

## Renal involvement in Fabry disease

### Acometimento renal na doença de Fabry

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#### ABSTRACT

Every cell in the human body has globotriaosylceramide accumulation (Gb3) in Fabry disease due to the mutation in gene of the enzyme  $\alpha$ -galactosidase A. It is a disease linked to sex. The main clinical features are: cutaneous angiokeratomas; acroparesthesias and early strokes; decreased sweating and heat intolerance; ocular changes; myocardial hypertrophy, arrhythmias; gastrointestinal disorders and renal involvement. Renal involvement occurs due to Gb3 accumulation in all types of renal cells. Therefore, patients may present glomerular and tubular function disorders. Podocytes are particularly affected, with pedicels effacement and development of proteinuria. The diagnosis is made by detection of reduced plasma or leukocyte  $\alpha$ -galactosidase activity and genetic study for detecting the  $\alpha$ -galactosidase gene mutation. Treatment with enzyme replacement contributes to delay the progression of kidney disease, especially if initiated early.

**Keywords:** enzyme replacement therapy, Fabry disease, podocytes.

#### RESUMO

Todas as células do corpo humano apresentam acúmulo de globotriaosilceramida (Gb3) na doença de Fabry devido à mutação que ocorre no gene da enzima  $\alpha$ -galactosidase A. Trata-se de uma doença ligada ao sexo. Os achados clínicos são: angioqueratomas cutâneos; acroparestesias e acidentes vasculares encefálicos precoces; sudorese diminuída e intolerância ao calor; alterações oculares; hipertrofia miocárdica, arritmias; alterações gastrointestinais e renais. O envolvimento renal ocorre devido ao acúmulo do Gb3 em todos os tipos de células renais. Portanto, os pacientes podem apresentar distúrbios das funções glomerulares e tubulares. Os podócitos são particularmente acometidos, com apagamento dos pedicélio e desenvolvimento de proteinúria. O diagnóstico é feito por meio da detecção de reduzida atividade plasmática ou leucocitária da  $\alpha$ -galactosidase e pela detecção da mutação do gene da  $\alpha$ -galactosidase. O tratamento com reposição enzimática contribui para o retardo da progressão da doença renal, principalmente se instituído precocemente.

**Palavras-chave:** doença de fabry; podócitos; terapia de reposição de enzimas.

#### INTRODUCTION

Fabry disease (FD) is caused by deficient or absent activity of enzyme  $\alpha$ -galactosidase A, a lysosomal enzyme whose substrate is glycosphingolipid globotriaosylceramide (Gb3/Gl-3). GB3 accumulates in the lysosomes of every cell in the bodies of individuals with this condition, thus compromising the function of multiple organs. FD follows a pattern of inheritance linked to chromosome X and involves every ethnic group. The incidence of the disease

has been poorly defined, and ranges between 1/100000 and 1/500000 inhabitants. More than 600 mutations have been described for the  $\alpha$ -galactosidase A gene.<sup>1</sup>

#### CLINICAL MANIFESTATIONS

Males present the classical form of the disease, which includes skin involvement characterized by angiokeratomas - clusters of small purple keratinized lesions located preferentially on the buttocks and thighs (bathing trunk distribution) (Figure 1);

Submitted on: 12/11/2015.

Approved on: 02/25/2016.

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DOI: 10.5935/0101-2800.20160034

peripheral and central nervous system impairment, with acroparesthesia and early stroke; reduced sweating and heat intolerance; eye disorders, such as deposits on the corneas (cornea verticillata), seen through examination with a slit lamp; myocardial hypertrophy, arrhythmia; gastrointestinal distress with nausea, vomiting, and diarrhea; and renal involvement - the manifestations targeted in this paper.<sup>2</sup>

**Figure 1.** Angiokeratomas (image courtesy of Dr. Cassiano Braga Silva).



Although heterozygous, females may also develop the disease and present severe cardiac, renal, and cerebral manifestations. This fact has not been fully understood, but the cells in female patients are believed to have only one active chromosome X, while the other chromosome X is randomly inactive. Therefore, clinical manifestations might depend on the number of cells with chromosome X alterations present in each given patient.<sup>3,4</sup>

The clinical signs of FD may vary significantly even in male patients, as a consequence of the type of mutation affecting the  $\alpha$ -galactosidase A gene and the levels of residual activity of the enzyme. Some male patients suffer from cardiac and renal impairment later in their lives, in what is called late-onset variant of Fabry disease.<sup>5,6</sup>

The clinical manifestations of FD usually escalate with age. Increases in disease severity have been correlated with the intensity of Gb3 cell deposition.

