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Arterial involvement in Fabry disease: state of the art and future diagnostic purposes

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Abstract. – Anderson-Fabry disease (FD) is a rare genetic, progressive, and multi-systemic condition, with X-linked inheritance. This is caused by pathogenic variants in the GLA gene, coding for the lysosomal enzyme called alpha-galactosidase A (aGLA), responsible for the cleavage of globotriaosylceramide (Gb3). The reduced or absent activity of aGLA causes the intracellular accumulation of Gb3, particularly in smooth and endothelial muscle cells, which causes cellular dysfunction. The main organs involved are the central nervous system, heart, and kidneys. However, being a ubiquitous enzyme, FD disease must be considered a systemic disease involving the peripheral nervous system, ocular and audio-vestibular systems. Also, the vascular district is damaged but the pathophysiology of vasculopathy in FD is not yet entirely understood. In literature, many vascular diagnostic tests were used to evaluate this specific involvement in FD, i.e., carotid intima media thickness (cIMT), arterial stiffness (AS), flow-mediated dilation (FMD) and atherosclerotic plaques; evaluation of vascular calcifications in FD patients is not presently available. In this review, we examined the current available literature on vascular aspects in FD. Moreover, we presented our global vascular evaluation, based on Radio Frequency Duplex Ultrasound (RF-DU), plaques, and vascular calcifications, to apply to FD patients.

Key Words:
Fabry disease, Vasculopathy in Fabry disease, Radio frequency duplex ultrasound, Vascular calcification, Atherosclerosis.

Introduction

Anderson-Fabry disease (FD) is a rare genetic, progressive, and multi-systemic condition, with X-linked inheritance. This is caused by pathogenic variants in the gene *GLA*, coding for the lysosomal enzyme called alpha-galactosidase A (aGLA), responsible for the cleavage of globotriaosylceramide (Gb3). The reduced or absent activity of aGLA causes the intracellular accumulation of Gb3, affecting endothelial cells in particular, inducing cellular dysfunction[1]. However, the aGLA gene expresses itself in a ubiquitous way causing damage to many organs and systems, such as the central and peripheral nervous system, heart, kidneys, eyes, lungs, and audio-vestibular system. Compared to other lysosomal storage disorders, FD has a slower evolution and, although the onset of symptoms can present in childhood, it is considered a pathology of adulthood[2]. Progressive lysosomal Gb3 deposition causes oxidative stress, and therefore cellular apoptosis[1]. In the late stages of the disease, irreversible fibrosis with cellular dysfunction and organ failure occurs. Some authors demonstrated the presence of microcirculation damage[3,4] and a pro-thrombotic state[5]. Moreover, a work published in 2017 showed a higher incidence of autoantibodies in FD patients[6]. Rozenfeld et al[7] have supposed that progressive accumulation of Gb3 could trigger inflammatory processes, contributing to FD pathogenesis. In fact, the accumulation of GB3 at the lysosomal level and higher release of glycosphingolipids, recognized as antigens by the natural killer cells, could lead to an altered capacity for autophagocytosis with altered release of different inflammatory mediators, as seen for other lysosomal storage disorders[6]. Despite these breakthroughs, there is no clear evidence for an autoimmune mechanism underlying FD and the pathogenesis of FD requires further clarification.

