RESEARCH PAPER

LOPED study: looking for an early diagnosis in a late-onset Pompe disease high-risk population

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ABSTRACT

Objective A multicentre observational study was aimed to assess the prevalence of late-onset Pompe disease (LOPD) in a large high-risk population, using the dried blood spot (DBS) as a main screening tool.

Design/methods 17 Italian neuromuscular centres were involved in the late-onset Pompe early diagnosis (LOPED) study. Inclusion criteria were: (1) age ≥5 years, (2) persistent hyperCKaemia and (3) muscle weakness at upper and/or lower limbs (limb-girdle muscle weakness, LGMW). Acid α-glucosidase (GAA) activity was measured separately on DBS by fluorometric as well as tandem mass spectrometry methods. A DBS retest was performed in patients resulted positive at first assay. For the final diagnosis, GAA deficiency was confirmed by a biochemical assay in skeletal muscle, whereas genotype was assessed by *GAA* molecular analysis.

Results In a 14-month period, we studied 1051 cases: 30 positive samples (2.9%) were detected by first DBS screening, whereas, after retesting, 21 samples were still positive. Biochemical and molecular genetic studies finally confirmed LOPD diagnosis in 17 cases (1.6%). The median time from the onset of symptoms/signs to diagnosis was 5 years. Among those patients, 35% showed presymptomatic hyperCKaemia and 59% showed hyperCKaemia+LGMW, whereas 6% manifested with LGMW.

Conclusions LOPED study suggests that GAA activity should be accurately screened by DBS in all patients referring for isolated hyperCKaemia and/or LGMW. A timely diagnosis was performed in five patients with presymptomatic hyperCKaemia, but two had already manifested with relevant changes on muscle morphology and MRI. Consequently, enzyme replacement therapy was started in 14/17 patients, including the 2 patients still clinically presymptomatic but with a laboratory evidence of disease progression.

INTRODUCTION

Pompe disease (glycogen storage disease type II, GSD II) is a rare autosomal recessive disorder due to Acid α-glucosidase (GAA) deficiency leading to glycogen accumulation in several tissues, with predilection of skeletal muscle and heart.[1] Clinically, it is associated with a range of phenotypes, with variable organ involvement, age of onset and severity degree.[2]

Late-onset Pompe disease (LOPD) is a slowly progressive form with juvenile or adult presentation, characterised by progressive muscle weakness and, often, respiratory impairment.[3]

Diagnosis of LOPD is still challenging and often quite delayed. This has been hypothesised to be due to several reasons such as rarity of the disorder, wide clinical spectrum, overlap of signs and symptoms with other neuromuscular disorders or variable diagnostic approach in different countries.[4]

Recently, Kishnani *et al*,[5] using data from the International Pompe Registry, calculated the time interval between onset and diagnosis, 'a diagnostic gap', in different categories of patients with Pompe disease and still found this delay consistent.

Since 2006, enzyme replacement therapy (ERT) represents the first disease-specific treatment. In patients with LOPD, ERT was less effective in older juveniles and adults than in infants, likely because of delayed diagnosis.[4 6]

These considerations suggested development of more rapid diagnostic tools, such as the dried blood spot (DBS), to detect GAA activity, which could result in an earlier LOPD diagnosis.[7–10]

This study, based on measuring GAA activity by DBS, reports on the results of a multicentre research project looking for the prevalence of LOPD in a large high-risk population, in order to start ERT in a timely manner.

PATIENTS AND METHODS

The study was conducted in accordance with GCP and ethical principles deriving from the Declaration of Helsinki, and from regulations in force for observational studies.

It was approved by the Ethics Committees of the Coordinator Center (Messina) and participant centres.

The patients, recruited from 17 Italian neuromuscular centres, were all admitted for diagnostic purposes.

Inclusion criteria were: (1) age ≥5 years, (2) persistent hyperCKaemia (considered as 1.5-fold the upper normal limit, ie, creatine kinase (CK) >250 UI/L in females and >350 UI/L in males), evidenced at least twice with a minimum interval of 1 month, and/or (3) muscle weakness affecting upper and/or lower limbs (limb-girdle muscle weakness, LGMW).

