Observational clinical study of 22 adult-onset Pompe disease patients undergoing enzyme replacement therapy over 5 years

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Abstract

Pompe disease is an autosomal recessive disease resulting from deficiency of the acid alpha-glucosidase (GAA). The late-onset Pompe Disease (LOPD) patients develop muscular and respiratory complications later in life. We describe a retrospective observational cohort study including 22 patients with LOPD. The cohort was assessed at baseline before Enzyme Replacement Therapy (ERT) with alglucosidase alpha (20 mg/kg biweekly) was com-menced and subsequently relevant information was collected at 2, 4 and 5 years later. The median age of the patients at study entry was 44 years (16–64 years), with median disease duration of 11.5 years (4–31 years). At baseline, 10 patients (45%) could walk without support, 12 (55%) could walk with unilateral or bilateral sup-port including 3/12 were wheelchair bound. Mean predicted FVC % was 55.7 (95% CI 45–66) of predicted normal at baseline and showed no significant change after 5 years (54.6 (95% CI 43–66)), (all $p = 0.9815$). Mean FVC %supine was 41.8 (95% CI 33.8–49) of predicted normal at baseline and remained significantly unchanged at 5 years (48.4 (95% CI 37–59.6)), (all $p = 0.8680$). The overnight non-invasive ventilator dependence increased by 18.2% as compared with baseline and requirement of mobility aids increased during this period by 5.2% as compared with the baseline. Mean walking distance at 6 min walk test was 411.5 (95% CI 338–485) at baseline, 266.5 (95% CI 187–346) m at 2 years, 238.6 (95% CI 162–315) m at 4 years and 286.8 (95% CI 203–370) m at 5 years ($p = 0.1981$; ANOVA was completed only for 14 patients). A gradual decline in FVC% predicted was noted only in four cases and a decline in FVC% supine in two other. Only one patient showed a decline in both pulmonary function tests. In all remaining cases (17/22) respiratory function remains stable. In conclusion overall pulmonary function tests and mobility remained stable for 5 years in majority of patients on ERT. However, in some patients they continued to decline in spite of ERT resulting in increased number of patients requiring ventilation and increase wheel chair dependence at the end of 5 years.

Keywords:
Late-onset Pompe disease
LOPD
Enzyme replacement therapy
Alpha glucosidase deficiency
Myozyme

1. Introduction

Pompe disease (OMIM 232300) is an autosomal recessive disease caused by deficiency of acid alpha-glucosidase (GAA) (EC 3.2.1.20), an enzyme responsible for the degradation of lysosomal glycogen by hydrolysis of alpha-1,4, and 1.6 links of glycogen [1]. The loss of GAA enzyme activity causes an accumulation of lysosomal glycogen leading to cellular dysfunction. Patients with the late-onset Pompe disease (LOPD) retain some residual GAA activity. An inverse correlation is usually observed between the amount of residual GAA activity and disease severity [2]. Patients with LOPD display a wide range of phenotypes with some having early presentation and significant respiratory involvement leading to respiratory failure [1, 3]. The clinical progression of LOPD [4, 5, 6] demonstrates a progressive deterioration in impaired motor function including swallowing and walking difficulties. In advanced disease patient gets wheel chair dependent and presents diaphragmatic dysfunction and requires invasive ventilation or non-invasive ventilation (NIV) (12 or more hours daily) [7]. There are reports of basilar dolichoectasia [8, 9], cerebral microbleeds [10], aneurysms [11, 12], intraparenchymal haemorrhage [10] and aneurysmal thrombosis [13] in patients with LOPD. Microscopic glycogen-laden molecules deposits in arteries is thought to be underlying pathology. There is no firm evidence that LOPD leads to cardiac involvement.

Patients with LOPD are expected to have shortened life span and in the majority of these patients respiratory failure and chest infection is the cause of mortality. Monitoring of the pulmonary function is essential in LOPD in order to evaluate the need for mechanical ventilation [4, 6, 14]. Various pulmonary function tests have been used to assess it, FVC % upright and supine being the most commonly used. Enzyme replacement therapy (ERT) seems to have a beneficial effect on survival that is related to its positive effect on pulmonary function [15]. Current

licensed treatment with human recombinant GAA (rh-GAA) (commercial name Myozyme) infusions has been shown to improve respiratory dysfunction and overall survival rate in late- onset patients [15].

Randomized control trials of ERT in adults were initiated in 2005 and included 90 patients [6]. Over a period of 78 weeks, treatment with alglucosidase alpha resulted in an improved walking distance on the 6MWT and stabilization of pulmonary function, meeting both primary endpoints of the trial [6]. ERT maintained efficacy for 2 years [5] that resulted in stabilization of VCmax following ERT for 3 years and longer in LOPD patients [16, 17, 18]. There are additional reports showing significant improvement in pulmonary function, preventing or delaying ventilator dependency and prolonging survival [6, 18, 19–24, 25] and led to fewer hospital admissions [24]. ERT also demonstrated improvements in muscle strength, pulmonary function and survival [15].They reported less fatigue and improvement in motor function and quality of life to a variable extent [6, 19, 24]. However, there seemed to be up to a third of patients still declining in their motor performance and lung function [6, 18, 19, 24]. The long-term effects of ERT on pulmonary function remain unclear. The aim of the study was to assess the response of respiratory function and mobility in a cohort of patients with LOPD undergoing ERT at a single centre.