Earlier manifestations may occur in children between the ages of three and ten<sup>7</sup> and include acroparesthesia, heat intolerance, and gastrointestinal distress.

## DIAGNOSIS

Patients are diagnosed with FD based on clinical manifestations, family history, or when screened for risk factors such as dialysis, myocardial hypertrophy without apparent cause, and early stroke.<sup>8</sup> Detection of low  $\alpha$ -galactosidase A activity in plasma or leukocytes confirms the diagnosis.<sup>9</sup> However, the activity of the enzyme may be within the range of normality in females with FD. In such cases, genetic tests are warranted to detect mutations in the  $\alpha$ -galactosidase A gene.<sup>10</sup> Substrate (Gb3<sup>11</sup> and globotriaosylsphingosine - lyso-Gb3<sup>12</sup>) levels in plasma, urine, and tissues may also help diagnose individuals with FD, but these tests are more useful in assessing response to treatment. Lyso-Gb3 has been used more frequently.

## RENAL INVOLVEMENT

Renal involvement is caused by the accumulation of Gb3 in virtually all renal cell types, including podocytes and endothelial, mesangial, and tubular cells. Therefore, patients with FD may present glomerular and tubular disorders. Glomerular manifestations follow a course similar to that of diabetic nephropathy, with early hyperfiltration, albuminuria, massive proteinuria, and progressive deterioration of renal function. Although less common, tubular manifestations include Fanconi syndrome, distal renal tubular acidosis, and isosthenuria caused by impaired renal concentration ability secondary to collecting duct involvement. Renal involvement is one of the main causes of death and disability among patients with FD.<sup>2</sup>

Albuminuria sets in between the second and third decades of life, and further contributes to the progression of renal involvement as in other forms of renal disease.<sup>13</sup> Advanced chronic kidney disease requiring dialysis is usually seen between the fourth and fifth decades of life of untreated patients.<sup>14</sup>

Some patients have oval bodies in their urine, which present a Maltese cross pattern under polarized light in a microscope typically seen in cases of nephrotic syndrome, and a lamellar pattern showing Gb3 deposits.<sup>15</sup> Urine samples under scanning electron microscopy show typical osmiophilic lamellar bodies in urinary cells (Gb3 deposits).

Branton *et al.*<sup>16</sup> shed light on the natural history of FD. The authors of this paper reviewed the charts of 105 patients with FD seen at the Warren Grant Magnuson Clinical Center of the National Institutes of Health in Bethesda, Maryland, USA, from 1970 to 2000. The patients were diagnosed based on typical signs of FD (angiokeratomas, neuropathic pain, ocular disorders, early stroke, proteinuria, and kidney failure) and the observation of low  $\alpha$ -galactosidase A activity in leukocytes (values under 15% of the levels seen in healthy controls).

None of the patients with FD in this study lived to be 60 years of age. By the age of 60, 82.5% of the patients had proteinuria (24-hour urinary protein > 200 mg and < 3 g), 32.4% had high blood pressure, 41.5% had chronic kidney disease (serum creatinine > 1.5 mg/dL), 22% had end-stage renal disease (glomerular filtration rate < 12 mL/min or need for dialysis or transplantation), and 17% died with a mean age of  $50 \pm 8$  years.

Proteinuria set in when patients had a mean age of  $34 \pm 10$  years (14-55 years); 50% of the patients had proteinuria at the age of 35 years; and 100% of the patients who survived had proteinuria at the age of 52 years. Nephrotic proteinuria was seen in 18% of the patients with renal involvement, starting at the age of  $40 \pm 7$  years (26-55 years); however, full-fledged nephrotic syndrome with hypoalbuminemia and hypercholesterolemia was not commonly observed. Urine protein electrophoresis almost unanimously revealed the presence of protein of a glomerular nature (albuminuria  $\geq 50\%$ ), regardless of the degree of proteinuria.

