Screening of Fabry Disease of patients in renal replacement therapy in a population from Lazio (Italy)

G. MARRONE[1], R. ANGELICO[2], M. DI LAURO[1], E. SARGENTINI[1],
T.M. MANZIA[2], G. TISONE[2], A.P. MITTERHOFER[1,3],
D. DELLA MORTE CANOSCI[1,4,5], M. TESAURO[1], N. DI DANIELE[6,7], A. NOCE[1,3]

[1]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[2]Department of Surgical Sciences, HPB and Transplant Unit, University of Rome Tor Vergata, Rome, Italy
[3]Nephrology and Dialysis Unit, Policlinico Tor Vergata, Rome, Italy
[4]Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy
[5]Department of Neurology, Evelyn F. McKnight Brain Institute, Miller School of Medicine,
 University of Miami, Miami, FL, USA
[6]University of Rome Tor Vergata, Rome, Italy
[7]Fondazione Leonardo per le Scienze Mediche Onlus, Abano Terme, Italy

Abstract. – OBJECTIVE: Fabry's disease (FD) is a genetic disorder of lysosomal storage characterized by the intralysosomal accumulation of globotriaosylceramide (Gb3). This genetic mutation causes a total or partial deficit of the α-galactosidase (GAL) enzyme activity. FD has an incidence of 1:40000-60000 born alive. Its prevalence is higher in specific pathological conditions like chronic kidney disease (CKD). The aim of this study was to evaluate the FD prevalence in Italian renal replacement therapy (RRT) patients from Lazio region.

PATIENTS AND METHODS: 485 patients in RRT (hemodialysis, peritoneal dialysis, and kidney transplantation) were recruited. The screening test was performed on venous blood sample. The latter was analyzed using specific FD diagnostic kit, based on the analysis of dried blood spots on filter paper.

RESULTS: We found 3 cases of positivity to FD (1 female and 2 males). In addition, 1 male patient was identified with biochemical alteration indicative of GAL enzyme deficiency with a genetic variant of the GLA gene of unknown clinical significance. The FD prevalence in our population was 0.60% (1 case out 163), it rises to 0.80% (1 case out of 122) if the genetic variant of unknown clinical significance is considered. Comparing the three subpopulations, we observed a statistically significant difference in GAL activity in transplanted patients compared to dialysis patients ($p<0.001$).

CONCLUSIONS: Considering the presence of an enzyme replacement therapy able to modify FD clinical history, it is essential to try to implement FD early diagnoses. However, the screening is too expensive to be extended on large scale, due to the low prevalence of the pathology. The screening should be performed on high-risk populations.

Key Words:
 Fabry disease, Renal replacement therapy, Kidney transplantation, End stage renal disease, Lysosomal storage disease, Alpha-galactosidase, Lyso-Gb3.

Introduction

Fabry disease (FD) is a genetic disorder of lysosomal storage with recessive X-linked transmission, characterized by the intralysosomal accumulation of globotriaosylceramide (Gb3) due to the mutation, in chromosome Xq22.1, of the *GLA* gene[1]. This genetic mutation causes a total or partial deficit of the α-galactosidase (GAL) enzyme activity, which leads to an alteration of the metabolism of some glycosphingolipids with consequent intralysosomal accumulation of Gb3 in various tissues and organs[2]. This process results in a multisystem disease that mainly involves heart, kidneys, nervous system, skin and eyes[3].

From an epidemiological point of view, FD is an extremely rare condition with an incidence of 1:40000-60000 born alive[4]. More recent studies, based on neonatal molecular screening programs, show a higher incidence, up to 1:3100 live births[5]. Furthermore, the prevalence appears to be higher in specific high-risk subpopulations such as young patients with cryptogenic stroke[6], patients

without other causes of hypertrophic cardiomyopathy[7] or patients with chronic kidney disease (CKD) by unknown cause[8].