2. Methods

2.1. Study design and ethical consideration

This was a retrospective observational cohort study. Patients had clinical features of LOPD disease and had confirmed reduced GAA activity in leukocytes as per protocol described elsewhere [24]. In all patients GAA was measured on leucocytes except one where it was on dried blood spot. Genetic testing was also performed and results were available for the majority. A few patients had muscle biopsy performed for histopathology prior to the confirmation of diagnosis by the referring clinician (Table 1: patients 2, 4, 6 and 22). Patients who had been on ERT (20 mg/kg biweekly) for a minimum of 5 years were included. During this period the dose was adjusted if there had been changes in the weight of the individuals. All patients gave consent prior to their inclusion in the study.

2.2. Clinical and instrumental examinations

Gender, age at onset, age and disease duration at first infusion, use of ambulation devices, need for respiratory support, co-morbidities and genetic results were collected from all patients.

Patients had 6-min walk test (6MWT) as previously described [26, 27]. Muscular strength was evaluated by manual muscle testing using the Medical Research Council (MRC) grading scale. Use of walking aid was recorded at each visit.

Pulmonary function testing (PFT) was measured by % predicted forced vital capacity (FVC) in the upright and supine position, and SNIP (Sniff Nasal Inspiratory Pressure). Majority of these patients had PFTs performed in the same laboratory at intervals however for some patients the results obtained from their local laboratories. Results were expressed as a percentage of the predicted normal value. Values lower than 80% of predicted normal values were considered to be abnormal. The use of respiratory support was recorded at each visit (at baseline and during the study period). Not all patients could perform every test because of degree of motor or respiratory involvement.

All patients had biochemical tests including creatine kinase (CK), vitamin D, renal functions, liver function measurements at regular intervals. *Anti*-human recombinant GAA (anti rh-GAA) and IgG antibody titre were determined by a non-commercial assay established in the Genzyme Clinical Specialty Laboratory (Genzyme Corp., Framingham, Massachusetts). Anti rh-GAA antibody titre and GAA enzyme activity were denoted for most patients.

Finally, all patients reported their subjective impression of quality of life using a standardized assessment tool (SF-36) [28].

2.3. Statistical analysis

Descriptive statistics mean (SD) and median (range) were used to describe patients' demographic and clinical characteristics for

Table 1
Patients characteristics.

Patient characteristics										
Pt No.	Gender	Age at diagnosis (years)	Disease duration	Age at the start of ERT (20 mg/kg biweekly)	Anti rh-GAA antibodies titre	Genetic mutation	Enzyme (μmol/g/h)	NIV before ERT	NIV after ERT	Mobility aids use before and after ERT
1	F	37	23	38	1:800	c.1040C>G/c.1559A>	0.3 (3−20)	+	+	+
2	M	40	31	63	1:6400	c.-32-13T>G/c.2135T>C	0.36 (3–20)	+	+	–
3[a]	M	22	13	26	1:6400	n. a.	0.15 (2−10)	+	+	+
4	M	37	10	41	neg	c.1437G>A/	2.1 (3–20)	–	–	+
5	F	38	26	40	neg	c.-32-13T>G/c.482_483del	0.8 (3–20)	–	–	–
6	M	36	11	36	neg	c.1437G>A/	1.2 (2–10)	–	–	–
7	F	49	25	50	1:1600	c.-32-13T>G/c.525delT	0.78 (3–20)	–	–	–
8	F	41	7	48	1:1600	c.482_483del/c.692+5G>T	0.23 (2–10)	–	–	+
9	M	16	1	16	n. a.	n. a.	2.4 (3–20)	+	+	+
10[a]	M	45	27	58	1:3200	c.-32-13T>G/C.258dupC	0.39 (3–20)	–	–	+
11	F	39	21	53	1:400	c.1548G>A/c.2799+4A>G	0.41(3–20)	–	+	+
12	M	28	4	28	n. a	n. a.	0.33(3–20)	–	+	–
13	M	46	6	47	1:25,699	c.-32-13T>G/c.482_483del	0.52(3–20)	–	–	–
14	F	42	4	41	1:800	n. a.	0.93(3–20)	–	–	+
15	F	48	33	48	1:1600	c.1437G>A	0.48(3–20)	–	+	–
16	F	37	21	37	neg	c.-32-13T>G	0.18(3–20)	–	–	+
17	M	34	17	34	1:800	c.-32-13T>G/c.896T>C	0.75(3–20)	–	+	–
18[a]	M	60	42	60	1:1600	n. a.	0.67(3–20)	+	+	–
19	M	54	35	54	1:6400	c.-32-13T>G c.2242dup	0.41(3–20)	+	+	+
20	F	46	26	46	1:12,800	c.-32-13T>G/c.2242dup	0.64(3–20)	+	+	–
21	M	56	35	56	1:1600	c.-32-13T>G/c.1128_1129delinsC	0.57(2–10)	+	+	+[b]
22[a]	F	40	18	40	1:12,800	c.-32-13T>G/c.258dup	2.77 pmol/punch/h (7.3–39)	+	+	+

NIV — non-invasive ventilation; n. a. — not available.
[a] Patients who discontinued ERT.
[b] One patient developed wheelchair dependence while being on ERT.

continuous variables. Percentages were calculated for categorical variables. The diversity of patient disease states was responsible for the fact that not all tests could be done on every patient.