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Clinically, FD patients present various signs and symptoms depending on the district involved. The progressive accumulation of Gb3 at the level of the myocardium occurs with EKG abnormalities in its initial phase, such as short PR interval and increased voltages, and then, with bi-ventricular concentric hypertrophy, valve abnormalities, angina, dyspnea, syncope, and severe arrhythmias that often require pacemaker implantation. The first manifestations of kidney involvement are microalbuminuria and increase in the glomerular filtration rate, with the subsequent appearance of proteinuria and renal failure, until end stage renal disease (ESRD)[4]. The Central Nervous System (CNS) may be affected by the progressive accumulation of Gb3. FD patients can exhibit non-specific symptoms, such as headaches, and present high risk of ischemic strokes or transient ischemic attack (TIA) at young age[8]. The presence of white matter lesions is usual at radiological imaging, and sometimes it can resemble multiple sclerosis[9,10]. Regarding the peripheral nervous system (PNS), the progressive accumulation of Gb3 causes damage to the small nerve fibers C and A-delta with progressive reduction of the nerve endings, impairing transmission of sensory and nociceptive signals. Pain is one of the most frequent symptoms and can be classified into four types: acute, chronic, neuropathic, and acute pain attacks, also called "Fabry crises", which are often caused by exposure to thermal and physical stress[11]. The accumulation of Gb3 also determines an impairment of the sympathetic nerve fibers and sweat glands with altered regulation of heat dispersion and sweating. Clinically, hypo-anhidrosis and severe heat intolerance are the main symptoms. The involvement of the peripheral nervous system also presents with gastro-intestinal symptoms, such as diarrhea, vomiting, abdominal pain, and dyspepsia[12]. Aguilera-Correa et al[13] showed that the accumulation of Gb3 could lead to a change in gut microbiota with a higher prevalence of Bacterioides *spp.* and a lower production of short chain fatty acids, such as butyric acid, performing a trophic function on the intestinal mucosa. Another possible clinical manifestation of FD is fever of unknown origin (FUO). On a retrospective analysis, our group found that about 21% of FD patients presented unexplained fever attacks during their childhood[14]. For this reason, a consensus of experts has suggested to include FD among the possible causes of FUO[15]. Ocular involvement determines the appearance of the cornea verticillata caused by GB3 deposits and retinal vascular tortuosity. The main dermatological manifestations are angiokeratomas, distributed at the periumbilical and genital regions. The audio-vestibular system is also affected, leading to tinnitus, dizziness, and sudden hearing loss with progressive deafness. These manifestations can be ascribed to damage caused to the cochlear region but also to vascularization defects of the acoustic nerve. Non-coronary circulation is also involved in FD, although poorly studied in literature[16]. However, FD patients must be considered at high cardiovascular risk[17].

Diagnosis of FD is difficult due to the presence of non-specific symptoms, especially in its early disease stages. For this reason, the diagnostic delay of this rare pathological condition is often considerable, helping to fuel FD's reputation as a "Great Imposter"[18]. In males, the diagnosis is based on the determination of the enzymatic activity on leukocytes or fibroblasts on whole blood, which is absent or markedly reduced. Molecular analysis, then, confirms FD diagnosis, revealing the presence of mutations on the *GLA* gene. In females, the diagnosis is based exclusively on a genetic test which highlights the heterozygous state, as the determination of enzyme activity is not considered reliable, producing potentially misleading results.

The treatment of FD is based on two main options: enzyme replacement therapy (ERT) with the infusion of recombinant enzymes, agalsidase alfa and agalsidase beta, and oral chaperone therapy[19]. Agalsidase alfa is administered at a dosage of 0.2 mg/kg, while agalsidase beta is administered at 1 mg/kg. Both molecules are administered intravenously every 14 days. The goal of intravenous therapy is to provide the body of the affected patient with the missing enzyme. On the other hand, chaperone therapy is taken orally at a dosage of 123 mg every other day. This treatment is only eligible for patients with amenable mutations (i.e., mutations with residual enzyme activity). It is estimated that only 30% of patients are candidates for chaperon therapy[20]. It is essential to underline the importance of early treatment, before non-reversible damage has occurred, regardless of the therapeutic choice[21].

Vascular Involvement in Fabry Disease

In August 2020, we performed a literature search through PubMed, Medline, and Google Scholar to understand the latest information about

FD and related vascular features. We used many combinations of keywords: Fabry Disease, vasculopathy, Intimal-Medial Thickness, Flow-Mediated Dilatation OR Pulse Wave Velocity OR micro-angiopathy OR calcifications. These search terms had to be identified anywhere in the article text. We selected main scientific studies conducted on humans, published in international journals in English on any date[16,22,23]. We excluded single patient case reports focusing on studies with a larger number of patients, always considering that FD is a rare disease. Abstracts were reviewed to assess if the article met the inclusion criteria and, in case this could not be determined from the abstract, we reviewed the full article when it was available online.