The disease, having an X-linked recessive transmission, presents different clinical manifestations according to the residual activity of the GAL enzyme and to the gender. Male patients with total deletion of the *GLA* gene present the classic clinical manifestation of the disease, while female patients may have different clinical pictures, depending on the random inactivation of the X chromosome (Lyonization)[9]. In fact, patients with the classic phenotype have mutations that cause the complete absence of GAL, while patients with missense mutation have a residual enzyme activity from 2 to 25% and, consequently, have a more "nuanced" phenotype. The last is characterized by an increased risk of developing cardiovascular diseases (CVDs) and/or, in case of CKD, to develop a rapid progression towards end-stage renal disease (ESRD).

Therefore, based on the GAL residual enzyme activity, we can recognize a classic manifestation of FD (type 1) and a late-onset variant (type 2).

Classic FD occurs in pediatric age with episodic pain in the extremity, fever of unknown origin, abdominal pain, and diarrhea after meals with a high lipid content[10]. During the adolescence, the first pathognomonic signs of the disease, such as angiokeratomas, namely vascular skin anomalies characterized by small red or blue papules, appear[11]. Angiokeratomas are frequently multiple and with diffuse distribution (angiokeratoma corporis diffusum)[12]. Ophthalmological manifestations are common and the main among these is represented by the vortical keratopathy with sub-epithelial corneal spiral accumulation (cornea verticillata)[13]. As it is universally acknowledged, the disease may progress with additional symptoms, resulting from alterations in the nervous, cardiovascular, or renal systems with an increased risk of developing complications, such as the heart failure, the arrhythmic pathology, the hypertrophic cardiomyopathy[14], the ischemic stroke with possible evolution in the hemorrhagic stroke[15] and a rapid progression towards ESRD[16].

On the other hand, late-onset FD is characterized by the involvement of only one organ (kidney or heart) and by the onset of pathognomonic symptoms in adulthood. In these cases, chronic insults related to FD, added to the traditional risk factors, lead to early cardiac and renal complications, and could cause misdiagnosis and subsequent failure to a therapeutic treatment[17,18]. The clinical manifesta-

tions are due to the chronic intralysosomal accumulation of not completely catabolized α-D-galactose fragments such as Gb3, which seem to induce a state of chronic cellular toxicity[19-21].

Furthermore, blood values of globotriaosyl-sphingosine (lyso-Gb3), the deacetylated form of Gb3, are up to 50 times higher in FD patients than in healthy controls[22]. Lyso-Gb3 is a soluble molecule that can be easily metabolized from the cells, and it is generated by the activity of the acid ceramidase, a lysosomal enzyme[23]. Lyso-Gb3 is an inhibitor of GAL A and B enzymes and a promoter of vascular smooth muscle cells replication, and thus, it can have a key-role in increasing myo-intimal thickness[24]. Although the action of lyso-Gb3 in the FD pathogenesis remains unclear, it is useful for monitoring the disease severity and the therapeutic response[25].

This study aimed to evaluate the prevalence of FD in the Lazio region (Italy) population in renal replacement therapy (RRT) and secondly to analyze the possible differences in GAL enzyme activity in different RRTs in ESRD patients without FD.

Patients and Methods

Enrolled Patients

In the present study, 485 patients in RRT (such as hemodialysis, peritoneal dialysis, or kidney transplantation) were recruited from UOC of Internal Medicine-Centre of Hypertension and Nephrology Unit and UOC of Transplantation of University Hospital Policlinico Tor Vergata (PTV) out-patients, from May 2018 to May 2020. The study protocol followed the Helsinki declaration and was approved by the University Hospital PTV Independent Ethics Committee (trial number 8466/2018, Clinical Experimentation Register of PTV). All enrolled patients signed an informed consent before starting the study. The inclusion criteria were patients in RRT, age ≥18 years old, both sex, and acceptance of informed consent. The exclusion criteria were the presence of HIV, HbsAg+ and HCV+.

At the time of the enrollment, all anamnestic data were collected for each patient. In addition, a physician provided general information about FD in each patient.

