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SELECTIVE SCREENING VS. NEONATAL SCREENING IN INFANTILE ONSET POMPE DISEASE: A CASE SERIES

V. GRAGNANIELLO[1], D. GUERALDI[1], A. PUMA[1], C. LORO[1], E. PORCÙ[1], D. VELASQUEZ[1], E. MAINES[2], A. LUPI[3], A. PEPE[3], G. DI SALVO[4], L. SALVIATI[5], A. BURLINA[1]

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[1]Division of Inherited Metabolic Diseases, Department of Women's and Children's Health, University Hospital of Padua, Padua, Italy
[2]Division of Pediatrics, S. Chiara General Hospital, Trento, Italy
[3]Institute of Radiology, Department of Medicine - DIMED, University Hospital of Padua, Padua, Italy
[4]Division of Paediatric Cardiology, Department of Women's and Children's Health, University Hospital of Padua, Padua, Italy
[5]Clinical Genetics Unit, Department of Women's and Children's Health, University of Padua, Padua, Italy

Corresponding Author
Alberto Burlina, MD; e-mail: alberto.burlina@unipd.it

***ABSTRACT* – Objective:** The study presents a comprehensive clinical history of patients with infantile-onset Pompe disease (IOPD) followed by the Center of Inborn Errors of Metabolism, Padua University Hospital, over the last 20 years.

Case presentation: The article presents six cases of patients with IOPD diagnosed via selective screening or through a newborn screening (NBS) program and treated with enzyme replacement therapy (ERT), including next-generation ERT.

Results: Three patients were diagnosed before 2015 based on clinical and biochemical features, further confirmed by enzymatic and genetic testing; the remaining three patients were diagnosed through NBS assay out of 257,236 neonates screened. All the patients were treated with ERT alglucosidase alfa at different initial dosages, up to 40 mg/kg weekly. Furthermore, one patient was treated with avalglucosidase alfa and one with the combination of cipaglucosidase alfa and miglustat. ERT treatment started at 4 months of age (range 1–6 months) for patients diagnosed via selective screening *vs.* 5–19 days for patients diagnosed via NBS. Two of the patients diagnosed before NBS died for respiratory failure at the age of 9 and 4.5 years, respectively; all the other patients are currently alive.

Conclusions: The study shows that the association between NBS and early treatment, plus the improvement in ERT dosages and the development of next-generation ERT approaches, can potentially improve the outcome of patients with IOPD.

Keywords: Pompe disease, Glycogenosis storage disease type II, Newborn screening, Alglucosidase alfa, Avalglucosidase alfa, Cipaglucosidase alfa.

List of abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; CPK: creatine phosphokinase; ECG: electrocardiography; EF: ejection fraction; EOW: every other week; ERT: late-onset PD; GAA: α-glucosidase; IOPD: infantile-onset PD; IVIG: intravenous immunoglobulins; LOPD: late-onset PD; LVMI: left ventricular mass index; MS/MS: tandem mass spectrometry; NBS: newborn screening; Rh-GAA: recombinant human α-glucosidase; RUSP: Recommended Uniform Screening Panel; TLC: thin-layer chromatography.

INTRODUCTION

Pompe disease (OMIM #232300) (PD) is an autosomal recessive lysosomal storage disease caused by a deficiency of the enzyme acid α-glucosidase (GAA) that results in the accumulation of glycogen in various tissues, especially in cardiac and skeletal muscle[1].

Clinical manifestations are broad. Patients with classic infantile-onset PD (IOPD) present in the first months of life with hypertrophic cardiomyopathy and muscular hypotonia and typically die within the first 2 years due to cardiorespiratory failure, if left untreated (Figure 1). Patients with late-onset PD (LOPD) may manifest at any age with progressive muscle weakness[2]. Since 2006, enzyme replacement therapy (ERT) with recombinant human GAA (rh-GAA) has significantly changed the natural history of the disorder. In patients with IOPD, ERT significantly improved survival and cardiac and motor outcomes[3]; however, an early diagnosis and treatment are essential.

