesity (9.6%), rheumatoid arthritis (9.2%), and polycystic kidney disease (6.7%). However, underlying causes of CKD were way less frequent (13.4%) in FD-positive patients (Fig. 7). HBP was the most frequent (10.1%) followed by DM, obesity and rheumatoid arthritis (1.1% each). There were no cases of Polycystic Kidney or Berger's Disease in FD-positive patients.

Most of the FD-negative patients with HBP were males (59.9%; Fig. 8B). In FD-positive individuals only a small part (9; 10.1%; Fig. 7) of the patients had HBP, most of them were female (52.1%) (Fig. 8A).

HBP in FD-negative patients was observed in all age groups, in about the same proportion in both sexes (Fig. 9A), and starts to become more common from 40y. In FD-positive female

patients, the number of individuals with HBP increased significantly from 30 y (Fig. 9B) while in FD-positive men the number of individuals with HBP was quite high in the age groups of 20-29 y and above 69 y.

In FD-negative patients, DM is observed in both sexes and in all age groups, but it starts to become more common above 40-49 y. However, the number of FD-positive patients with DM was very small (one female).

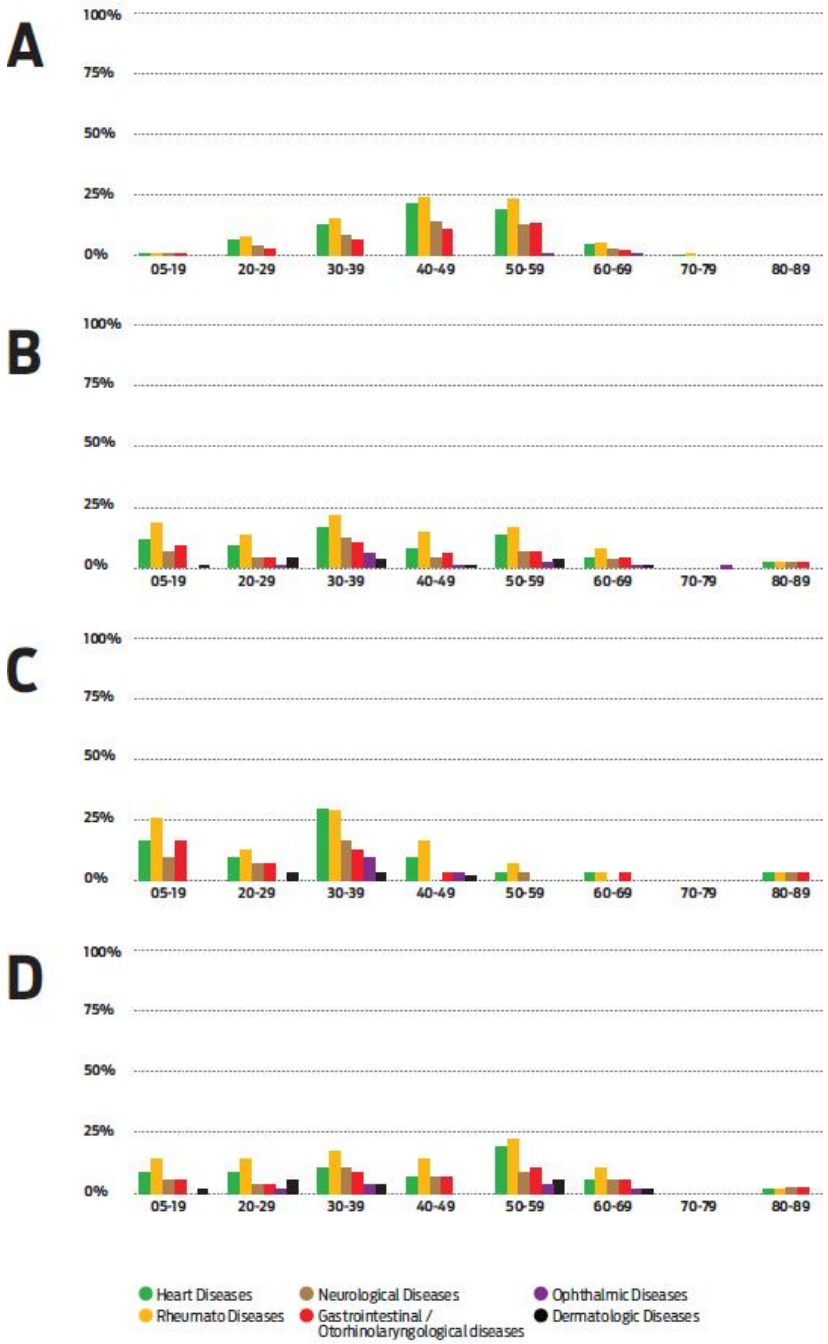

Fig. 6. Frequency of FD symptoms by gender, age group, and FD diagnosis. A) FD-negative; B) FD-positive; C) FD-positive male; D) FD-positive female