At present, the pathophysiology of vasculopathy in FD remains uncertain. As reported above, the lysosomal accumulation of Gb3 in the cells of the arterial wall is considered the basis of this process and its main complications, including stroke, hypertrophic cardiomyopathy, and renal failure. On the contrary, venous involvement in FD is not known in literature, and is certainly less involved than arteries. Frustaci et al[24], describing a case of a 54-year-old man affected by FD, underwent a double aorto-coronary by-pass, one from the saphenous vein (SV) and the other from the left internal mammary artery (LIMA). It was concluded that veins are more suitable for grafts than arteries in FD patients, due to the histological absence of Gb3 accumulation in the veins. Usually, in patients with advanced or severe disease, ERT is not able to stabilize FD nor prevent its further progression. These facts suggest that the accumulation of Gb3 is not the unique way of vascular damage, but that there are other mechanisms, such as the formation of reactive oxygen species (ROS). We know that the accumulation of Gb3 occurs in various layers of vascular tissue, causing different alterations in endothelial and smooth muscle cells, with thickening of the neo-intimal fibrotic structures, consequent loss of vascular compliance, and endothelial dysfunction[25]. The result is an enhanced activation of the local renin-angiotensin system with overexpression of adhesion molecules, cytokines, chemokines and pro-thrombotic factors, and a decrease in the synthesis of nitric oxygen (NO). Some authors suggest that storage of Gb3 is sufficient to induce ROS, activate Rho-Kinase (ROCK), and dysregulate the activity of endothelial NO synthase (eNOS)[1,26,27]. This mechanism predisposes to muscle hyper-contractility and vasospasm, and it may represent the initial phase in the cascade that leads to FD vasculopathy. In the end, calcium/vitamin D metabolism could also interfere with cardiovascular health. Although an alteration in serum calcium levels is not described, FD patients are at high risk for vitamin D deficiency. In fact, they tend to have low exposure to sunlight due to heat intolerance and are at risk of malabsorption or reduced nutritional intake due to the FD itself. Drechsler et al[28], in 2014, found a vitamin D deficiency in 73% of FD patients. We know that vitamin D deficiency has a negative impact on cardiac outcomes. In 2010 Pilz et al[29] showed that vitamin D metabolites may have anti-hypertrophic and anti-proliferative actions, and its deficiency is related to worse phenotype of FD cardiomiopathy[29]. Moreover, Chen et al[30] evidenced a higher expression of Vitamin D Receptor (VDR) in cardiac myocytes and fibroblasts of hypertrophic hearts. Teitcher et al[31] evidenced a correlation between FD and the presence of vitamin D receptor (VDR) polymorphisms, showing a potential protective effect of some haplotypes on cardiovascular outcome. We know that vitamin D plays a protective role for the vascular system. Its deficiency is associated with early atherosclerotic damage and with increased cardiovascular-related morbidity and mortality, although in absence of clear evidence[32,33].

Both large and small vessels can have morphological and structural alterations, i.e., vascular tortuosity of the retina[24], skin[34], and dolichoectasia of intracranial arteries[35,36]. The accumulation of glycosphingolipids in the endothelial and smooth muscle cells in FD causes microcirculatory dysfunction and Raynaud phenomenon. Some recent studies noticed an increased frequency of Raynaud phenomenon in FD patients, using capillaroscopy to assess the presence of capillary changes in affected subjects. These studies[22,37,38], although numerically limited, highlighted the presence of capillaroscopic abnormalities in FD patients. Costanzo et al[39] examined a cohort of FD patients without left ventricular hypertrophy by carotid ultrasound, FMD, and nail capillaroscopy. Compared to the healthy subjects, FD patients had higher mean values of IMT, significantly lower FMD, and irregular nailfold architecture. They found significant presence of dystrophic capillaries, especially ramified dystrophic capillaries. FD vasculopathy is basically different from prema-

ture atherosclerosis. In FD there is more fibrotic structure formation and the smooth muscle cells are primarily involved with enhanced arterial stiffness. Rombach et al[16] reviewed twenty-four studies about histopathology of arteries in FD and only three investigations indicated atherosclerosis as a major finding. In FD arteries, smooth muscle cell involvement with stored Gb3 is one of the most prominent and early features. Instead, in females and atypical cardiac variants, no significant endothelial storage is found. The smooth muscle cells are also hypertrophic; the proliferation of smooth muscle cells and Gb3 storage results in higher carotid Intimal-Medial Thickness (cIMT).