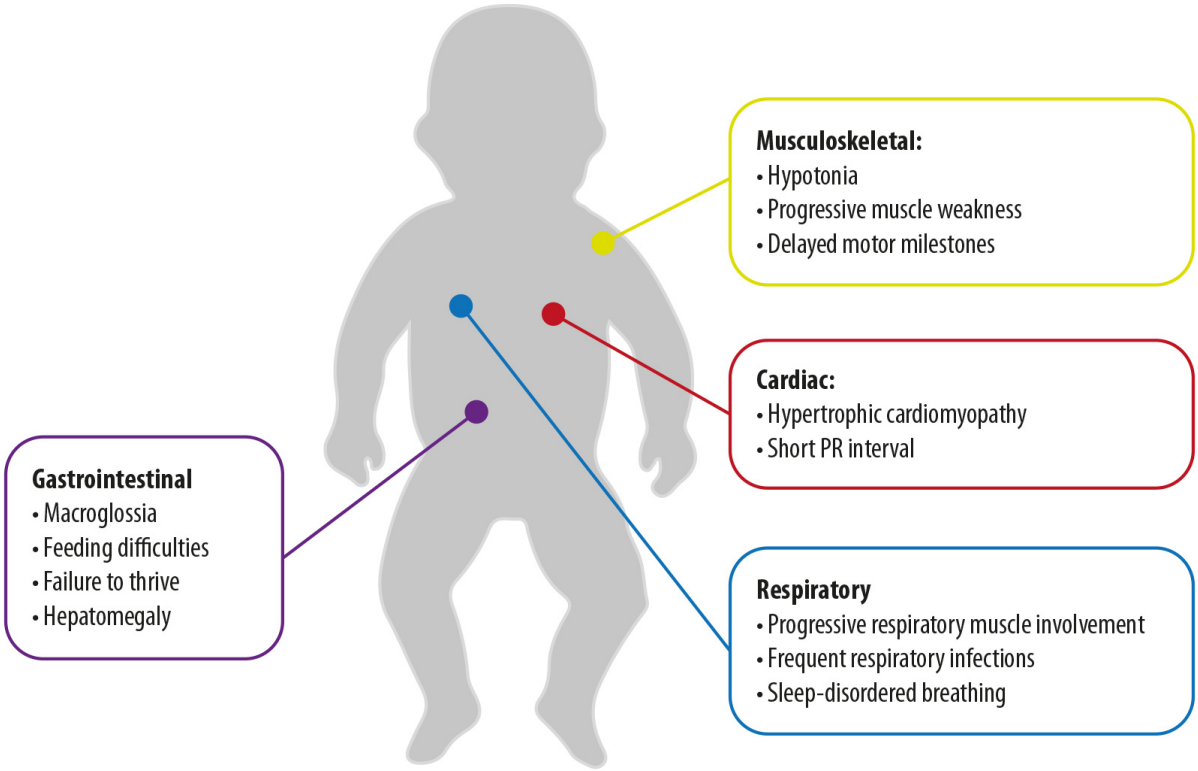

Figure 1. Main manifestations of infantile-onset Pompe disease.

Over the past two decades, newborn screening (NBS) programs have been progressively implemented. The first NBS pilot study was launched in Taiwan in 2005 using a fluorometric assay[4]. Their results showed that favorable outcomes are strongly associated with very early treatment initiation. Delays of even a few days can influence outcomes[5,6].

Subsequent improvements in screening technologies led to the development of multiplexed enzyme assays using fluorescence-based digital microfluidics or tandem mass spectrometry (MS/MS) and the implementation of NBS worldwide[7]. In the US, pilot projects were launched between 2013 and 2014 in Missouri, New York and Illinois[8,9]. PD was included in the Recommended Uniform Screening Panel (RUSP) in February 2015. Little data are available on the follow-up of screening-positive newborns to determine the effect of NBS on the outcomes of patients with IOPD[10].

In 2015, in northeast Italy, a NBS program using MS/MS assays for lysosomal storage disorders, including PD, was established. In 2022, we reported our first 7 years' experience, including more than 200,000 screened newborns[11].

This study aimed to evaluate the comprehensive clinical status of patients with IOPD in a single Italian center over the past 20 years. Our experience, based on selective screening for many years and recently on NBS and early treatment with ERT at different dosages and recent next-generation enzymes, aimed at further improving the outcome of IOPD.

METHODS

Study Design and Patients

This monocentric, retrospective, and observational study was conducted in the Center of Inborn Errors of Metabolism, Padua University Hospital. The study was approved by the Ethics Committee for Clinical Trials of the Province of Padua (n. 0661384, dated 30 September 2021). Parents/legal representatives of the patients signed an informed consent form to participate in the study and allowed publication of their data. The study aimed to collect screening, diagnostic, and outcome data of patients diagnosed with IOPD and followed at the center over the past 20 years. Patients diagnosed through either selective screening (before 2015) or the NBS program (from 2015 to 2023) were included in the study.