Methods

The screening test was performed on venous blood sample. The latter has been analyzed using specific FD diagnostic kit, based on the analysis
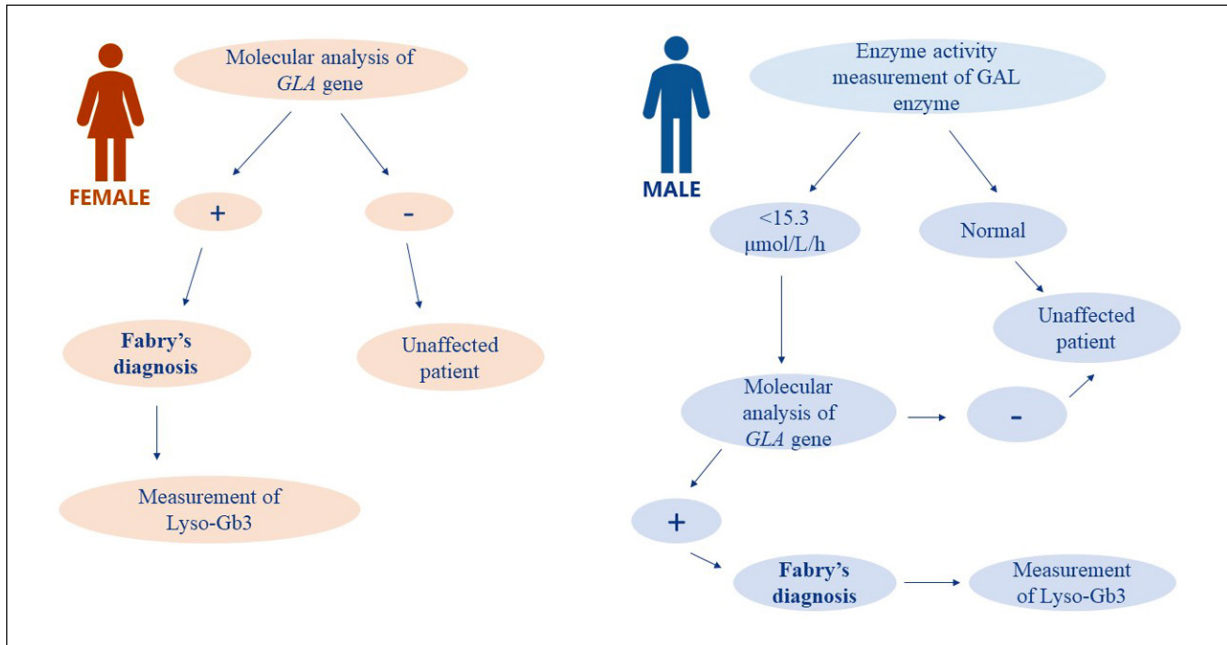
Figure 1. Fabry's disease diagnostic protocol.

of dried blood spots. In particular, the latter had been left to dry on a filter paper at room temperature for two hours. The sample analysis has been performed at CENTOGENE, (CENTOGENE AG laboratories, Rostock, Germany).

The study design is illustrated in Figure 1. For male patients, the analysis firstly evaluated the GAL enzyme activity (normal values ≥15.3 µmol/L/h with immunofluorimetry)[26] and then, for subjects with pathological enzyme activity, it was performed the genetic assessment of the *GLA* gene (NGS-Illumina®, CENTOGENE, Rostock, Germany) to detect known pathogenic mutations, new genetic variants, or those with unknown clinical significance.

The method used to analyze *GLA* gene in next generation sequencing (NGS) is based on the developing and sequencing of amplicons. Amplicons occupy all coding regions and highly conserved splicing junctions between exons and introns. This method ensures a minimum coverage of more than 20x for each amplicon. It was also performed lyso-Gb3 analysis to evaluate the disease severity (normal values between 0.08-1.13 nmol/L with liquid chromatography-MS)[26]. Female patients directly underwent the genetic test, as the enzyme activity is non-diagnostic for the X-chromosome Lyonization.

FD positive patients underwent a further specific genetic test in order to identify their own allelic variant. Moreover, we extended the FD screening to the relatives of the positive patients.

Statistical Analysis

All data was initially entered into an Excel spreadsheet (Microsoft, Redmond, WA, USA) and the analysis was performed using the Windows Statistical Package for Social Science, version 15.0 (SPSS Inc., Chicago, IL, USA). The descriptive statistics consider the mean ± standard deviation (SD) for the parameters with normal distribution (after confirmation with histograms and the Kolgomorov-Smirnov test), while for the non-normal variables, they consider the median and the interval (minimum-maximum).