The increase in cIMT is probably the most specific FD vessel wall alteration compared to the more traditional cIMT changes seen in premature atherosclerotic diseases[16]. Boutouyrie et al[40,41] showed that FD patients had a higher cIMT and distensibility of the radial artery than healthy controls. Barbey et al[42] provided evidence for a marked thickening of the cIMT wall in FD patients, compared with 120 age-matched controls with a low cardiovascular risk profile. The smooth muscle cells hypertrophy occurred to the same extent in men and women, which indicates that the vascular remodeling is independent of residual alfa-galactosidase A activity. Atherosclerotic plaques were not observed in the common carotid artery (CCA) of any FD patient, while ultrasound signals revealed homogeneous thickening of cIMT. Most of the FD patients have one or more traditional cardiovascular risk factor, which could contribute to the marked thickening of the CCA wall. cIMT was only correlated with age, confirming data on elastic arteries previously reported by Boutouyrie et al[40,41]. Kalliokoski et al[43] showed a statistically significant increase not only in cIMT, but also in the brachial artery and abdominal aorta IMT in 17 FD patients, compared to 34 health controls matched by age, sex, and smoking.

Barbey et al[42] have shown a correlation between cIMT and left ventricular hypertrophy in the absence of arterial hypertension. Based on these results, the authors hypothesized that the presence of common pathogenic factors underlying both processes suggest the use of cIMT as a clinically relevant indicator of LV mass; moreover, they suggest cIMT as a potential marker for clinical follow-up and intervention studies. As indicated above, increased cIMT in both sexes is not related to residual alfa-galactosidase activity. This suggests that there was an additional pathogenic mechanism other than the mere accumulation of Gb3, as confirmed by some histological surveys[44].

In another study[45] conducted in male patients with typical FD, brachial FMD was decreased (p=0.01) and cIMT was increased (p=0.01) compared to healthy matched controls. Pulse wave velocity (PWV) was not different. In this study, IMT and flow-mediated dilation FMD have not shown remarkable improvement after ERT.

Puccio et al[46] showed an impaired FMD after reactive hyperthermia in FD patients. Collin et al[47] also demonstrated an increase in aortic stiffness, expressed as PWV, in FD patients prior to the onset of ERT. In 2013, Bensalah et al[48] used cardiovascular magnetic resonance (CMR) to analyze the stiffness of the aortic arch in 29 FD males, highlighting a significant increase of pulse wave velocity (PWV) compared to 58 healthy controls. Patients with FD also had a marked decrease in distensibility and an increase in the beta-stiffness index in the ascending aorta. Instead, Kalliokoski et al[23] found that patient FDs have FMD values that are superimposable compared to healthy controls, although FD patients have higher minimal coronary resistance values and decreased coronary reserve than healthy controls. This data seems to be concordant, but to our knowledge, cIMT, arterial stiffness (AS), plaques, and vascular calcification have never been evaluated together by ultrasound radiofrequency (RF-US) data technology in FD patients. We summarized the results of our investigation in Table I.

Diagnosis of Subclinical Atherosclerosis by (RF-US) Data Technology in Different Scenarios

Cardiovascular diseases (CVD) are a major health problem in Western Countries, due to their slow and often asymptomatic progression, and the unavoidable impact on morbidity and mortality[49]. Every possible means should be used to prevent CV events, ranging from the identification and control of the cardiovascular risk factors, to the early diagnosis of subclinical atherosclerosis. As stated by American Heart Association since 2009, a noninvasive assessment of subclinical atherosclerosis is advisable even in children and adolescents, because precise and reliable non-invasive tests for atherosclerosis in youth could improve our ability to estimate future risk of heart attack and stroke[50].

Table I. Principal studies in Literature which analyzed vascular aspects of Fabry Disease.