Procedures and Data Analysis

After diagnosis, patients were followed as per standard hospital procedures for patients with IOPD, including administration of ERT once this therapeutic approach became available. Before starting ERT, cross-reactive immunological material (CRIM) status was determined either by genotype prediction or western blot analysis on lymphocytes, and an immunomodulation protocol was performed accordingly[11].

Patients were then monitored closely with clinical, biochemical, and instrumental assessments that included plasma and urine biomarkers (creatine phosphokinase (CPK), aspartate transaminase (AST), alanine transaminase (ALT), urinary Glc4), cardiac testing (ECG and echocardiogram), and pulmonary and feeding status; psychomotor development was monitored using age-appropriate scales. Anti-rh-GAA antibodies were monitored by ELISA.

RESULTS

Selective Screening

Three patients were diagnosed with IOPD before the start of the NBS program (before 2015). These three patients (patients 1–3) were all female. The median age at onset of symptoms was 2 months (range 0–3 months), median age at diagnosis was 3 months (range 0-5 months), and median age at start of ERT was 4 months (range 1–6 months) (Table 1).

The diagnosis was suspected in the first 3 months of life based on the basis of clinical (hypotonia, hypertrophic cardiomyopathy) and biochemical (increased CPK) features. The diagnosis was confirmed by low GAA activity (absent) in dried blood spots, lymphocytes or fibroblasts, and gene analysis. Furthermore, urinary glucose tetrasaccharide (Glc4), a molecule derived from glycogen breakdown, was measured using a qualitative thin-layer chromatography (TLC) assay and subsequently by quantitative MS/MS assay. In this setting, urinary Glc4 was used as a specific biomarker in the context of reduced GAA activity or other signs and symptoms of disease progression.

Patient 1 and Patient 3

Patients 1 and 3 presented within 3 months of life with poor growth and hypotonia. They needed a nasogastric tube to feed. At 4 and 5 months, they were diagnosed with hypertrophic cardiomyopathy (left ventricular mass index (LVMI) 204–183 g/m^2) and impaired cardiac function (ejection fraction (EF) 19–30%), respectively. On electrocardiography (ECG), they both had short PR intervals (0.08 and 0.07 seconds, respectively). Blood tests showed hyperCKemia (575–648 U/L), oligosaccharides positive in the TLC assay. The diagnosis of PD was confirmed by reduced levels of GAA on muscle (absent) in patient 1 and dried blood spots (0.24 µmol/l/h) in patient 3, and by molecular examination of the GAA gene (patient 1: homozygous variant c.1564C>G, p.Pro522Ala; patient 3: compound heterozygous variant c.236_246del, p.Pro79Argfs *13 + c.2560C>T, p.Arg854*).

Patient 1 was born in 2008. The CRIM status was not determined, and she began therapy with alglucosidase alfa 20 mg/kg every other week (EOW) at 5 months of life. The therapy led to improvement in the heart disease (at 21 months of life, no hypertrophy, EF 45%), but severe hypotonia persisted, and the patient never achieved head control. She was fed via a percutaneous endoscopic gastrostomy tube and needed mechanical ventilation from 2 years of age. Anti-rh-GAA antibodies remained at low titers (1:6400), and she was never immunomodulated. The patient died for respiratory failure at the age of 9 years.

Table 1. Demographic and clinical characteristics of patients diagnosed through selective screening.

Patient No.	Year of birth	Sex	Ethnic origin	GAA activity	Age at diagnosis (months)	Symptoms	uGlc4 (0–5 months <16.3 mmol/mol creatinine)	CPK U/L (nv 0–295)	ECG PR interval (s)	Echo LVMI g/m^2	EF (%)	Gene variant 1	Gene variant 2	Protein 1	Protein 2	Diagnosis	Age at start ERT (months)	ITI protocol
1	2008	F	Europe	Absent on muscle biopsy	3	Hypotonia, poor growth	Positive	575	0.08	204	19	c.1564C>G	c.1564C>G	p.Pro522Ala	p.Pro522Ala	IOPD	5	No
2	2008	F	Europe	Absent on lymphocytes	0.5	Hypotonia	Positive	731	0.08	171	62	c.1933G>A	c.1933G>A	p.Asp645Asn	p.Asp645Asn	IOPD	0.5	No
3	2016	F	North Africa	0.24 µmol/l/h on DBS	4	Hypotonia, poor growth	Positive	648	0.07	183	30	c.2560C>T	c.236_246 delCCACA CACTGC	p.Arg854*	p.Pro79 Argfs*13	IOPD CRIM neg	6	Yes