The first signs of kidney failure (serum creatinine  $\geq 1.5$  mg/dL) were observed when patients had a median age of 42 years (19-54 years); they were diagnosed with end-stage kidney disease at a median age of 47 years (25-56 years). It took a mean of  $4 \pm 3$  years (1-13 years) for patients to move from kidney failure to end-stage kidney disease. Fabry nephropathy progresses rapidly, with annual decreases of 12.2 mL/min in the glomerular filtration rate after the onset of kidney failure, while other conditions produce annual decreases of 4 mL/min in renal function.<sup>17</sup> Every patient who reached the age of 55 years progressed to end-stage renal disease. Fourteen patients were started on hemodialysis and six

on peritoneal dialysis. The patients on hemodialysis and the individuals on peritoneal dialysis not offered kidney transplantation survived for up to 17 years and over four years, respectively.

Fourteen patients had 15 kidney transplants with good outcomes, 14 from deceased donors and one from a living related donor. Late biopsies (13 and 7 years after transplantation) did not reveal the presence of Fabry deposits. Scanning electron microscopy images of graft biopsies from transplant patients with FD showed Fabry deposits in the vascular endothelium, possibly arising from the colonization of the graft vasculature by recipient endothelial cells.<sup>18</sup> Recent studies revealed that kidney transplantation normalizes only the urine, and not the plasma, levels of  $\alpha$ -galactosidase A. Therefore, kidney transplantation has no effect on the progression of non-renal manifestations of FD. Post-transplantation graft and patient survival rates are similar to those of other renal diseases.<sup>19</sup>

The severity of renal involvement in patients with FD has been correlated with  $\alpha$ -galactosidase A activity in leukocytes. Individuals with activity levels below 1% were diagnosed with renal failure at the age of 22 years, whereas patients with activity levels ranging between 1% and 12% were diagnosed with renal failure at the age of 47 years. Histology findings indicated more severe involvement in patients with enzyme activity levels below 1%. Enzyme replacement therapy may have a role in helping patients in whom the enzyme is missing or altered.

The type of mutation has been correlated with the severity of renal involvement. Conservative mutations, in which one amino acid is replaced by another amino acid of the same class in certain exons, has been associated with fewer occurrences of kidney failure. Non-conservative mutations, in which amino acids are replaced by amino acids of different classes and deletions and insertions occur, have been associated with more severe renal disease.

MacDermot *et al.*<sup>20</sup> looked into a series of 98 males with FD selected from the genetic registry of the United Kingdom from 1985 to 2000, most of which diagnosed with low  $\alpha$ -galactosidase A activity in leukocytes. The patients included in this study were started on dialysis at a mean age of

36.7 years and offered renal transplantation at a mean age of 40 years.

## RENAL BIOPSY FINDINGS

The morphologic alterations seen in patients with FD characterized by Gb3 cell deposits may be found in renal biopsy specimens, even in cases of early onset disease in children;<sup>21,22</sup> this finding carries both diagnostic and prognostic value for patients with FD.<sup>23</sup> In individuals with the disease, it is used to follow the progression of the disease and assess treatment efficacy.<sup>21,24,25</sup> Gb3 deposits in the kidneys have been correlated with severity of morphologic and renal function alterations.<sup>16</sup>

Gb3 deposits are found on the renal parenchyma in the four compartments of the kidney: vascular, glomerular, tubular e interstitial. They are initially observed in glomerular podocytes, cells with terminal differentiation in the capillary loop, and play an important role in the permeability of the filtration barrier. Podocyte involvement causes proteinuria/nephrotic syndrome. The deposits become more prominent as the disease progresses, and are found not only in the podocytes, but also in the Bowman's capsule epithelium, in the mesangial and endothelial cells of the glomeruli, in the endothelial cells of peritubular capillaries and arteries, in the smooth muscle cells of arteries and arterioles, in the distal tubule, and less often in the proximal tubule and in the cells of the interstitial compartment.

In more advanced cases of disease, the mesangial matrix may become enlarged with segmental or global glomerulosclerosis and present interstitial fibrosis, tubular atrophy, and arteriosclerosis.<sup>23</sup> Gb3 deposits and other injuries in the different compartments may be quantified and scored, as proposed by the International Study Group of Fabry Nephropathy (ISGFN).<sup>24</sup> The score is then used for diagnostic, prognostic, and treatment follow-up purposes.<sup>21,26</sup> Automated morphometry may be used to quantify Gb3 deposits in renal biopsy specimens.<sup>27</sup>