Authors	Design study	Subjects included (case/control)	Age (years) mean ± SD or range and group	Gender (case *vs.* control)	Vessel type	Outcome	Results (case *vs.* control %)	Used Method (instrument)
Rombach et al[16] 2012	Case control	67/55	38.4 ± 14.3 M 45.7 ± 13.3 F	27M-40F *vs.* 20M-35F	CCA and femoral arteries	Increased IMT in CCA increased PWV reduction in FMD	IMT: +9% M, +8% F PWV: +7% M, +4% F FMD: -30% M, -5%	DUS (B-mode DICOM), Sphygmo Cor
Wasik et al[22] 2009	Obser- vational	25	37.1 years (mean age) M 41.8 years (mean age) F	17M-8F	Nailfold Capillaries	Morphological and functional microangiopathy +	Bushy capillaries (62% *vs.* 10%) presence of others pathological patterns	Capillaroscopy (Fluorescence video microscopy)
Costanzo et al[39] 2014	Case control	19/19	30.1 ± 14.8	3M-16F *vs.* 6M-13F	CCA Nailfold capillaries	Increased IMT in CCA, reduction in FMD, alteration of capillaries	IMT: +23% FMD: -32% Significative micro-angiopathy of nailfold capillaries in case	DUS (GE Vivid E) and capillaroscopy
Boutouyrie et al[40] 2001	Case control	21/21	31 ± 13	21M *vs.* 21M	Radial artery	Increased IMT in radial artery	+2.3-fold higher (case)	DUS (high precision NIUS 02)
Boutouyrie et al[41] 2002	Case control	21/24	32 ± 13 M	21M *vs.* 24M	CCA Radial artery	Increased IMT in CCA and radial artery	CCA: +18% Radial artery: +2.3-fold higher (case)	High definition echotracking systems (not reported)
Barbey et al[42] 2006	Case control	53/120	45.0 ± 1.7 M 55.0 ± 2.2 F	24M-29F *vs.* 83M-37F	CCA	Increased IMT in CCA, no atherosclerotic plaques	+13% M +18% F	DUS (not reported)
Kalliokoski et al[43] 2006	Case control	17/34	38 ± 14	7M-10F *vs.* 16M-18F	CCA, aortic and brachial artery	Increased IMT in CCA, aortic and brachial artery, reduction in FMD	CCA IMT: +11% Aortic IMT: +27% Brachial IMT: +16% FMD: -33%	DUS (Acuson)

Continued

Table I *(Continued)*. Principal studies in Literature which analyzed vascular aspects of Fabry Disease.

Authors	Design study	Subjects included (case/control)	Age (years) mean ± SD or range and group	Gender (case *vs.* control)	Vessel type	Outcome	Results (case *vs.* control %)	Used Method (instrument)
Barbey et al[44]	Case control	68/324	40.7 ± 10.9 M 44.7 ± 2.8 F	30M-38F *vs.* 208M-116F	CCA	Increased IMT in CCA	+13% M +8% F	DUS (Toshiba PowerVision 6000)
Moore et al[45] 2002	Case control	7/8	22-42 years	7M *vs.* 8M	Radial artery	Increased IMT Increased PWV	IMT: +32% PWV: +2%	Intra- arterial pressure transducer
Puccio et al[46] 2005	Case control	6/12	Mean age 37 years	4M-2F *vs.* 8M-4F	Brachial artery	Reduction in FMD	FMD: -44%	DUS (instrument not reported)
Collin et al[47] 2012	Case control	46/30	33 ± 12	43M-3F vs. 28M-2F	CCA, Radial artery, Aorta	Increased IMT in CCA and radial artery, increased PWV in aorta	CCA IMT: +23% Radial artery IMT: +28% PWV: +29%	DUS (WTS, Pie MEdical)
Bensalah et al[48] 2013	Case control	29/58	Not reported	29M *vs.* 58M	Aortic Arch	Increased PWV	+24%	CMR
Kalliokoski et al[23] 2005	Case control	15/30	35 ± 12 years	9M-6F *vs.* 30M	Brachial artery	Reduction in FMD	No difference in rest and after hyperaemia	Acuson XP 128/10 US

CCA: Common Carotid Artery, DUS: Duplex UltraSound, IMT: Intimal-media Thickness, FMD: Flow mediated dilation, PWV: Pulse Wave Velocity, CALC-Score: Carotid Aortic Lower-limbs Calcification Score, CMR: Cardiac Magnetic Resonance.

The importance of obtaining a complete evaluation of the atherosclerotic burden was recently provided from Näslund et al[51]. For these authors, an open dialogue with both primary care physicians and individuals about silent atherosclerosis may be useful for improving patient's adherence to a healthy lifestyle, enhancing primary prevention of CV diseases. In this pragmatic, open-label, randomized, and controlled trial the simple presentation of an atherosclerosis picture to patients could reduce the CV disease burden by the 1-year follow-up.

Coronary Artery Calcification (CAC) score, introduced by Agatston et al[52], is the gold standard to predict future cardiovascular events. A limitation of CAC score is that CT imaging follow-up is not widely suitable due to the risk related to radiation exposure; moreover, it may have a considerable economic impact on public health costs. Currently, flow-mediated dilation, arterial stiffness, intima media wall thickness, ankle-brachial index, atherosclerotic plaques, and vascular calcification evaluation are the most widely used diagnostic tools to assess preclinical atherosclerosis[53,54].