Abbreviations - CPK: creatine phosphokinase; CRIM: cross-reactive immunological material; DBS: dried blood spots; ECG: electrocardiography; ERT: enzyme replacement therapy; F: Female; EF: ejection fraction; GAA: acid α-glucosidase; IOPD: infantile-onset; neg:negative, nv: normal value, Pompe disease; ITI: immune tolerance induction; LVMI: left ventricular mass index; M: Male; uGlc4: urinary tetrasaccharide.

Patient 3 was CRIM-negative and was immunomodulated with rituximab, methotrexate, and intravenous immunoglobulins (IVIG) before starting ERT at the age of 6 months. She was treated with alglucosidase alfa 40 mg/kg/week in the first 18 months of life, with benefit. Cardiac function normalized (at 18 months, LVMI 58.9 g/m^2, EF 63%), and the patient was able to feed independently and achieved a sitting position. When she was 18 months old, the family relocated outside Italy, and the patient maintained therapy with alglucosidase alfa 40 mg/kg EOW and continued follow-up at our center. After a long period of no follow-up due to the COVID-19 pandemic occurring in 2020, she returned to our center at the age of 4 years. She presented marked hypotonia with the inability to feed independently and worsening respiratory failure, which required tracheostomy and mechanical ventilation. The urinary tetrasaccharide was 210 mmol/mol creatinine. Anti-rh-GAA antibody titer was low (1: 6400); due to the clinical worsening, she was immunomodulated with bortezomib, rituximab, and IVIG. ERT frequency was increased to 40 mg/kg weekly, but the patient progressively worsened. The patient died for respiratory failure at the age of 4.5 years.

Patient 2

Patient 2 was born at term by emergency cesarean section because of fetal bradycardia. At birth, she presented a short PR interval (0.08 seconds), hypertrophic cardiomyopathy (LVMI 171 g/m^2 with normal EF), and hypotonia. Biochemical examination showed increased CPK (731 U/L) and positive urinary Glc4 by TLC assay. An enzyme assay was performed in peripheral blood lymphocytes, revealing the absence of GAA activity. GAA mutational analysis showed that the patient was homozygote for the c.1933G>A p.Asp645Asn variant. At 20 days of age, she started ERT with alglucosidase alfa 20 mg/kg EOW. A gradual improvement in left ventricular hypertrophy was observed, with normalization of cardiac structure and function within 24 months (LVMI 35 g/m^2, EF 68%). Furthermore, the patient had normal growth parameters and psychomotor development. She never developed anti-rh-GAA antibodies. After 8 years of ERT, frequent respiratory infections and worsening motor performances were observed. Indeed, she climbed stairs with difficulty, and her gait was unstable. Thus, alglucosidase alfa dosage was increased to 40 mg/kg EOW and then to 40 mg/kg weekly. In the following 5 years, the patient presented a progressive motor and respiratory worsening, with severe scoliosis and a moderate-severe restrictive pattern at spirometry. The biomarker showed CPK 546 U/L, urinary Glc4 25.5 mmol/mol creatinine (nv <1.1) (samples collected before the infusion). In November 2021, at 13 years old, she was enrolled in the avalglucosidase alfa compassionate use project. At baseline, she had a rich language, although her speech was dyslexic. She had a hypomimic face, palpebral ptosis, increased lumbar lordosis and severe scoliosis. Muscle hypotrophy was more evident in the lower limbs, and she had a waddling gait with a wide base and extra rotation of her feet. At the 6-Minute Walking Test (6MWT), she managed 170 m. Heart ultrasound showed no hypertrophy, and EF was 60%. Spirometry evidenced severe restrictive ventilatory dysfunction in the standing position (vital capacity: 1.30 L; forced expiratory volume 1: 1.13 L; 45% of predicted), which was reduced by a further 20% in the supine position. At the last follow-up, after 30 months of therapy with avalglucosidase alfa 40 mg EOW, she could walk with braces, and the 6MWT showed a mild improvement from 175 to 180 and 240 meters. At spirometry, parameters remained stable, and scoliosis, despite severe, did not worsen. Biomarkers improved (CPK 387U/L, urinary Glc4 12.2 mmol/mol creatinine; samples collected before the infusion).