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Exclusion criteria were: (1) patients suffering from systemic diseases associated with hyperCKaemia (ie, hypothyroidism, electrolyte imbalance, etc), (2) relatives of patients with diagnosed Pompe disease, (3) relatives of patients with known neuromuscular disorders (ie, myotonic dystrophy).

Informed consent was obtained from all patients, or from parents, if patients were underage.

Blood samples were collected, immediately spotted on filter paper (DBS) and dried at room temperature.

DBS samples were sent to two different laboratories specialised in metabolic disorders, both experts in measuring GAA activity, to be examined, respectively, by fluorometry or tandem mass spectrometry (TMS) techniques.

For each patient, clinical history and laboratory data were collected in a Case Report Form (CRF).

GAA activity in DBS was assessed by a fluorometric method using the substrate 4-methylumbelliferyl-α-D-glucoside as previously described.[11] The cut-off target range was considered as the interval between the 99th centile activity of the disease range (5.78 nM/h/mL) and the 1st centile activity of the normal controls (6.10 nM/h/mL), and the cut-off for α-glucosidase activity at 6 nM/h/mL on DBS. Patients with GAA activity below 6 nM/h/mL were considered positive.

TMS method was based on an online trapping and clean-up Liquid chromatography-tandem mass spectrometry (LC-MS/MS) test.[8] Cut-off ranges, calculated as 1st and 99th centiles of normal population (n=1563), were 1.86–21.9 nmol/h/mL. Patients with GAA activity below 1.86 nmol/h/mL were considered positive. A second DBS test (retest) was performed in all patients positive at the first assay, according to guidelines for the diagnosis of Pompe disease.[10]

Those patients positive at retest underwent a confirmatory step by determination of GAA enzymatic activity on skeletal muscle.

After biochemical confirmation, a molecular genetic analysis was performed by *GAA* gene sequencing to assess the genotype of patients with LOPD.

Then, the newly diagnosed patients with LOPD were evaluated according to the following protocol: clinical presentation, neurological examination, CK levels, electromyography, motor and sensory conduction velocities (NCVs), muscle morphology, including acid phosphatase (AP) staining as marker of lysosomal alteration, muscle GAA residual activity, *GAA* genotype, functional measures such as 6 min walk test (6MWT), heart and respiratory assessment, and muscle MRI.

Heart assessment comprised ECG and echocardiography. Respiratory function was evaluated by per cent forced vital capacity (FVC) in supine and upright position and polysomnography.

Muscle MRI included axial and coronal T1-weighted images and T2 sequences on a 1.5 T system of paraspinal, pelvis, thigh and leg muscles.

Statistical analysis

All statistical tests were performed using the SPSS software package V.21.0 (SPSS Inc, Chicago, Illinois, USA). Shapiro-Wilk test was used for testing the normality of data distribution. Results are presented as mean (SD) or median (IQR), as appropriate. The association between categorical variables was evaluated using Fisher's exact test. Differences between the two groups were assessed using Student t test. Pearson's or Spearman's correlation coefficients were used, as appropriate, to test correlations between quantitative variables for significance.

The statistical significance for all calculations was considered achieved when two-tailed p value was less than 0.050.

RESULTS

In a 14-month period, we studied 1051 cases, consecutively admitted to the 17 Italian neuromuscular centres.

According to inclusion criteria, the main clinical features of the selected cases and their distribution in different groups are shown in table 1.

DBS results

Using the two different methods, reduced GAA enzyme activity in DBS was found in 30 participants (2.9%). Twenty-six samples were detected either by TMS method or by fluorometric assay but not fully overlapped (table 2).

DBS test on abnormal samples was repeated (retest). After retesting, 21 samples resulted positive.

Biochemical assay

A confirmatory biochemical test performed on skeletal muscles of the 21 patients resulted positive at the retesting; GAA deficiency was confirmed in 17/21 patients (1.6%).

GAA molecular genetic analysis

GAA gene sequencing defined the LOPD genotypes in the 17 new cases. In fact, all these patients harboured the common mutation c.−32−13T>G on one allele, whereas, on the second allele, different known causative mutations were detected in all but two participants, who were homozygous for the c.−32−13T>G mutation.