The comparison of the data of normal variables with the non-parametric ones were carried out with a one-way ANOVA. Regarding occurrences (percentages), the chi-square test, possibly corrected by Fisher's exact test, was performed. A *p*-value <0.05 was considered statistically significant.

Results

The epidemiological findings of the study population are illustrated in Table I. The mean age of the all-enrolled patients was 57.6±11.6 years.

Of the 485 patients analyzed, 155 (31.9%) were female while 330 (68.1%) were male. One

Table I. Epidemiological distribution of the study population based on the type of renal replacement therapy.

	Hemodialysis	Peritoneal dialysis	Kidney Transplantation	Total population
Patients (n)	114	49	322	485
Age (years)	55.7±11.0	59.7±10.2	58.3±12.1	57.6±11.6
Male patients (n)	79	30	221	330
Female patients (n)	35	19	101	155
Arterial hypertension (%)	85.1	92	74.3	-
Diabetes mellitus (%)	13	14	6.80	-
Presence of angiokeratomas (%)	0.60	0	1.30	-

hundred fourteen patients underwent hemodialysis, including 35 females and 79 males, 49 patients underwent peritoneal dialysis as RRT, 19 of which were female and 30 males.

The two groups of patients in dialysis (namely in hemodialysis or in peritoneal dialysis) were homogeneous for gender ($p=0.863$) and age ($p=0.939$). In hemodialysis group, 29 patients (25%) underwent biopsy and histological examination of the native kidneys, while in the peritoneal dialysis group, only 9 patients (18%) received this diagnostic procedure.

At the moment of the venous sample, patients with functional kidney transplant were 322 (65.8%), with an average age of 58.3±12.1, of which 101 females and 221 males. As stated before, the kidney transplanted patients presented a statistically significant gender disproportion ($p=0.007$). One-hundred eighty-nine patients (58.7%) who underwent kidney transplantation did not perform histological examination of the native kidneys.

Among the renal transplanted patients, 233 underwent hemodialysis treatment, before the surgical procedure. Sixteen of the kidney-transplanted patients reported a medical history of type 2 diabetes mellitus, while 176 of them reported systemic arterial hypertension.

Within the 485 enrolled patients, 3 cases of positivity to FD were found (1 female and 2 males). Moreover, 1 male patient was identified with biochemical alterations indicative of GAL enzyme deficiency with a genetic variant of the *GLA* gene of unknown clinical significance. In detail, we observed the variant c.937G> T (p.Asp313Tyr), detected in exon 6 of the *GLA* gene, that causes an amino acid change from Asp to Tyr in position 313 (p.D313Y). According to a systematic review[27], which analyzed 35 studies, the patients with GLA (p.D313Y) showed a low prevalence in typical FD clinical manifestations and a higher enzyme activity compared to patients with the FD

classic phenotype. Indeed, according to a study conducted by Lenders et al[28], this variant could represent a possible risk factor for neurological disorders, such as white matter lesions. Moreover, this variant should be evaluated in case of absence of other classic risk factors for neurological disease. This observation was confirmed in a meta-analysis conducted by Palaiodimou et al[29]. They highlighted that the variant p.D313Y seems to be related to milder FD clinical manifestations and to a late development of the phenotype, in which prevail the neurological disorders.

The patient with an ascertained diagnosis was treated with hemodialysis and subsequently transplanted, while 2 FD patients were treated with peritoneal dialysis, and 1 FD with hemodialysis, before the kidney transplantation.

Two of 3 FD positive patients received a previous diagnosis as cause of ESRD instead of FD, namely a probable nephroangiosclerosis and a lupus nephritis.

The prevalence of FD in our population was 0.60% (1 case out of 163), it could rise to 0.80% (1 case out of 122) if the genetic variant of unknown clinical significance was considered. The median age of patients with FD diagnosis was 49 (45-60) years.

Discussion

Data concerning the values of lyso-Gb3 and GAL enzyme activity of the study population, divided according to RRTs, were reported in Table II. Comparing the three subpopulations for these parameters, we observed a statistically significant difference in GAL enzyme activity in transplanted patients compared to dialysis patients ($p<0.001$). Moreover, comparing the lyso-Gb3 values among the three subpopulations, we did not observe any statistical significance.