Newborn Screening

The NBS assay is based on assessing GAA activity by multiplex MS/MS using the NeoLSD® assay system from Perkin Elmer (Turku, Finland). Between 2015 and 2023, 257,236 neonates were screened for IOPD; 13 newborns had positive results and were referred to the Center of Inborn Errors of Metabolism for confirmatory tests. Confirmatory testing included clinical evaluation, cardiologic assessment (ECG and echocardiogram), biochemical tests (blood CK, AST, ALT, LDH; urinary Glc4), and molecular analysis. Three patients (patients 4–6) were diagnosed with IOPD after a positive NBS (Table 2).

Patients were referred to the Center of Inborn Errors of Metabolism between days 3 and 14 of life for confirmatory tests. Patients 5 and 6 had a prenatal diagnosis of hypertrophic cardiomyopathy, while patient 4 was apparently asymptomatic. At first evaluation, all presented hypotonia and cardiac involvement with short PR intervals (0.07–0.08 seconds) and hypertrophic cardiomyopathy (LVMI 128–232 g/m^2). Pa-

Table 2. Demographic and clinical characteristics of patients diagnosed through newborn screening.

Patient No.	Year of birth	Sex	Ethnic origin	GAA activity on DBS (µmol/L/h)	Age at diagnosis (days)	Symptoms	uGlc4 (0–5 months <16.3 mmol/mol creatinine)	CPK U/L (nv 0–295)	ECG PR interval (s)	Echo LVMI g/m^2	EF (%)	Gene variant 1	Gene variant 2	Protein 1	Protein 2	Diagnosis	Age at start ERT (months)	ITI protocol
14	2017	F	Euro-pean	0.45	9	Hypotonia	26.5	990	0.08	187	78	c.1933G>A	c.2237G>A	p.Asp645Asn	p.Trp746*	IOPD CRIM pos	11	No
5	2018	M	West Africa	0.49	3	Hypotonia	71.2	1063	0.07	232	20	c.2560C>T	Deletion exons 4–8	p.Arg854*	/	IOPD CRIM neg	5	Yes
6	2020	M	North Africa	0.73	14	Hypotonia	29.6	653	0.08	128	63	c.236_246 delCCACA CACTGC	c.236_246 delCCACA CACTGC	p.Pro79 Argfs*13	p.Pro79 Argfs*13	IOPD CRIM neg	19	Yes

Abbreviations - CPK: creatine phosphokinase; CRIM: cross-reactive immunological material; DBS: dried blood spots; ECG: electrocardiography; ERT: enzyme replacement therapy; F: Female; EF: ejection fraction; GAA: acid α-glucosidase; IOPD: infantile-onset; neg: negative, nv: normal value, Pompe disease; pos: positive; ITI: immune tolerance induction; LVMI: left ventricular mass index; M: Male; uGlc4: urinary tetrasaccharide.

tient 5 also presented with heart failure (EF 20%) at birth and needed invasive ventilation and circulatory support. All had increased levels of muscle necrosis markers (CPK 653–1063U/L) and urinary Glc4 (26.5–71.2 mmol/mol creatinine, nv <7.4). CRIM status was tested on the peripheral blood mononuclear cells and confirmed through molecular analyses (patient 4: CRIM-positive c.1933G>A, c.2237G>A—p.Asp645Asn, p.Trp746*; patient 5: CRIM-negative, compound heterozygous c.2560C>T, p.Arg854* and deletion exons 4–8; patient 6: CRIM-negative homozygous c.236_246del—p.Pro79Argfs*13).

All patients started ERT (alglucosidase alfa) between day 5 and day 19 of life. CRIM-negative patients received a dosage of 40 mg/kg weekly, simultaneously with an immune tolerance induction (ITI) protocol (methotrexate, rituximab, IVIG); the CRIM-positive patient was treated with an initial dosage of 40 mg/kg EOW without ITI protocol, based on the best evidence at that time. Based on recent evidence, the dosage was then increased to 40 mg/kg weekly when she was 3 years old.