In children with FD, the accumulation of Gb3 in podocytes has been associated with foot process effacement and degree of proteinuria. Early podocyte injury may play a role in the development and progression of Fabry nephropathy.<sup>22</sup> Podocyte

injury has been described in patients with FD and normoalbuminuria.<sup>28</sup>

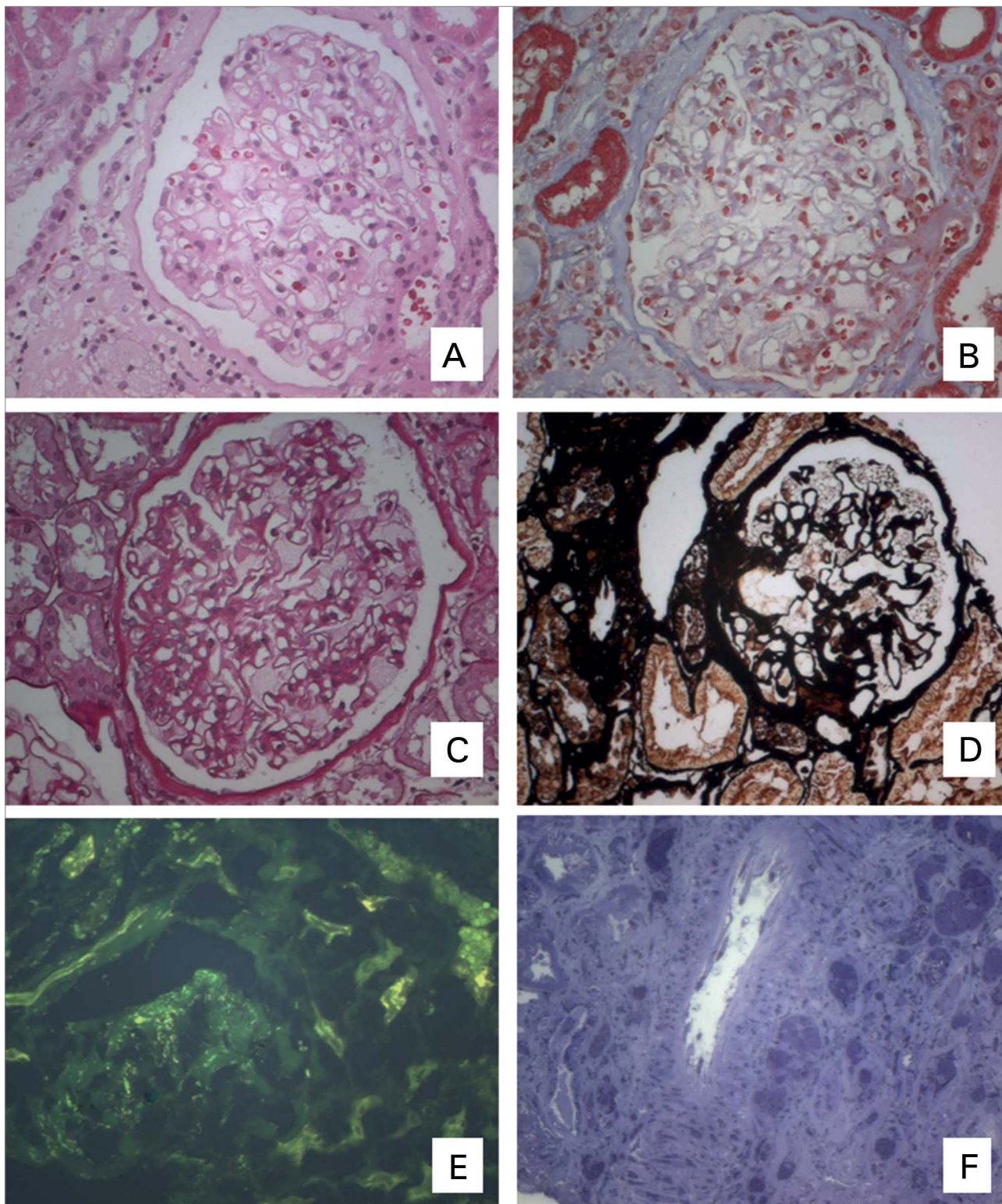
Three techniques are used in the analysis of renal biopsy specimens acquired from the vascular, glomerular, tubular, and interstitial compartments: (a) light microscopy for paraffin-embedded specimens; (b) immune complex analysis from fresh frozen core biopsy specimens through immunofluorescence; and (c) scanning electron microscopy. The three techniques show renal alterations in every compartment of patients with FD. The specimens analyzed with a light microscope can be fixed in a number of different solutions, including formaldehyde, paraformaldehyde, Bouin's fixative, or methacarn, to name a few. Specimens have to be prepared before they are embedded in paraffin and sliced in 2  $\mu$ m-thick sections. They are first dehydrated and diaphonized in alcohol and xylene solutions, which dissolve lipid deposits and Gb3.