The FD prevalence in our study population, excluding the variant of uncertain significance, was

0.60%. This exclusion has been performed in order to compare our data to those obtained in similar studies, already present in literature. In particular, studies carried out on a larger population than ours, such as in Japan[30] or in Turkey[31], reported a lower prevalence, namely around 0.31%. Similar data has also been highlighted by studies[32] conducted in other countries, such as Brazil (0.32%)[32] and China (0.18%)[33]. While another study[34] conducted in Spain in RRT patients of European origin, reported a similar prevalence (0.55%) compared to the one obtained from our study.

Moreover, our study confirmed the literature data according to which the prevalence of FD in patients undergoing RRT was far higher than that observed in the general population, which is around 1:40000-60000 (0.000025-0.000016%)[35].

As observed in other studies, it has been shown that in most cases patients who, after molecular screening, received FD diagnoses, had previously other aetiological diagnoses of CKD and rarely performed biopsies on native kidneys[36].

In particular, in our population, 1 of the 3 FD positive patients had previously undergone biopsy with histological diagnosis.

Another interesting and unexpected finding of our study was obtained by the statistical analysis of the enzyme activity levels of GAL, stratified according to the type of RRT. In fact, the enzyme activity levels are significantly higher (p-value=0.001) in the kidney-transplanted population than in the ESRD population on peritoneal dialysis or on hemodialysis. Currently, in literature, there are no further data on this parameter, namely an evaluation about GAL enzyme activity in RRT patients without FD.

The higher GAL enzyme activity in kidney transplanted recipients could be related to the protective role of immunosuppressive drugs, administered after transplantation. Although underlying biochemical mechanism is still under evaluation, preliminary data suggest that high dosages of immunosuppressive drugs, such as prednisolone, tacrolimus, and mycophenolate mofetil, which represented also the post-transplant immuno-suppressive regimen of our cohort, can decrease the title of anti-GAL IgG and might prevent the enzyme inhibition[37]. This pathway could explain the increased enzyme activity levels observed in our kidney transplanted population. Furthermore, Lenders et al[37] showed that patients with FD, who were started enzyme replacement therapy (ERT) after transplantation, did not develop new antibodies against ERT in long-term follow up, supporting the protective effect of immuno-suppression on FD. Interestingly, the efficacy of the immunosuppression to counteract the ERT inhibition, decreased when the immunosuppressive drug blood levels were downtitrated. Additionally, the optimal immunosuppressive regimen and ideal dosages against formation of anti-GAL antibodies need to be further investigated.

We speculate that uremic toxins induce a possible inhibiting action on GAL enzyme. In order to support this hypothesis, further clinical studies could be necessary to evaluate how the uremic toxins and the oxidative stress impact on GAL enzyme activity. In fact, CKD is characterized by several clinical manifestations and by numerous metabolic dysfunctions, not all well-known yet. It could be hypothesized that CKD itself impacts on various enzymatic pathways and among these, GAL enzyme activity. Currently, the only study that examined the GAL enzyme activity in kidney transplanted patients without FD supports our findings, namely this high-risk population presents lower GAL enzyme activity compared to general population[38].

A limitation of our monocentric study is the low sample size. To overcome this, it is essential to conduct similar studies in order to broaden the sample and make the evidence more significant. Considering that it is a genetic pathology, it will be important to collect new data both on Italian population and on other countries' populations, from all over the world. It is notable to detect the possible differences of FD prevalence related to ethnicity, in order to select high-risk populations, not only based on comorbidities but also on ethnicity.

Table II. Lyso-Gb3 and GAL values of the screened population, divided according to RRT.

	Hemodialysis	Peritoneal dialysis	Kidney Transplantation	Total population
Lyso-Gb3 (ng/ml)	1.49±1.50	1.16±0.40	1.44±0.56	1.39±0.55
GAL (ng/ml/h)	18.28±14.36	18.82±14.45	21.33±15.16	18.99±16.34

Conclusions

Considering the presence of an ERT able to modify the clinical development of FD, it is essential to try to implement FD early diagnoses. However, the screening is too expensive to be extended on large scale, due to the low prevalence of pathology (1: 20000-40000). The screening should be performed on high-risk populations, for example on CKD patients under conservative therapy or in RRT (hemodialysis, peritoneal dialysis, kidney transplantation). In particular, this screening should be conducted on CKD patients in early stages in order to slow down the progression of CKD itself and to permit, in the case of missed diagnosis, the identification of the actual CKD primary cause.