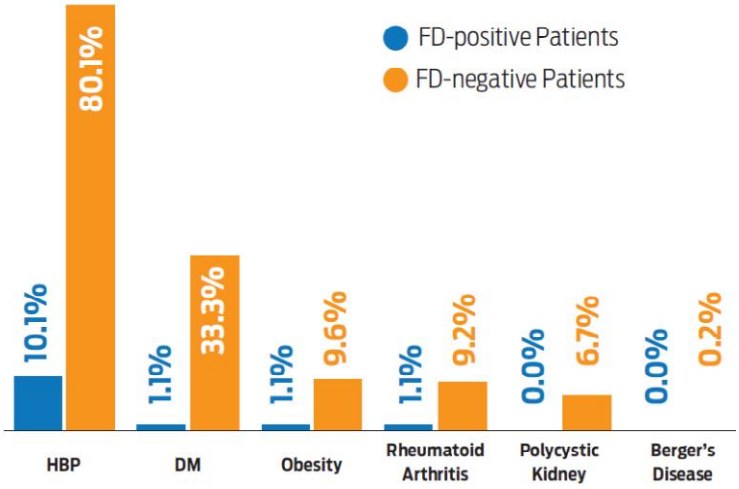

Fig. 7. Frequency of underlying causes of CKD in FD-positive and negative patients

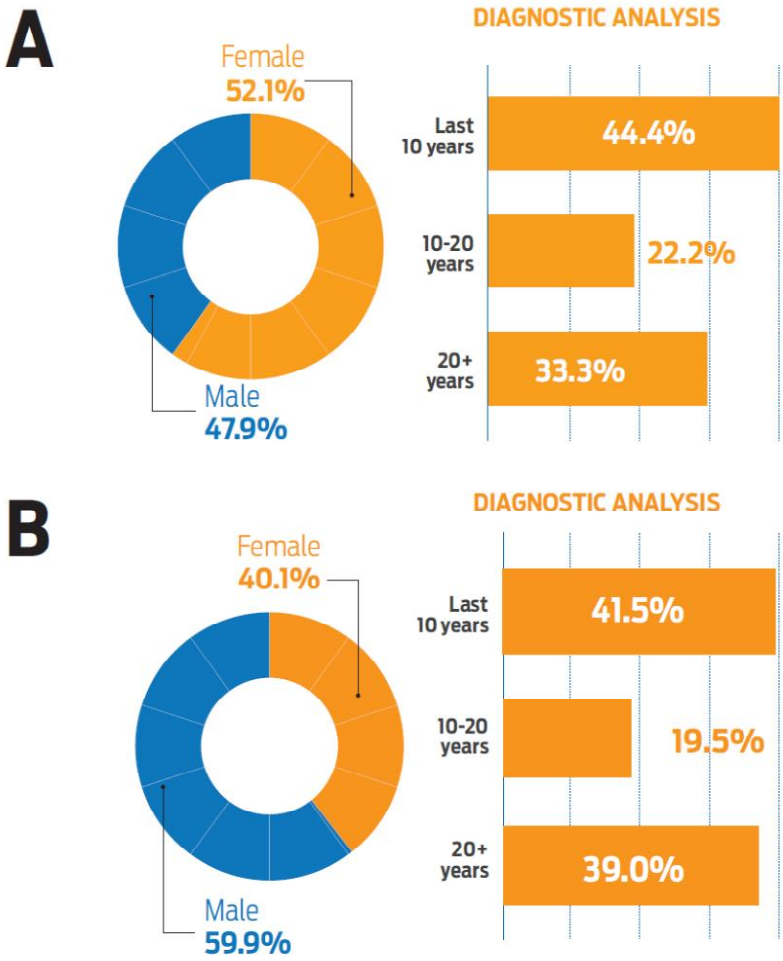

Fig. 8. Diagnosis time and distribution of HBP by gender in FD patients. A) FD-positive; B) FD-negative