The sites once occupied by Gb3 in the cytoplasm of the cells are empty, and the resulting vacuoles can be viewed with any of the staining techniques of choice (hematoxylin and eosin, Masson's trichrome, PAS - periodic acid-Schiff, PAMS - periodic acid silver methenamine stain, or picro-sirius red) (Figures 2A to 2D). Podocytes are the most frequently involved cells, and the empty spaces where they were once located have to be quantified.<sup>24</sup> Although most of the Gb3 dissolves and leaves behind optically empty spaces, immunohistochemistry testing with a monoclonal antibody reactive with ceramide trihexoside may find traces of Gb3 in paraffin-embedded specimens.<sup>29</sup>

Fresh frozen specimens are often used to test for immune complex deposits. However, patients with FD do not suffer from immune complex deposition, and test results are negative or non-specific, such as finding IgM or C3 in areas of sclerosis. Gb3 deposits are birefringent and produce a characteristic Maltese cross pattern when viewed under polarized light, in addition to presenting natural fluorescence with shades of yellowish green under ultraviolet light; therefore, Gb3 cell deposits can be seen in "negative control" slides (Figure 2E). Fresh frozen specimens can be stained for lipids with Sudan to elicit Gb3 deposits.<sup>23</sup>



**Figure 2.** Specimen embedded in paraffin, 2- $\mu$ m sections under common light (A to D); the optically empty vacuoles seen in the cytoplasm of the podocytes originated from the dissolution of intracellular Gb3 as the specimen was exposed to alcohol and xylene solutions; hematoxylin and eosin (A), Masson's trichrome (B), picro-sirius red (C), and PAMS (D). Specimen frozen in liquid nitrogen, negative control section, no antisera, seen in a microscope under ultraviolet light for immunofluorescence; Gb3 deposits present yellowish green fluorescence in the glomerular, tubular, vascular, and interstitial compartments (E). Specimen embedded in resin, 0.5  $\mu$ m semi-thin section, stained with toluidine blue; Gb3 deposits stained in a stronger shade of blue in tubular cells and on the wall of the interlobular artery, on smooth muscle cells and endothelium (F). (A to F: magnification: 20X objective; 2.0X optovar).



Specimens analyzed on a scanning electron microscope are first fixed with glutaraldehyde or Karnovsky fixative, then with osmium, and are later processed and embedded in blocks of EPON, Epoxy, or another resin. The lipid deposits in this processing technique are osmiophilic, thus making it easier to visualize Gb3 both in semi-thin sections stained with toluidine blue viewed on a light microscope, and in ultra-thin sections viewed on a scanning electron microscope. Therefore, the first 0.5µm semi-thin section stained with toluidine blue will show Gb3 deposits strongly stained blue inside the cells viewed on an ordinary light microscope, particularly in podocytes, followed by the distal tubule and other cells (Figure 2F, Figure 3A).

Once they are easy to visualize, Gb3 deposits can be quantified in semi-thin sections;<sup>24</sup> some authors have even resorted to automated morphometry.<sup>27</sup> The second section, an ultra-thin 60-nm slice, is viewed on a scanning electron microscope (Figures 3B to F). Gb3 deposits are observed inside the lysosomes of different cell types, and in glomeruli, tubules, and vessels, and in the peritubular capillaries of the endothelial cells in the interstitial compartment.

The lysosomal deposits are lamellar electron-dense structures intercalated with electron-lucid lamellas. Depending on the orientation of the section in the lysosome vis-à-vis the deposits, a concentric shape called “myelin figure,” “onion skin” or “zebra bodies” is shown. Larger magnifications (250,000X) allow the observation of thin electron-dense layers in the electron-dense lamella (Figure 3F). Podocytes are cells with terminal differentiation, and are thus the ones in which more deposits are found.