The screening on ESRD patients has important implications both directly on their health status, and indirectly on their family. Once the ESRD has been reached, even counteracting the accumulation of FD catabolites, the renal recovery is marginal. Therefore, limiting the amount of waste products can mostly reduce either the onset of the comorbidities related to CKD or the systemic manifestations.

In addition, in FD kidney transplanted patients, ERT may slow down the accumulation of catabolites related to FD and therefore the CKD progression.

Moreover, the diagnosis of FD, as it is a genetic pathology, allows to obtain an important impact on the health of the relatives, who could thus receive an early diagnosis. Consequently, an adequate genetic counselling is important to identify any possible carrier of the mutation, who should undergo further investigations.

References

1) Opitz JM, Stiles FC, Wise D, Race RR, Sanger R, Von Gemmingen GR, Kierland RR, Cross EG, De Groot WP. The Genetics of Angiokeratoma Corporis Diffusum (Fabry's Disease) and Its Linkage Relations with the Xg Locus. Am J Hum Genet 1965; 17: 325-342.

2) Schiffmann R. Fabry disease. Pharmacol Ther 2009; 122: 65-77.

3) Germain DP. Fabry disease. Orphanet J Rare Dis 2010; 5: 30.

4) Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA 1999; 281: 249-254.

5) Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. High incidence of later-onset fabry disease revealed by newborn screening. Am J Hum Genet 2006; 79: 31-40.

6) Reisin RC, Mazziotti J, Cejas LL, Zinnerman A, Bonardo P, Pardal MF, Martinez A, Riccio P, Ameriso S, Bendersky E, Nofal P, Cairola P, Jure L, Sotelo A, Rozenfeld P, Ceci R, Casas-Parera I, Sanchez-Luceros A, Investigators A. Prevalence of Fabry Disease in Young Patients with Stroke in Argentina. J Stroke Cerebrovasc Dis 2018; 27: 575-582.

7) Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, Elliott PM. Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation 2002; 105: 1407-1411.

8) Nishino T, Obata Y, Furusu A, Hirose M, Shinzato K, Hattori K, Nakamura K, Matsumoto T, Endo F, Kohno S. Identification of a novel mutation and prevalence study for fabry disease in Japanese dialysis patients. Ren Fail 2012; 34: 566-570.

9) Echevarria L, Benistan K, Toussaint A, Dubourg O, Hagege AA, Eladari D, Jabbour F, Beldjord C, De Mazancourt P, Germain DP. X-chromosome inactivation in female patients with Fabry disease. Clin Genet 2016; 89: 44-54.

10) Ries M, Gupta S, Moore DF, Sachdev V, Quirk JM, Murray GJ, Rosing DR, Robinson C, Schaefer E, Gal A, Dambrosia JM, Garman SC, Brady RO, Schiffmann R. Pediatric Fabry disease. Pediatrics 2005; 115: e344-355.

11) Macrae WG, Ghosh M, McCulloch C. Corneal changes in Fabry's disease: a clinico-pathologic case report of a heterozygote. Ophthalmic Paediatr Genet 1985; 5: 185-190.

12) van Mullem PJ, Ruiter M. Fine structure of the skin in angiokeratoma corporis diffusum (Fabry's disease). J Pathol 1970; 101: 221-226.

13) Sodi A, Ioannidis A, Pitz S. Fabry Disease: Perspectives from 5 Years of FOS. Ophthalmological manifestations of Fabry disease. Oxford Pharma-Genesis 2006.

14) Weidemann F, Breunig F, Beer M, Sandstede J, Stork S, Voelker W, Ertl G, Knoll A, Wanner C, Strotmann JM. The variation of morphological and functional cardiac manifestation in Fabry disease: potential implications for the time course of the disease. Eur Heart J 2005; 26: 1221-1227.