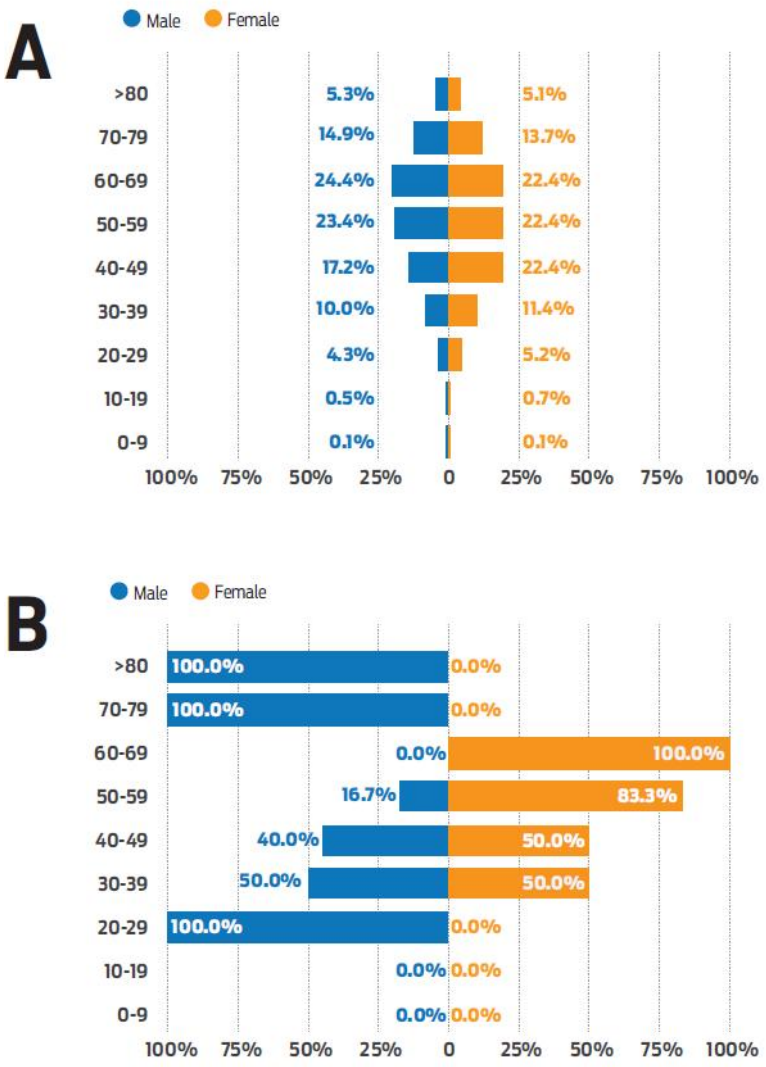

Fig. 9. Frequency of HBP by gender and age group in FD patients. A) FD-negative; B) FD-positive

The distribution of underlying causes of CKD according to age group revealed that the highest prevalence occurs between 40-49 y for FD-positive patients and between 50-69y for negative patients; FD-positive patients showed higher frequency before 50 y (81.8% *versus* 48.4% in FD-negative patients), (*P* (Fisher) = 0.03). Similar findings occurred with HBP (88.9% versus 33.8%; *P* (Fisher) = 0.001). Comparing FD-negative and positive patients regarding to HBP, FD-negative patients showed higher frequencies (χ^2 = 218.15; *P* < 0.0001). The small number of FD-positive patients with DM, Obesity and Rheumatoid Arthritis prevented this kind of analysis.

4. DISCUSSION

As far as we know, this is the first study with such a large sample carried out in a continental multiethnic country like Brazil, in order to identify FD patients among dialysis patients enrolled in dialysis centers from all over the country. FD has a vast phenotypic spectrum, lacking a clear genotype-phenotype correlation [4,7,13]. Thus, an algorithm based on combinations of signs and symptoms, age and gender (Fig. 4, Table 2) was proposed and used to screen for FD-suspected patients in a population of 25,223 dialysis patients. In this study, this strategy allowed to reduce to 88.3% the number of dialysis patients for FD enzymatic and genetic testing. The

prevalence of 0.3% (0.2% female, 0.1% male) is in line with previous studies carried out in hemodialysis centers. Porsch et al. [25] evaluated 558 southern Brazilian male patients with ESRD and only two had low α-Gal A activity and were diagnosed with FD (0.36%). Other FD screening studies performed in Brazilian patients with CKD undergoing hemodialysis in Paraná and in patients on dialysis treatment in Rio de Janeiro found a prevalence of 0.24% [26,27]. In Latin American countries, FD prevalence in Peru was 0.3% and in Colombia, 0.4% [28,29]. A review encompassing all screening studies [16] showed 55 patients (44 males/11 females) detected in a total of 18,837 hemodialysis patients; mean prevalence of 0.29% (0.23% female/0.06% male).

The decline in the life expectancy of FD patients [12] not associated to underlying causes of CKD (Figs. 7,9), reinforces previous findings that FD is a devastating disorder [2,3,12]. Life expectancy of male FD patients, according to The United States Fabry Registry (58.2 y male) [12] was diminished to about a quarter of the average in the Brazilian general population (74.9 y) [30]. Lack of diagnosis may have contributed to the early deaths due to FD in Brazilian dialysis patients, since FD progresses quickly and kidney failure may occur by the third or fourth decade of life [2,12].