Podocytes have traits of epithelial and mesenchymal cells, with a rich cytoskeleton harmoniously organized to maintain their primary, secondary, and tertiary processes, with the foot processes as their last branches, adhered to the basement membrane and forming the slit diaphragm between foot processes. This cell type has been targeted in a number of studies.<sup>30-32</sup> Gb3 deposits impair the cytoskeleton and cause the effacement of foot processes, changing permeability and inducing proteinuria. Studies with morphometric analysis have looked into foot processes and podocyte volume, and one showed an arrangement

of foot processes in a mosaic pattern in a female patient with FD.<sup>33</sup>

Although renal biopsy is a diagnostic method and scanning electron microscopy a reliable tool to identify deposits, they are not pathognomonic/specific, as similar deposits are found in individuals with silica nephropathy<sup>34</sup> and pseudolipidosis induced by drugs such as amiodarone, chloroquine and hydroxychloroquine.<sup>23,35</sup>

Tøndel *et al.*<sup>21</sup> performed renal biopsies in eight children with FD aged between four and 16 years without albuminuria and with normal renal function and acroparesthesia. The authors reported they had podocyte Gb3 deposits and some presented foot process effacement. Given the early onset of renal involvement, treatment must be started promptly for patients with this condition.

## TREATMENT

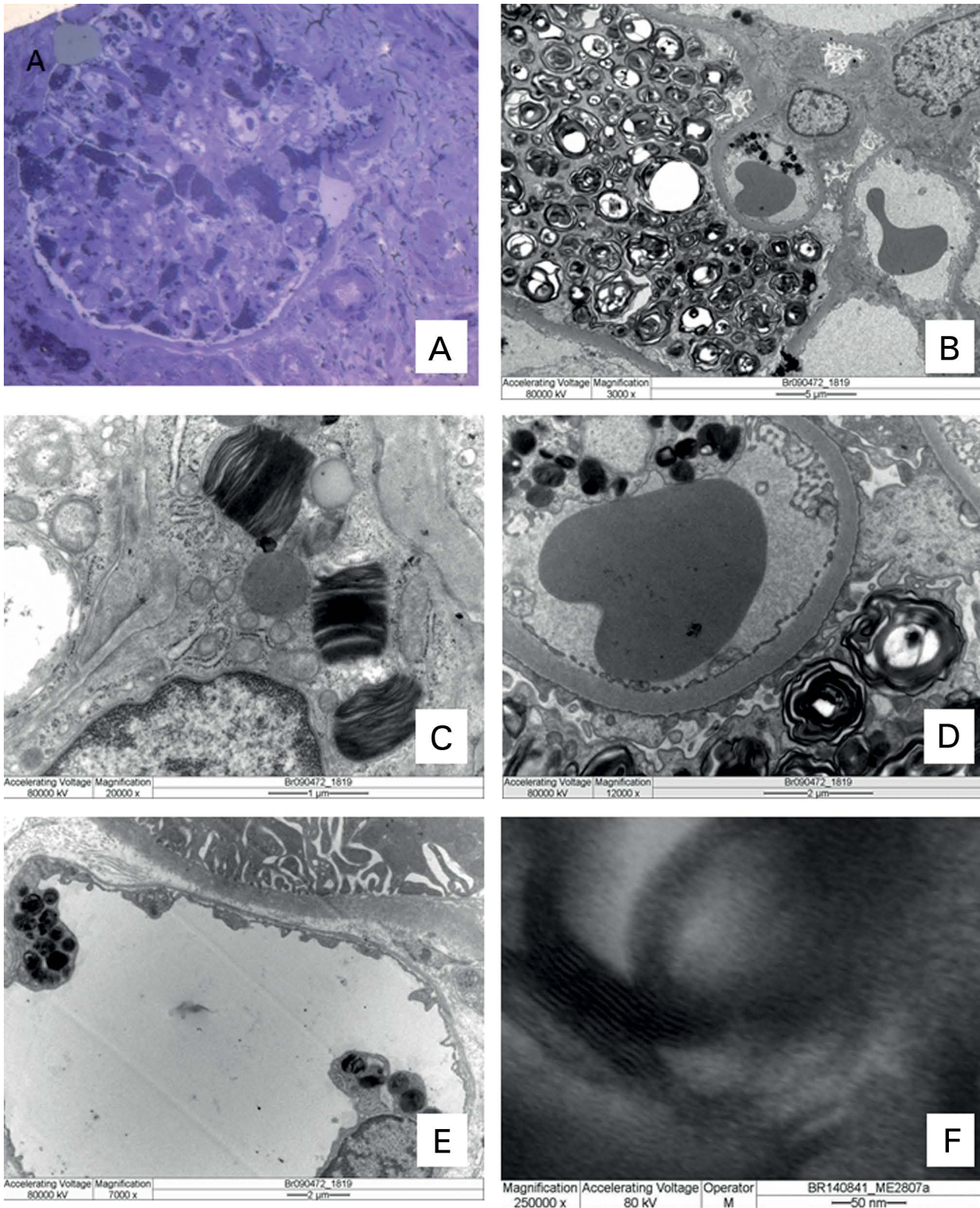
The treatment of FD is designed to prevent Gb3 deposition or remove intracellular Gb3 deposits by means of enzyme replacement therapy with recombinant forms of  $\alpha$ -galactosidase A. Additionally, patients must be offered specific therapies for each involved organ, which may include antiproteinuric medication, angiotensin-converting-enzyme inhibitors, or angiotensin II receptor blockers in cases of renal involvement.