Of the four patients positive at the retest but not confirmed by the biochemical test, three were heterozygous for the c.−32−13T>G mutation whereas the fourth patient carried a p.P522A change.

As for the other nine positive cases at first DBS but not confirmed at the retest: six of them were recruited because of LGMW+hyperCKaemia and three owing to isolated hyperCKaemia.

For these patients, the final diagnoses were: inflammatory myopathy,[2] sarcoglycanopathy,[2] neurogenic atrophy,[1] traumatic peripheral nerve lesion (11th cranial nerve) mimicking an upper limb myopathy[1] and undefined asymptomatic hyperCKaemia.[3]

However, we further investigated them from a genetic perspective, looking for the most common Caucasian *GAA* gene mutation (c.−32−13T>G) and for the pseudodeficiency allele (p.G576S), but the molecular results were negative for changes.

Clinical and laboratory features of the 17 newly diagnosed patients with LOPD

Among the 17 patients with LOPD, 9/17 were women (47%); the mean age at disease onset was 40±14.3 years, while the

Table 1 Summary of demographic and clinical characteristics of examined participants

	Patients (n=1051)
Age, years, mean (SD)	47.7±11.2
Gender, n (%)	
Female	418 (40)
Male	633 (60)
Clinical presentation, n (%)	
Isolated hyperCKaemia	545 (52)
HyperCKaemia+ LGMW	427 (40)
LGMW	79 (8)

LGMW, limb-girdle muscle weakness.

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Table 2 Table showing the distribution of DBS results

	Test		Retest	
	DBS TMS	DBS F	DBS TMS	DBS F
Positive	26	26	19	19
Positive individuals at one test	30 (23 with both methods)		21 (15 with both methods)	
Confirmed positive			17	15

DBS, dried blood spot; F, fluorometry; TMS, tandem mass spectrometry.

median age at disease diagnosis was 47±14 years. According to these data, it has to be outlined that the median time from disease onset to the final diagnosis was 5 years.

Sixty-five per cent of patients (11 individuals) presented with hyperCKaemia+LGMW, 29% (5 individuals) with isolated hyperCKaemia and 6% (1 individual) with LGMW.

Clinical and laboratory data of patients with LOPD as CK, muscle GAA residual activity, body mass index, heart assessment, 6MWT, and per cent predicted FVC and muscle MRI are detailed in table 3.

All patients were ambulant and able to perform a 6MWT (mean 452±99.2).

Blood CK levels were increased in all but one patient, ranging from 323 to 1378 UI/L (mean 600±302.7).

From the morphological point of view, 11/17 of patients showed a vacuolisation of myofibers with variable glycogen storage; the other 6 patients showed unspecific changes as fibre size variability and/or increased centralised nuclei, but in 2 of them AP staining was increased. In fact, AP staining, evaluated in all biopsies at a semiquantitative rate[12] of intensity, was defined as 'low' in six patients, 'medium' in five patients and 'high' in two patients, whereas the remaining four patients did not show any relevant lysosomal alteration. Residual GAA activity in muscle was low in all patients, ranging from 5% to 20% (mean 9.5%±3.9%).

Table 3 Summary of demographic and clinical features of the 17 patients with LOPD

	Patients (n=17)
Age, years, mean (SD)	40±14.36
Gender, n (%)	
Female	9 (52)
Male	8 (47)
Clinical presentation, n (%)	
Isolated hyperCKaemia	5 (29.4)
HyperCKaemia+LGMW	11 (64.7)
LGMW	1 (5.9)
Timing of diagnosis, years, median (IQR)	5 (2.5–10)
BMI, mean (SD)	25.2±5.8
Serum CK, mean (SD)	600±302
Muscle biopsy, n (%)	
Vacuolar myopathy	11 (64.7)
Unspecific changes	6 (35.2)
Muscle GAA %, mean (SD)	9.4±3.8
EMG, n (%)	
Myogenic pattern	10 (58.8)
Normal	7 (41.1)
MRI findings, n (%)	
Abnormal	14 (82.3)
Normal	3 (17.6)
%Predicted FVC, mean (SD)	73±11
6MWT, mean (SD)	452±9
ERT, n (%)	
Yes	14 (82.3)
No	3 (17.6)

6MWT, 6 min walk test; BMI, body mass index; CK, creatine kinase; EMG, electromyography; ERT, enzyme replacement therapy; FVC, forced vital capacity; GAA, acid α-glucosidase; LGMW, limb-girdle muscle weakness; LOPD, late-onset Pompe disease.