Patient 4 and Patient 6

Patients 4 and 6 showed an early response to ERT. Hypertrophic cardiomyopathy regressed, with normalization of LVMI after 9 and 2.5 months of ERT, respectively. Hypotonia and psychomotor delay progressively improved to an age-appropriate motor development starting at 1 year of life. They never needed feeding or respiratory assistance. Urinary Glc4 normalized after 1 month of therapy, and CPK after 2.5–4 months. To date, patients are alive and in active follow-up. They are 6 and 4 years old and have age-appropriate motor development with no signs of cardiomyopathy and normal biochemical testing, including CPK and urinary Glc4. They continue alglucosidase alfa 40 mg/kg weekly and have not developed anti-rh-GAA antibodies or experienced adverse events.

Patient 5

The patient, a male of African descent, was born at 37 weeks from an emergency delivery due to fetal heart failure. At birth, echocardiography showed hypertrophic cardiomyopathy (LVMI 232 g/m^2) with severe biventricular dysfunction (EF 30%). The ECG showed a short PR interval (0.07 seconds). Moreover, the patient presented with severe hypotonia and needed feeding (nasogastric tube) and respiratory assistance. The biochemical assay showed elevated serum CPK (1063 U/L) and brain natriuretic peptide (9169 U/L, nv 0–100). NBS was positive for GAA deficiency (0.49 µmol/L/h). The diagnosis of PD was confirmed by increased urinary Glc4 (71.2 mmol/mol creatinine, nv <16.3) and GAA molecular analysis (patient 5: CRIM-negative, compound heterozygous c.2560C>T, p.Arg854* and deletion exons 4–8). At 5 days of life, the patient was treated with alglucosidase alfa; simultaneously, an immunomodulation protocol with rituximab, IVIG, and methotrexate was started due to the CRIM-negative status. In the first months of ERT, he progressively improved and was able to eat and breathe independently. At 1 year, he showed improvement in cardiac mass (LVMI 116.6 g/m^2) and function (EF 50%), and motor function. Urinary Glc4 reduced to 20.2 mmol/mol creatinine. Unfortunately, the patient subsequently developed a high anti-ERT antibody titer (1:102,400), followed by clinical (psychomotor delay, hypertrophic cardiomyopathy with LVMI 229 g/m^2, EF 50%) and biochemical worsening (elevated serum CPK up to 6795 U/L, elevated urinary Glc4 up to 50 mmol/mol creatinine, brain natriuretic peptide levels 237 U/L). The patient underwent a new immunomodulation cycle with bortezomib, rituximab, sirolimus, and IVIG, with a reduction in the antibody titer to 1:6400 after 1 year, but with only partial clinical and biochemical improvements (persistent psychomotor delay, hypertrophic cardiomyopathy with LVMI 118 g/m^2, EF 48%, urinary Glc4 35 mmol/mol creatinine, CPK 4728 U/L). At 2.5 years of life, he started a compassionate use therapy that combined a new ERT (cipaglucosidase 30 mg/kg/week) with a chaperone (miglustat 115 mg) (ATB200-15 Program), with clinical benefit. After 3 months, the patient could walk independently, biomarkers were reduced (CPK 2989 U/L, urinary Glc4 32.6 mmol/mol creatinine), and cardiac mass (LVMI 82 g/m^2) and function (EF 67%) improved, despite an evolution in non-compaction myocardium (NC/C index 2.6). The benefits were persistent until the age of 4 years. At that time, he presented delayed psychomotor development, but he walked unsupported, had stable biomarkers and cardiac parameters (LVMI 98 g/m^2, EF 66%, CPK 3115, urinary Glc4 30.4 mmol/mol creatinine, CPK 2750 U/L), and did not need respiratory or feeding assistance.

At the age of 4 years, the anti-rh-GAA antibodies became positive again (1:102,400). At the same time, due to an influenza A infection, he was admitted to intensive care and mechanically ventilated. Urinary Glc4 increased up to 210 mmol/mol creatinine, while CPK was low (540 U/L), probably because the patient was sedated. Cardiac function remained stable. Therefore, we decided to return to conventional therapy

with alglucosidase alfa 40 mg/kg/week and start an immunomodulation protocol with rituximab, bortezomib, methotrexate, and IVIG. After 2 months, the patient showed a reduction in antibody titer (1:6400) and good recovery with the reacquisition of independent breathing and feeding. He did not recover the ability to walk but was able to sit without support and stand with support. At the age of 6 years, he is stable (LVMI 90 g/m^2, FE 58%, stable motor ability, CPK 3180 U/L, urinary Glc4 34.4 mmol/mol creatinine). Of note, he has cognitive impairment with the absence of language and relational difficulties. Brain MRI shows widespread bilateral and symmetrical hyperintensities of centrum semiovale white matter.