Two recombinant forms of  $\alpha$ -galactosidase A are available in Brazil: agalsidase alfa (Replagal®, Shire) and agalsidase beta (Fabrazyme®, Genzyme/Sanofi). The two preparations are administered intravenously every two weeks. The main difference between them is the dosage prescribed for the treatment of FD for each medication: agalsidase alfa with 0.2 mg/kg and agalsidase beta with 1 mg/kg. Once the recommended dosage of agalsidase alfa is lower, the time of infusion is also lower when compared to that of agalsidase beta (40 minutes vs. 120 minutes, respectively).

The dosage of agalsidase beta was defined in a phase-1 and phase-2 trial by Eng *et al.*<sup>36</sup> In this study, 15 patients with FD were given five doses of agalsidase beta of 0.3, 1.0 or 3 mg every two or fourteen days. The scheme with agalsidase beta 1 mg/kg every 14 days offered the best efficacy-to-safety ratio. Gb3 clearance in the kidneys, heart, skin, and plasma was observed. Endothelial cells were preferentially benefitted by the removal of deposits.



**Figure 3.** Specimen embedded in resin, 0.5  $\mu\text{m}$  semi-thin section, stained with toluidine blue, viewed on a light microscope: Gb3 deposits stained in a stronger shade of blue, particularly on the podocytes in the glomerulus (A). Transmission electron microscope (B to F): intralysosomal Gb3 deposits in different cells in the glomerulus: podocyte and endothelial cell (B and D), mesangial cell (C) and endothelial cell of a peritubular capillary (E). Gb3 deposits consist of electron-dense layers intercalated with electron-lucid layers, sometimes in a concentric pattern (B and D) and sometimes following a zebra body pattern (B and C); under higher magnification (F - 250,000X) the various thin layers making each lamella can be seen.



After this short-course therapy patients reported improvements in pain relief, ability to sweat, and quality of life. The treatment was well tolerated. Four patients had mild to moderate adverse events possibly due to hypersensitivity to the drug, but all cases were managed conservatively. Eight patients developed IgG antibodies against agalsidase beta, but they were not neutralizing antibodies.

The phase-3 trial<sup>37</sup> with agalsidase beta enrolled 58 patients confirmed with FD aged 16 years and older, with a mean serum creatinine level of 0.8 mg/dL. This multicenter placebo-controlled study ran for 20 weeks. Twenty-nine patients were given agalsidase beta 1 mg/kg every 15 days and 29 were offered placebo (mannitol) every 15 days. Afterwards, all patients were treated with agalsidase beta for six months.

The primary endpoint, total Gb3 clearance from the endothelium of the renal microvasculature, was reached in 20 of the 29 patients given agalsidase beta and in none of the individuals offered placebo. After the six-month course of agalsidase beta, 98% of the patients reached the primary endpoint. The patients were followed based on this protocol for 54 months<sup>38</sup> and were subsequently followed as part of the Fabry registry for a mean of 10 years<sup>39</sup> on agalsidase beta 1 mg/kg every 15 days.

Six patients were excluded from the 10-year follow-up analysis for not having data with the Fabry Registry. Eighty-one percent of the 52 patients included in the analysis did not present severe clinical events and 94% were alive at the end of the follow-up period. Thirty-two of the 52 patients were categorized as having minor renal involvement (proteinuria  $\leq$  0.5 mg/g of creatinine and glomerulosclerosis  $<$  50%) and 20 as having major renal involvement in relation to baseline (proteinuria  $>$  0.5 mg/g of creatinine and glomerulosclerosis  $\geq$  50%).