In 14/17 of patients with LOPD (82%), muscle MRI revealed an involvement of the affected muscles with muscle fatty degeneration, predominantly at pelvic and thigh muscles. It is noticeable that, among the five patients with asymptomatic hyperCKaemia, two unexpectedly displayed an adipose substitution of paraspinal and thigh posterior compartment muscles whereas the other three had a normal MRI.

According to percentage of predicted FVC in supine and upright positions as well as polysomnographic registrations, a respiratory involvement was demonstrated in 14/17 patients (82%) with a mean FVC of 74±12% of that predicted (range 44–99%) in the upright position and 55±26% of that predicted (range 24–95%) in the supine position.

Two of the 17 patients required nocturnal non-invasive ventilation and 3/17 had obstructive sleep apnoea (18%).

In 2/17 patients, echocardiography showed a mild cardiac hypertrophy of left ventriculum or septum.

Considering the 17 patients in relationship to their specific phenotype (group 1: LGMW+hyperCKaemia; group 2: isolated hyperCKaemia; and group 3: LGMW), no statistically significant differences were found regarding muscle GAA residual activity, CK levels, morphological aspects, pulmonary function or genotype. On the other hand, abnormal MRI occurred more frequently in individuals with LGMW+hyperCKaemia than in those with isolated hyperCKaemia (p=0.029).

AP staining intensity did not correlate with age at onset, age at biopsy, GAA residual enzyme activity, gender or clinical presentation.

Treatment

After the diagnostic assessment, ERT was started in 14/17 patients: 11 symptomatic patients with LGMW+hyperCKaemia, 1 patient with LGMW with normal CK and also in 2 of the 5 patients with hyperCKaemia who, despite an apparently presymptomatic condition, showed altered muscle morphological and MRI features. The other three presymptomatic patients with hyperCKaemia, normal neurological examination as well as muscle biopsy and MRI, were monitored every 6 months.

DISCUSSION

Pompe disease is a rare disorder having an estimated worldwide incidence of 1 in 40 000 live births. However, several studies have suggested that incidence rates may vary among different ethnic populations with a fluctuating range from 1 in 14 000 to 1 in 300 000.[13]

Since the introduction of ERT, either among neuromuscular experts or general practitioners, the awareness of this disease has considerably increased; but, unfortunately, the delay of diagnosis is still consistent, especially in LOPD.[5]

Recent studies tried to improve the approach to a rapid and correct diagnosis using the DBS method in targeted populations. Currently, there are two different techniques to analyse DBS samples: fluorometry or TMS. These methods, applied in different laboratories, both had appropriate results for Pompe diagnosis, even when performed for a newborn screening (NBS).[8–10]

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In Taiwan, in 2005, a nationwide NBS programme for Pompe disease was initiated with highly successful results. So far, a large number of newborns have been screened for Pompe disease (over 400 000 infants). Of those screened, 6 infants were found to carry the infantile-onset Pompe disease (IOPD—prevalence 1/57 000), and 20 cases of LOPD and 294 cases of pseudodeficiency were also detected.[14 15]

On the other hand, considering as key features specific signs or symptoms, usually represented by isolated hyperCKaemia and/or unclassified LGMW, Pompe disease has been screened by DBS, although in a limited number of patients and in different ethnic populations.

Recently, Spada *et al*[11] screened 137 patients with unclassified hyperCKaemia and found a 2.2% prevalence of Pompe disease. In 2013, Preisler *et al*,[16] using the DBS method to evaluate the prevalence of Pompe disease in undetermined patients with limb-girdle muscle dystrophy, identified three patients with Pompe disease in 38 individuals screened (8%).

Consequently, to the best of our knowledge, the present study likely has the largest series of patients screened and studied as a LOPD high-risk population. In a period of 14 months, we were able to collect 1051 samples from patients with suspected neuromuscular disorders because of proximal LGMW with or without hyperCKaemia or with isolated hyperCKaemia.