15) Sims K, Politei J, Banikazemi M, Lee P. Stroke in Fabry disease frequently occurs before diagnosis and in the absence of other clinical events: natural history data from the Fabry Registry. Stroke 2009; 40: 788-794.

16) Del Pino M, Andres A, Bernabeu AA, de Juan-Rivera J, Fernandez E, de Dios Garcia Diaz J, Hernandez D, Luno J, Fernandez IM, Paniagua J, Posada de la Paz M, Rodriguez-Perez JC, Santamaria R, Torra R, Ambros JT, Vidau P, Torregrosa JV. Fabry Nephropathy: An Evidence-Based Narrative Review. Kidney Blood Press Res 2018; 43: 406-421.

17) Schiffmann R, Forni S, Swift C, Brignol N, Wu X, Lockhart DJ, Blankenship D, Wang X, Grayburn PA, Taylor MR, Lowes BD, Fuller M, Benjamin ER, Sweetman L. Risk of death in heart disease is associated with elevated urinary globotriaosylceramide. J Am Heart Assoc 2014; 3: e000394.

18) De Angelis S, Noce A, Di Renzo L, Cianci R, Naticchia A, Giarrizzo GF, Giordano F, Tozzo C, Splendiani G, De Lorenzo A. Is rasburicase an effective alternative to allopurinol for management of hyperuricemia in renal failure patients? A double blind-randomized study. Eur Rev Med Pharmacol Sci 2007; 11: 179-184.

19) Garman SC, Garboczi DN. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. J Mol Biol 2004; 337: 319-335.

20) Noce A, Fabrini R, Dessi M, Bocedi A, Santini S, Rovella V, Pastore A, Tesauro M, Bernardini S, Di Daniele N, Ricci G. Erythrocyte glutathione transferase activity: a possible early biomarker for blood toxicity in uremic diabetic patients. Acta Diabetol 2014; 51: 219-224.

21) Tesauro M, Nistico S, Noce A, Tarantino A, Marrone G, Costa A, Rovella V, Di Cola G, Campia U, Lauro D, Cardillo C, Di Daniele N. The possible role of glutathione-S-transferase activity in diabetic nephropathy. Int J Immunopathol Pharmacol 2015; 28: 129-133.

22) Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R, van Roomen C, Mirzaian M, Wijburg FA, Linthorst GE, Vedder AC, Rombach SM, Cox-Brinkman J, Somerharju P, Boot RG, Hollak CE, Brady RO, Poorthuis BJ. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. Proc Natl Acad Sci U S A 2008; 105: 2812-2817.

23) Ferraz MJ, Marques AR, Appelman MD, Verhoek M, Strijland A, Mirzaian M, Scheij S, Ouairy CM, Lahav D, Wisse P, Overkleeft HS, Boot RG, Aerts JM. Lysosomal glycosphingolipid catabolism by acid ceramidase: formation of glycosphingoid bases during deficiency of glycosidases. FEBS Lett 2016; 590: 716-725.

24) Rombach SM, Twickler TB, Aerts JM, Linthorst GE, Wijburg FA, Hollak CE. Vasculopathy in patients with Fabry disease: current controversies and research directions. Mol Genet Metab 2010; 99: 99-108.

25) Niemann M, Rolfs A, Stork S, Bijnens B, Breunig F, Beer M, Ertl G, Wanner C, Weidemann F. Gene mutations versus clinically relevant phenotypes: lyso-Gb3 defines Fabry disease. Circ Cardiovasc Genet 2014; 7: 8-16.

26) Malavera A, Cadilhac DA, Thijs V, Lim JY, Grabsch B, Breen S, Jan S, Anderson CS. Screening for Fabry Disease in Young Strokes in the Australian Stroke Clinical Registry (AuSCR). Front Neurol 2020; 11: 596420.

27) Effraimidis G, Rasmussen AK, Bundgaard H, Sorensen SS, Feldt-Rasmussen U. Is the alpha-galactosidase A variant p.Asp313Tyr (p.D313Y) pathogenic for Fabry disease? A systematic review. J Inherit Metab Dis 2020; 43: 922-933.