The individuals with less renal involvement offered treatment at younger ages benefitted more from enzyme replacement therapy, and had annual decreases in renal function of 1.89 ml/min/1.73 m<sup>2</sup> versus 6.82 ml/min/1.73 m<sup>2</sup> seen in the group with more severe renal involvement. In general, the sizes of the septum and the posterior wall of the left ventricle remained stable and within normality. Plasma levels of GB3 became normal after six months of treatment and remained stable since then. The patients had a mean age of 41.6 years

at the end of follow-up, and most remained free of clinical events.

The phase-4 trial<sup>40</sup> enrolled 82 patients with more advanced FD and serum creatinine levels ranging between 1.2 and 3.0 mg/dL (estimated creatinine clearance  $<$  80 ml/min). The patients were followed for 18.5 months. Two thirds of the subjects were given intravenous agalsidase beta 1 mg/kg every two weeks and a third of the individuals were given placebo. Although the combined risk of renal, cardiac, and cerebral events and death was reduced by 43%, no statistically significant differences were found between the groups.

A significant 61% difference was observed after the groups were adjusted for proteinuria. The subjects in the agalsidase beta group had higher levels of proteinuria at baseline. Once again, the individuals starting the study with lower serum creatinine levels ( $\leq$  1.5 mg/dL) and higher glomerular filtration rates ( $\geq$  55 ml/min) benefitted more from enzyme replacement therapy.

A randomized trial<sup>41</sup> with 26 patients diagnosed with FD was carried out to assess the use of intravenous agalsidase alfa 0.2 mg/kg every two weeks and its effects on neuropathic pain as an endpoint. This double-blind placebo-controlled study was carried out for six months. The patients given the recombinant enzyme had significant decreases in neuropathic pain scores and lesser reductions in creatinine clearance, but no differences were observed between the placebo and agalsidase alfa groups in terms of inulin clearance. Significant reductions of Gb3 levels were seen in plasma and urine, but not in the kidneys.

Several authors have investigated the dosage differences between the two forms of recombinant  $\alpha$ -galactosidase A. Tøndel *et al.*<sup>42</sup> analyzed 12 children prescribed enzyme replacement therapy with agalsidase alfa or beta submitted to serial renal biopsies for five years and found that higher cumulative dosages of recombinant enzyme were associated with greater Gb3 clearance from podocytes and lower levels of urine protein. Podocytes cannot divide and, therefore, require greater amounts of enzyme to clear Gb3 deposits.

Twelve patients with fast-declining renal function ( $-8.0 \pm 0.8$  ml/min/1.73 m<sup>2</sup>/year) on agalsidase alfa 0.2 mg/kg every two weeks had their prescriptions changed to 0.2 mg/kg every week and



saw the rate of loss of renal function decrease ( $-3.3 \pm 1.4$  ml/min/1.73 m<sup>2</sup>/year), showing the need to increase the supply of agalsidase alfa.<sup>43</sup>

The plant at which agalsidase beta is made had a viral contamination incident in 2009. The ensuing shortage of the medication affected patients all over the world for two years. Some were able to maintain their therapeutic regimens with 1 mg/kg of agalsidase beta every two weeks, while others had to wind down their use of the drug to 0.25 or 0.50 mg/kg every two weeks; some had to switch to agalsidase alfa 0.2 mg/kg every two weeks.

Many authors have analyzed the effects of recombinant enzyme dosage decreases. A Dutch study with 35 patients reported increased LysoGb3 levels.<sup>44</sup> Another study with 105 patients described increased proteinuria among patients on lower-dosage agalsidase beta and individuals switched to agalsidase alfa.<sup>45</sup> Histologic evidences of intensified foot process effacement and accumulation of Gb3 in patients on low-dosage recombinant enzyme protocols have also been reported.<sup>21</sup> However, authors of less robust studies (no more than 11 patients per study) and studies with patients with normal renal function failed to describe alterations secondary to decreased recombinant enzyme dosages.<sup>46-48</sup>

Unfortunately, a Canadian study<sup>49</sup> designed to enroll 362 patients and compare the incidence of renal, cardiac, and neurologic events and death of patients given agalsidase alfa or beta lost its statistical power after the shortage of agalsidase beta, and only 26 patients were included in the agalsidase beta arm of the study.

## CONCLUSION

Patients with FD are subject to early renal impairment involving all cell types, suffer from glomerular and tubular manifestations, and invariably progress to end-stage renal disease. The disease particularly affects podocytes. Properly prescribed early enzyme replacement therapy combined with renin-angiotensin-aldosterone system inhibitors have effectively dealt with the renal complications of FD.

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