In this group of patients, we used the DBS assay as main screening tool to check GAA activity: we found, after the first assay, 30/1051 samples with low GAA activity on DBS, but after the retest and biochemical confirmatory assay on skeletal muscle, LOPD diagnosis was assessed in 17 patients (1.6%). Among those patients, 35% showed presymptomatic hyperCKaemia, 59% showed hyperCKaemia and LGMW, whereas 6% manifested with LGMW.

Considering the subgroup of patients with isolated hyperCKaemia, 5/545 (1.1%) were early on identified as presymptomatic patients with LOPD. Although they have been found in a larger cohort, the prevalence is lower compared with previous studies. In 2006, Fernandez *et al*[17] retrospectively reviewed muscle biopsy specimens of 104 patients with hyperCKaemia and found Pompe disease in 4 patients (3.8%).

Although Vissing *et al*,[18] in a recent diagnostic review, indicated the 'blood-based' assays as prevalent diagnostic tool, muscle biopsy still had an important role. In fact, in the present study, the biochemical assay on skeletal muscle tissue was always able to confirm Pompe diagnosis.

In our cohort, 11 patients (65%) showed typical morphological findings suggesting Pompe disease, although 2 cases presented with presymptomatic hyperCKaemia. Regarding the role of AP staining, it has been found positive in 13/17 cases; this

Table 4 Molecular genetic aspects of the 17 newly diagnosed patients with Pompe disease

Patient	Sex	Age at onset/ diagnosis (δ)	Clinical presentation	Allele 1	Transcript	Effect (Pompe Erasmus database)	Allele 2	Transcript	Effect (Pompe Erasmus database)
1	M	14/16 (2)	HyperCK	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1655T>C	p.L552P	Less severe
2	M	66/72 (6)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.525dT	p.E176fsX45	Severe
3	F	29/39 (10)	HyperCK	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.784G>A	p.E262K	Less severe
4	F	31/40 (9)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1564C>G	p.P522A	Potentially less severe
5	F	64/67 (3)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.525dT	p.E176fsX45	Severe
6	F	55/59 (4)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1561G>C	p.E521K	Potentially less severe
7	F	53/55 (2)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1927G>A	p.G643R	Less severe
8	M	30/35 (5)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2481+102_2646 +31del	p.G828_A882d	Severe
9	M	48/52 (4)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	IVS1-32-13T>G	Leaky splice mutation	Potentially mild
10	M	34/42 (8)	HyperCK	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	IVS1-32-13T>G	Leaky splice mutation	Potentially mild
11	M	24/47 (23)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2481+102_2646 +31del	p.G828_A882d	Severe
12	M	42/46 (4)	HyperCK	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1438-2A>G	p.V480_I517d	Probably severe
13	F	28/48 (20)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2189+1G>T	Loss of mutated allele	Very severe
14	M	50/66 (16)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2219-20dTG	p.Val740GfsX55	Severe
15	F	42/52 (10)	HyperCK +LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.1927G>A	p.G643R	Less severe
16	F	32/32 (0)	LGMW	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2481+102_2646 +31del	p.G828_A882d	Severe
17	F	38/39 (1)	HyperCK	IVS1-32-13T>G	Leaky splice mutation	Potentially mild	c.2237G>A	p.W746X	Severe

F, female; HyperCK, hyperCKaemia; LGMW, limb-girdle muscle weakness; M, male.

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suggests that it is still a morphological valuable tool to detect Pompe disease.

Muscle imaging was highly sensitive to detect skeletal muscle involvement: 14 patients (82%) evidenced abnormal MRI and a significant association was found between abnormal muscle MRI and presence of LGMW+hyperCKaemia (p=0.029).

All the patients were genetically defined: they showed, at least on one allele, the common c.-32−13T>G mutation (except for 2 patients who resulted homozygotes). The second mutation G828_A882d (Δ18) was found in 3/17 patients (17.6%) confirming its prevalence in the Italian LOPD population, as already reported.[19–21]

According to the Pompe Erasmus database, regarding the severity of mutations, we have matched clinical and molecular results in our cohort of patients but we did not find any genotype–phenotype correlation (table 4).