28) Lenders M, Duning T, Schelleckes M, Schmitz B, Stander S, Rolfs A, Brand SM, Brand E. Multifocal white matter lesions associated with the D313Y mutation of the alpha-galactosidase A gene. PLoS One 2013; 8: e55565.

29) Palaiodimou L, Stefanou MI, Bakola E, Papadopoulou M, Kokotis P, Vrettou AR, Kapsia E, Petras D, Anastasakis A, Xifaras N, Karachaliou E, Touloumi G, Vlachopoulos C, Boletis IN, Giannopoulos S, Tsivgoulis G, Zompola C. D313Y Variant in Fabry Disease: A Systematic Review and Meta-analysis. Neurology 2022; 99: e2188-e2200.

30) Saito O, Kusano E, Akimoto T, Asano Y, Kitagawa T, Suzuki K, Ishige N, Akiba T, Saito A, Ishimura E, Hattori M, Hishida A, Guili C, Maruyama H, Kobayashi M, Ohashi T, Matsuda I, Eto Y. Prevalence of Fabry disease in dialysis patients: Japan Fabry disease screening study (J-FAST). Clin Exp Nephrol 2016; 20: 284-293.

31) Yalin SF, Eren N, Sinangil A, Yilmaz VT, Tatar E, Ucar AR, Sevinc M, Can O, Gurkan A, Arik N, Alisir Ecder S, Uyar M, Yasar M, Gulcicek S, Mese M, Dheir H, Cakir U, Koksal Cevher S, Turkmen K, Guven B, Guven Taymez D, Erkalma Senates B, Ecder T, Kocak H, Uslu A, Demir E, Basturk T, Ogutmen MB, Kinalp C, Dursun B, Bicik Bahcebasi Z, Sipahi S, Dede F, Oruc M, Caliskan Y, Genc A, Yelken B, Altiparmak MR, Turkmen A, Seyahi N. Fabry Disease Prevalence in Renal Replacement Therapy in Turkey. Nephron 2019; 142: 26-33.

32) Abensur H. Fabry disease: patients at risk in Brazil! J Bras Nefrol 2021; 43: 5-6.

33) Silva CAB, Moura-Neto JA, Dos Reis MA, Vieira Neto OM, Barreto FC. Renal Manifestations of Fabry Disease: A Narrative Review. Can J Kidney Health Dis 2021; 8: 2054358120985627.

34) Herrera J, Miranda CS. Prevalence of Fabry's disease within hemodialysis patients in Spain. Clin Nephrol 2014; 81: 112-120.

35) Fabry Institute, Epidemiology of Fabry Disease. Accessed in December 2022, available at: https://fabry-institute.com/burden-of-disease/epidemiology-of-fabry-disease#.

36) Yenicerioglu Y, Akdam H, Dursun B, Alp A, Saglam Eyiler F, Akin D, Gun Y, Huddam B, Batmazoglu M, Gibyeli Genek D, Pirincci S, Ersoy IR, Uzum A, Soypacaci Z, Tanrisev M, Colak H, Demiral Sezer S, Bozkurt G, Akyildiz UO, Akyuz Unsal AI, Unubol M, Uslu M, Eryilmaz U, Gunel C, Meteoglu I, Yavasoglu I, Unsal A, Akar H, Okyay P. Screening Fabry's disease in chronic kidney disease patients not on dialysis: a multicenter study. Ren Fail 2017; 39: 104-111.

37) Lenders M, Oder D, Nowak A, Canaan-Kuhl S, Arash-Kaps L, Drechsler C, Schmitz B, Nordbeck P, Hennermann JB, Kampmann C, Reuter S, Brand SM, Wanner C, Brand E. Impact of immunosuppressive therapy on therapy-neutralizing antibodies in transplanted patients with Fabry disease. J Intern Med 2017; 282: 241-253.

38) De Schoenmakere G, Poppe B, Wuyts B, Claes K, Cassiman D, Maes B, Verbeelen D, Vanholder R, Kuypers DR, Lameire N, De Paepe A, Terryn W. Two-tier approach for the detection of alpha-galactosidase A deficiency in kidney transplant recipients. Nephrol Dial Transplant 2008; 23: 4044-4048.