DISCUSSION

We reported the long-term outcomes of patients with IOPD diagnosed through selective screening or using NBS in our Center of Inborn Errors of Metabolism, Padua University Hospital (Padua, Italy).

Three patients diagnosed through NBS are alive; they breathe and feed independently after an average of 5.3 years of follow-up. In comparison, among patients diagnosed before NBS, two died at ages 4.5 and 9 years, respectively, and only one is currently alive. The two deceased patients started ERT at 5 and 6 months of life, respectively, while patient 2 started ERT in the first month of life, like all patients diagnosed by NBS. This underlines how the outcome of patients with IOPD is strongly influenced by early diagnosis and early therapy, even in CRIM-negative patients. Unfortunately, the diagnosis is often late, as demonstrated by patients 1 and 3; despite the presence of clinical signs, such as hypotonia and significant cardiac involvement, a diagnostic delay is expected in the absence of NBS, as shown by the three patients diagnosed via NBS. Therefore, ERT, possibly associated with an ITI protocol, should be started as soon as possible after confirmation of the diagnosis and establishment of CRIM status.

Another difference in our patient population is represented by the dosage of ERT with alglucosidase alfa, which was administered at the standard dosage of 20 mg/kg EOW in the first years after its commercialization. This dosage was probably sufficient to allow regression of the hypertrophic cardiomyopathy, as demonstrated in patient 1, but the benefits on skeletal muscle were minimal. Currently, all our patients are being treated with a dosage of 40 mg/kg weekly. This approach is supported by a recent multicenter cohort study including 116 patients with IOPD, confirming that a high dosage of 40 mg/kg/week significantly improves patient survival and the proportion of patients achieving walking abilities[3].

Among the patients diagnosed via NBS, patient 5 had the worst outcome. He developed a high antibody titer despite early therapy (5 days of life) and preventive immunomodulation. This can probably be explained by the initial disease burden[12].

Another important finding was the high morbidity among the long-term survivors, experiencing secondary deterioration of motor and respiratory functions after an initial improvement with ERT. These results are consistent with real-world studies examining motor function in larger cohorts of patients with IOPD; an example is a recent retrospective European multicenter cohort study with 86 patients, which showed that about 50% at the age of 18 months had learned to walk while at the age of 15 years and older only 30% were still ambulant[13-16].

New Enzyme Replacement Therapies

Until recently, alglucosidase alfa ERT was the only approved treatment for PD[1,17]. However, its effectiveness is limited by its poor drug targeting to skeletal muscles, resulting in the need for higher drug doses. It may be related to the relatively low affinity of alglucosidase alfa for the cation-independent mannose-6-phosphate (M6P) receptor, the major route for internalizing alglucosidase alfa into cells[17]. As the long-term results of alglucosidase alfa are still suboptimal, new enzyme preparations, such as cipaglucosidase alfa and avalglucosidase alfa, have been developed to improve receptor targeting and enzyme uptake[17-19].

Avalglucosidase Alfa

Avalglucosidase alfa is a next-generation rh-GAA. The drug has received US approval for the treatment of patients aged 1 year and older with LOPD, while in the EU, it has received a positive opinion for all patients with PD (IOPD and LOPD)[20]. In Italy, it became commercially available in January 2024.