Diagnostic delay is quite common in Pompe disease, especially in LOPD, because of the rarity of the disorder and the variable clinical presentation or overlapping of symptoms with other neuromuscular disorders. A recent study showed that patients presenting with respiratory and musculoskeletal signs or symptoms were diagnosed sooner than other patients manifesting with isolated hyperCKaemia and/or myalgia. The 'diagnostic gap' was about 6 years in patients with adult onset.[5]

In our cohort, the median time from onset of symptoms/signs to diagnosis was 5 years. It is worthwhile to note that five patients were identified when still manifesting with isolated hyperCKaemia, confirming DBS validity and leading to an early diagnosis, even in the absence of clinical muscle damage.

The interpretation of DBS results needs great accuracy and experience: in fact, the results may indicate some false positives at the first assay.[9] This also happened in our study, considering that we first obtained 30 positive samples; however, after retesting, 21 samples again resulted positive and, finally, 17 patients were biochemically confirmed with a diagnosis of LOPD.

At the first assay, each laboratory identified 26 samples with 23 comparable results. After retesting, the number of positive samples decreased to 21 samples with both methods. Considering the final diagnosis, TMS was able to identify all the 17 positive patients but also 2 false-positive samples whereas fluorometric assay identified 15/17 patients with LOPD. A possible explanation of those slightly controversial results could be due to the quality of samples. It is well known that DBS specimen collection and storage can be crucial for a correct