Rheumatologic symptoms were the most frequent in general (Fig. 6A,6B). However, the frequency was much higher in FD-positives as these symptoms are common in FD since childhood [2,3,31]. Three mutations (c.1102G>A [A368T], c.325C>T [R118C] and c.870G>C [M290I]) were highly frequent in FD-positive patients (Table 3). However, no mutation previously confirmed with manifestation confined to the kidney or heart [15,32] was found in this study.

Heart diseases were the second most prevalent amongst FD-positive patients. Mutation c.352C>T (R118C) was found in 11 of these patients with heart disease. This same mutation was previously found in Italian male neonates [33]. It was frequent in unrelated hemodialysis patients in Spain [34] and in young Portuguese patients with stroke [35]. Historically, Brazil has received large numbers of Italian, Spanish, and Portuguese immigrants. The country itself was a colony of Portugal which may explain the high frequency of this mutation among Brazilian FD patients. This mutation has been described in Brazilian families suspected of FD [36]. R118C is considered by HMGD a disease causing mutation since it has been found in young adults with stroke, in a patient with apical left ventricular hypertrophy, and may be a cardiomyopathy phenotype modifier thought not to cause classic FD phenotype in a Medelian fashion [35,37-39].

Depression was the third most frequent symptom amongst FD-positive patients and mutations c.1102G>A (A368T) and c.352C>T (R118C) were frequent. Dementia, cognitive impairment, and depression occur in patients with FD [40,41]. However, additional studies are needed to establish a direct link of these morbidities to FD.

An analysis of a large cohort of 2,446 patients in the Fabry Registry (Fabryregistry.com) reported that stroke occurs in 6.9% of men and 4.3% of women [42]. Six female patients had cerebrovascular disease, four had c.1102G>A (A368T) mutation, making it highly prevalent; mutation that was previously reported in Brazilian hemodialysis patients [43,44]. Nevertheless, it is not considered a disease causing mutation by HMGD [4]. Despite of being considered a mutation that is associated with cerebrovascular disease, slight decrease of Alpha-galactosidase A activity, normal lyso-Gb3 and less severe typical signs and symptoms of FD, c.427G>A (A143T) mutation was not found in our patients. It seems to be most likely a neutral variant or a possible modifier instead of a disease-causing mutation [45,46].

Angiokeratoma, a classic sign of FD was frequent among FD-positive patients (14.6%). It's not directly related to kidney failure [2]. A previous study carried out in Brazil found 6.7% of FD patients after reviewing angiokeratomas' biopsies [47]. Another Brazilian study about FD patients' registry identified angiokeratomas in 8.7% of FD patients [48]. Despite of different methodologies, the percentage of FD patients with angiokeratomas in this study was higher. Six of the patients with angiokeratoma had M290I mutation that was originally identified as causing FD classic phenotype in 66 unrelated families [49]. On the other hand, cornea verticillata, the main ocular finding in FD [50], was present in only 11.2% of the patients. Its prevalence in FD ranges from 44% to 94.5% in men and 88.0% in women [51]. The striking low incidence found in this study may be explained due to the need of a more specific evaluation by an ophthalmologist for a more precise diagnosis.

Although the intronic mutations found in this study (Table 3) have been found in FD patients before [11,24], their effects on the *GLA* gene expression or in alpha-galactosidase protein remain unknown.

HBP and DM were highly prevalent in FD-negative patients while FD-positive patients presented much less underlying causes of CKD (Fig. 7). These results reinforce previous findings [52] that FD is the main cause of kidney failure in FD patients.

The algorithm (Fig. 4, Table 2) used in the present study to track FD-suspected allowed to reduce significantly (by 88.3%) the number of dialysis patients for genetic and enzymatic testing. The natural history and frequency of FD among dialysis patients in this study were in line with literature. This indicates the algorithm could be a helpful tool in screening studies set to identify FD patients among large numbers of dialysis patients.

5. CONCLUSION

The initial findings of this large long-term study ongoing in Brazil emphasize the importance of early diagnosis in order to detect and treat FD before it may causes irreversible renal, cardiac, and/or neurologic damages. Although the algorithm used for screening of FD-suspected patients would have been a useful tool, it still needs to be statistically validated (sensitivity, specificity, predictive value). The encouraging results obtained from this first 18 months abet us to move forward with this country-wide study since it will allow us to have a better understanding of FD natural history in Brazil. More importantly, it will contribute to the development of an optimized diagnosis strategy which can save resources from public health system and provide early disease identification for an appropriate timely treatment.

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CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this study. The patients invited to participate were informed about the study purposes and each subject freely signed an individual consent form agreeing to participate in the study.

ETHICAL APPROVAL

The study protocol was approved by the Research Ethics Committee of Campos Medical School (legal opinion #305·988; 06/28/2013). Patients and the health care team involved had their anonymity fully preserved according to Resolution no. 196/96 of the National Board of Health, 1988 Medical Ethics Code and 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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