Avalglucosidase alfa is a hydrolytic lysosomal glycogen-specific rh-GAA enzyme conjugated with multiple synthetic bis-M6P-tetra-mannose glycans (approximately 15 moles of M6P per mole of enzyme)[19]. *In vitro* and *in vivo* (PD mouse model) studies found that avalglucosidase alfa increased binding affinity for the M6P receptor compared with alglucosidase alfa (>95% binding *vs*. 15–30% binding, respectively), and its uptake into muscle cells was increased approximately fivefold (approaching saturation at 100 nmol/L *vs*. approximately 500 nmol/L)[18,21]. Avalglucosidase alfa reduced glucose tetrasaccharide (urinary Glc4) levels in the urine. It provided clinically meaningful improvements compared with alglucosidase alfa in terms of respiratory function and movement endurance measures in treatment-naïve patients with LOPD participating in phase III, randomized, double-blind, multinational COMET study[22]. In the Mini-COMET study, 16 patients with IOPD who had inadequate response or motor function regression on alglucosidase alfa were switched to avalglucosidase alfa. This study demonstrated improved or better-stabilized symptoms, as assessed by motor outcomes, cardiac parameters, and eyelid measures, with the avalglucosidase alfa 40 mg/kg dose providing additional benefits[23]. The ongoing open-label multicenter Baby-COMET study analyzed the efficacy, safety, pharmacokinetics, and pharmacodynamics of avalglucosidase alfa in treatment-naïve patients with IOPD. Patient 2 was treated for 3 years with avalglucosidase alfa 40 mg EOW. She was undergoing rapid deterioration and was very compromised. She achieved stabilization/improvement of motor/respiratory parameters after the therapeutic switch.

Cipaglucosidase Alfa

On 27 March 2023, cipaglucosidase alfa received its first approval in the EU for use in combination with miglustat for treating adults with LOPD[24]. It is under regulatory review in the US for the treatment of LOPD and is currently in phase III clinical development for this indication in numerous other countries worldwide. Cipaglucosidase alfa is a novel rh-GAA enriched with bis-phosphorylated (bis-M6P) N-glycans for high-affinity cation-independent M6P receptor binding. It is co-administered with miglustat, which acts as a stabilizer and prevents loss of enzyme activity during cipaglucosidase alfa infusion[24]. In phase I/II trial, cipaglucosidase alfa plus miglustat reduced biomarkers of muscle damage (creatine kinase) and glycogen accumulation (urinary Glc4) in patients with LOPD. Co-administration of cipaglucosidase alfa and miglustat was associated with a 48% increase in the distribution half-life of cipaglucosidase alfa and a 27% reduction in plasma clearance[25]. Phase III study on cipaglucosidase alfa plus miglustat (PROPEL trial and open-label extension OLE, OLE - ATB200-07; NCT04138277) did not achieve statistical superiority over alglucosidase alfa plus placebo for improving 6MWT in adult patients with LOPD. However, cipaglucosidase alfa plus miglustat provided potentially clinically meaningful improvements in motor and respiratory function compared with alglucosidase alfa plus placebo, significantly associated with improved quality of life and other patient-reported outcomes[26,27]. Recruitment is underway for two phase III trials evaluating the safety, efficacy, pharmacokinetics, pharmacodynamics, and immunogenicity of cipaglucosidase alfa plus miglustat in ERT-experienced and ERT-naïve pediatric patients (aged 0 to <18 years) with IOPD or LOPD[24]. Two expanded access programs are also ongoing in patients with PD. Fiege et al[28] reported the disease course of a patient with severe IOPD, who showed serious progression even with a high-dose standard of care ERT alglucosidase alfa. Changing the therapy to cipaglucosidase alfa/miglustat at compassionate use significantly improved respiratory failure, cardiomyopathy, and motor functions. The patient could be weaned from respiratory support and oxygen supplementation. Cardiac function was normalized. Furthermore, the patient, who had lost nearly all motor skills, acquired head control, learned to speak, and could move his wheelchair by himself.

Patient 5, after treatment with cipaglucosidase alfa/miglustat, showed normalization of ventricular mass and function and sustained improvement of the motor function. Unfortunately, after 2 years of therapy, anti-rh-GAA antibodies returned positive with a clinical worsening. This finding led us to hypothesize a possible cross-reactivity among several rh-GAA.

CONCLUSIONS

In summary, our experience shows that the association between NBS and early treatment has the potential to increase the efficacy of treatment in patients with IOPD. The improvement obtained with NBS, immunomodulation, higher first-generation ERT doses, and the development of next-generation enzymes could lead to better outcomes for patients with IOPD receiving ERT with recombinant GAA in the future.

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Authors' contributions:
Study conception and design: VG, ABB; collection and interpretation of data: all authors; statistical analysis: VG; manuscript drafting: VG; manuscript editing: all authors; approval to submit: all authors.

Availability of data and material:
All data generated or analyzed during this study are included in this published article.

Conflicts of interest:
The authors declare that they have no conflict of interest to disclose.

Consent for publication:
Consent to publication was obtained by patients and their parents.

Ethical approval:
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Informed consent:
All the patients signed an informed consent form to participate in the study and allowed publication of their data.

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