Evaluation of subclinical atherosclerosis by ultrasound radiofrequency data technology (RF-US) has gained popularity in experimental and clinical conditions recently[55,56]. The availability of vascular ultrasound instruments, implemented with RF-US, allows an automatic measurement of far-wall arterial QIMT (quality intima-media thickness), inter-adventitial diameter, and distension over the cardiac cycle (QAS, quality arterial stiffness). It provides feedback on the measurement's quality with high accuracy. RF-US technology allows precise and quantitative measurements of both IMT elevation and decreases in vascular elasticity. For example, in rheumatologic patients, some authors found stiffness values and cIMT measurements higher than in healthy controls, especially in phases of active diseases[57,58]. RF-US technology was also used to evaluate a cohort of children with increased body mass index (BMI), compared with healthy peers. These studies documented an increased Q-IMT in overweight and obese children compared to those with normal weight[59]. In conclusion, many reports contributed to providing evidence in the usefulness of atherosclerosis early diagnosis in different scenarios.

Flore et al[60] published by our group RF-US data technology, associated with a new ultrasound-based score for non-coronary arterial calcifications (CALCs), represented a non-invasive and complete method with great utility in subjects with subclinical atherosclerosis. In another report[61] we found, QAS, QIMT and CALCs were highly correlated in subjects at low cardiovascular risk without previous cardiovascular events.

Our hypothesis is that QIMT, QAS and CALCs together could also be a useful tool to evaluate the "vascular age" in FD patients. In Figure 1, 2, 3 several paradigmatic examples of these diagnostic purposes are presented (images from personal archive). We believe that such pictorial representations could maximize the chances of identification.

Conclusions

A comprehensive evaluation of the cardiovascular system must be performed in all subjects to identify initial changes, stratify the patient's CV risk, and lower the number of CV events in the general population. The importance of this goal is greater in FD patients. Determining

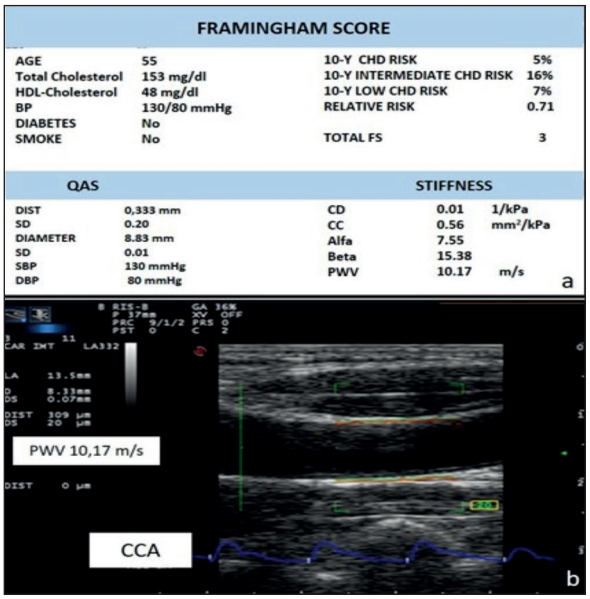

Figure 1. Framingham score of a Fabry patient at low cardiovascular risk and stiffness parameters. QAS: quality arterial stiffness; DIST: distensibility; SBP systolic blood pressure; DBP: diastolic blood pressure; CD Distensibility coefficient; CC: compliance coefficient; PWV: pulse-wave velocity (**a**). Quality arterial stiffness B-mode image. Pressure-dependent vessel wall movement amplification curve (blue dot) of common carotid artery have a standard deviation 20 which define the term "Quality" (**b**). (Images from personal archive, see 52 for better explanation).