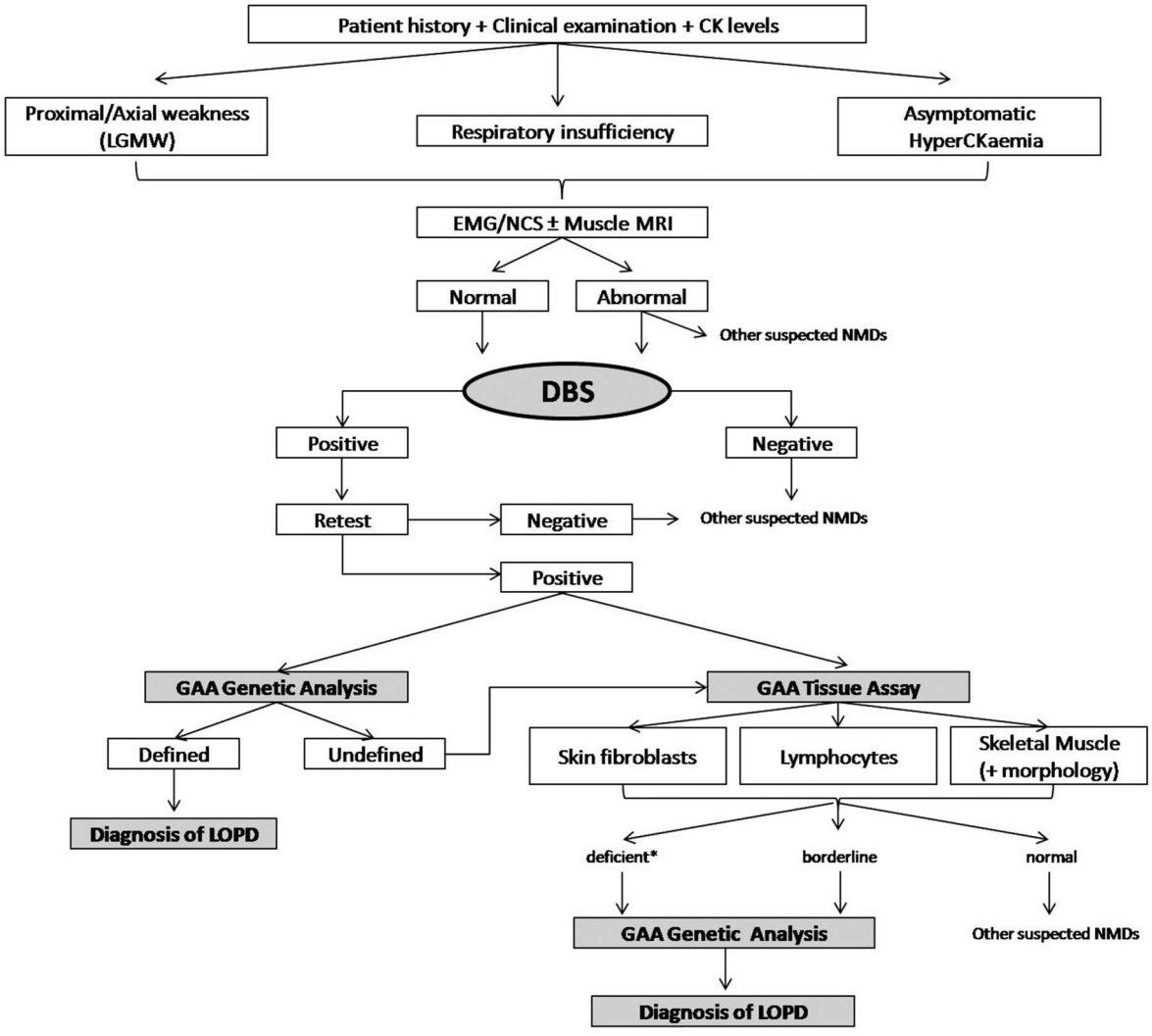

Figure 1 Algorithm for LOPD diagnosis. *GAA activity <35%, borderline 35–40% (CK, creatine kinase; DBS, dried blood spot; EMG/NCS, electromyography and nerve conduction studies; GAA, acid α-glucosidase; LGMD, limb-girdle muscle dystrophy; LGMW, limb-girdle muscle weakness; LOPD, late-onset Pompe disease; NMDs, neuromuscular disorders).

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analysis.[22] At the end of the retesting, we still found four 'false' positive cases presenting with presymptomatic hyperCKaemia, no muscle biopsy-specific abnormalities, residual GAA activity in skeletal muscle within normal range and only one mutation identified by *GAA* gene sequencing; these results suggest considering them as heterozygous individuals.

The two different methods presented good performances, even if some differences have been revealed. The false-positive rate was 9/1051 (0.85%) for TMS analysis and 11/1051 (1.05%) for fluorometry at first assay, and 4/1051 (0.38%) and 6/1051 (0.57%) for retesting, respectively. Even if not appropriate due to small numbers of investigated individuals, in this study, TMS identified all 17 patients with LOPD with a positive predictive value (PPV) at first test 0.65, whereas PPV was slightly lower by fluorometry (0.58). Between these two techniques, similar differences in false-positive rate and PPVs have already been reported in the literature in a larger population.[23]

In addition, we have here included a revised version of a diagnostic algorithm (figure 1), where it is indicated that a positive DBS must be confirmed by biochemical assays on different tissues and/or by a genetic analysis to complete the diagnostic panel.[4] However, in cases where the molecular analysis, first requested after a positive DBS, is not fully confirmatory, it will be necessary to perform the biochemical analysis to detect GAA activity levels.

Nowadays, when to start ERT in LOPD is largely debated. In 2012, American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) guidelines recommended starting ERT in symptomatic patients and in presymptomatic patients with proximal muscle weakness, detectable by manual muscle testing, or with a reduction in respiratory parameters.[24]

On the other hand, some reports have suggested that ERT should be started at the earliest onset of symptoms or objective signs, to obtain better therapeutic responses.[25–27]

In fact, it is well known that a progressive muscular derangement, caused by enlargement and rupture of glycogen-filled lysosomes as well as autophagic dysfunction, is responsible for irreversible damage of skeletal and respiratory muscles.

Therefore, ERT guidelines have been mainly addressed to symptomatic patients, without taking into consideration laboratory or instrumental investigations, such as GAA residual activity, morphological aspects or muscle MRI findings in presymptomatic patients. ERT was started in 12 patients showing LGMW+hyperCKaemia but also in 2/5 presenting with hyperCKaemia with no clinical symptoms but with clear-cut muscle damage demonstrated by MRI, and altered muscle morphological features.

Finally, we suggest reconsidering the guidelines in order to better define possible additional parameters that may influence the decision for a timely start of ERT. However, only a prolonged follow-up of early treated cases could confirm the opportunity to treat such patients at the presymptomatic stage.

Therefore, our data suggest that DBS plays a central role in the diagnostic work up of high-risk patients with presymptomatic hyperCKaemia and/or LGMW of unknown origin where LOPD is suspected.

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