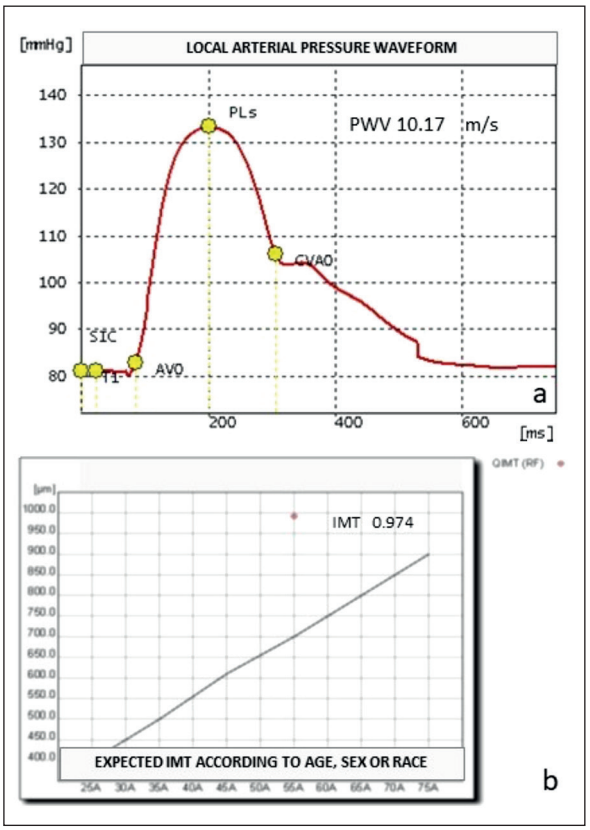

Figure 2. Pressure-distensibility arterial waveform SIC: start of isometric contraction; AVO: aortic valve opening, PLs Local Systolic Pressure, CVAO aortic valve closure. T1: inflection point (**a**). "Vascular age" based on Quality Intima-Media Thickness (QIMT) (**b**). (Images from personal archive, see reference 60 for better explanation).

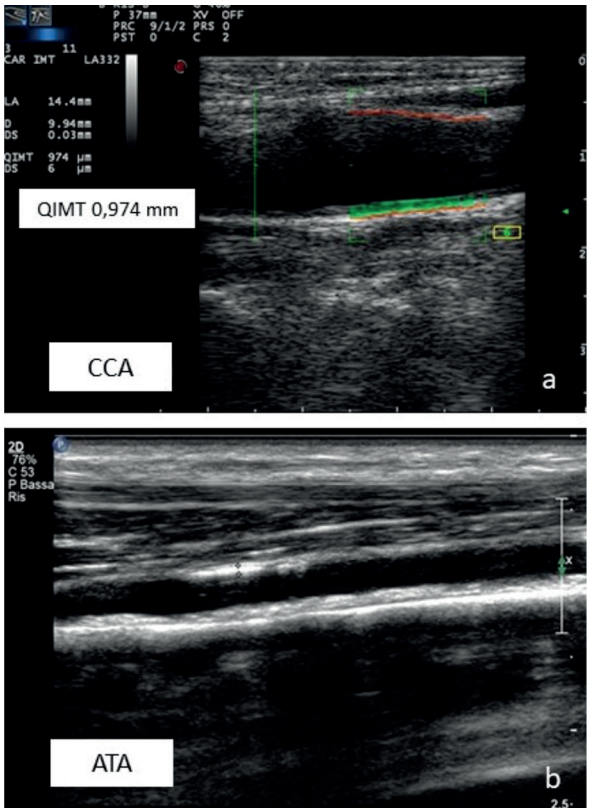

Figure 3. RF-DU image of augmented Intima-Media Thickness (IMT) in the same FP of Figure 2. The standard deviation 0.006 mm of IMT in 6 successive cardiac cycles define the term "Quality" (**a**). Augmented IMT and vascular calcifications in Anterior Tibial Artery (ATA) of the same Fabry Patient (**b**). (Images from personal archive, see 52 for better explanation).

CV risk in FD patient is very complex, since some authors claim that FD is itself a CV risk factor[16]. The reduction of the overall CV risk in this category of patients is one of the objectives to be pursued, considering the high risk that they have. A fortiori, FD patients should undergo periodic assessments of atherosclerotic involvement of the arteries, through non-invasive and economic techniques. Ultrasound could be the most accurate choice, although most studies describe the absence of characteristic plaque formation[20]. More frequently we find traces of subclinical atherosclerosis, such as an increase in IMT. Therefore, it is very important to find parameters that can constitute milestones in the evaluation of vascular involvement. Average intimal wall thickness and arterial stiffness have gained popularity in several scenarios and could be useful for this purpose. One of the future perspectives for this area is to perform simultaneous assessments of QAS, QIMT and CALC to study their role in the early diagnosis of atherosclerosis, and in the prediction of cardiovascular risk in FD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Passaro G, Sicignano LL, and Flore R conceived and designed the study. Passaro G, Sicignano LL, Flore R, Gerardino L, Verrecchia E, Crasti M, Santoro L, Massaro MG consulted literature and collected data. Passaro G and Sicignano LL wrote the paper. Sicignano LL and Massaro MG drew up the bibliography. MR and TP reviewed and edited the manuscript. All authors read and approved the manuscript.

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