For normally distributed variables, repeated measures analysis of variance (ANOVA) was performed for testing the significance of the main effect of therapy (pre- vs. post-measures). The overall change in pulmonary function tests and 6MWT was calculated using ANOVA. The test was used to determine whether there was any significant difference between the means of independent groups of variables all together. The results were presented as means with 95% confidence intervals (CIs) and as median (±range). Statistical tests were conducted using Analyse-it (v4.00.1). A p-value ≤0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

Twenty- two patients with LOPD treated with ERT were included in the study. 12/22 (55%) patients were males. The median age at start of ERT was 44 years (range 16–64). The median age at onset of symptoms was 35 years (range 13–44). The median age at diagnosis was 40 years (range 16–60). Patients' characteristics are summarized in Table 1. Median disease duration was 11.5 years (range: 4–31 years).

At baseline, 10 patients (45%) could walk without support, 12 (55%) could walk with unilateral or bilateral support including 3/12 were wheelchair bound. At study entry 13 patients (59%) did not require ventilator support, 7 patients (32%) required ventilation support for <12 h, and 2 (9%) required a ventilator for more than 12 h per day (including one who required it for 24 h).

3.2. GAA enzyme activity and molecular analysis

Both mutated GAA alleles were identified in 13/22 patients (59%) and 4 patients only one (18%). IVS1-13T>G was found in 11 cases (73%), always in heterozygous state. In 5 patients no mutations were available. Residual enzymatic activity was measured in leucocytes in 21 patients and in bloodspot in one case (not confirmed by leucocyte analysis). This patient was known to have two mutated GAA alleles. Results were below normal limits (Table 1). Some patients in the cohort were related.

3.3. Motor function

3.3.1. Six minute walk test (6MWT)

The 6MWT was available for 4 patients at baseline, 12 at 2 years, 15 at 4 years and 11 at 5 years. Mean walking distance was 411.5 (95% CI 338–485) at baseline, 266.5 (95% CI 187–346) m at 2 years, 238.6 (95% CI 162–315) m at 4 years and 286.8 (95% CI 203–370) m at 5 years. Results are also presented as median (±range) in Table 2. Mean walking distance showed no statistically significant differences at 2, 4 and 5 years ($p = 0.1981$; ANOVA was completed for 14 patients) (Table 2). 7 out of 15 patients (46%) increased and 8 patients (53%) decreased the distance in the 6MWT on the final test. The total number of patients requiring ambulatory aids baseline and after 5-years of ERT (12/22, 55%) remained unchanged. However, the wheelchair dependency in the group requiring ambulatory aids increased from 3/22 to 4/22 patients (by 5.2%).

3.4. Respiratory function

Standard spirometry was performed in the upright and supine position [29] at baseline and 2, 4, 5 years after ERT in 22 patients. Complete datasets were available in 22 patients (Table 2). In this cohort the mean FVC % sitting was 55.7 (95% CI 45–66) of predicted normal at baseline. After 2 years the mean FVC% was 57.8 (95% CI 45.8–68.7), at 4 years was 55.3 (95% CI 44–66), and after 5 years 54.6 (95% CI 43–66) of predicted normal. There were no significant changes in FVC% compared to baseline (all $p = 0.9815$) (Fig. 1). The mean FVC % supine was 41.8 (95% CI 33.8-49.9) of predicted normal at baseline. 2 years later it was 46 (95% CI 34.6–58.5), at 4 years was 48 (95% CI 36.6–59) and at 5 years was 48.4 (95% CI 37–59.6) (all $p = 0.8680$) (Fig. 2). The mean SNIP in 22 patients was 37.5 (95% CI 30.4–44.5) at baseline, after 2 years 45 (95% CI 35.8–54), at 4 years 46 (95% CI 37.5–54) and 50 (95% CI 40–60) after 5 years (all $p = 0.2893$,) (Fig. 3). Data is also presented in mean and median in Table 2.

The requirements for overnight non-invasive ventilation (NIV) increased by 18.2% (from 9/22 at baseline to 13/22 after 5 years). Over time one patient had to increase the length of time used on the ventilator from <12 h to above 12 h over the 5-year follow-up and three other patients developed NIV dependency for up to 12 h for the first time. In one case a significant reduction of hours of ventilation (from 24 to 12 h) was observed during 5 years of ERT. 27.2% (6/22) patients who were able to walk required NIV. As represented by Figs. 1, 2 and 3 a gradual decline in FVC% predicted was noted in four cases and a decline in FVC% supine in two cases. Only one patient (number 17) showed a decline in both pulmonary function tests. As expected, most patients who showed a decline either in FVC% predicted or in FVC% supine required NIV. However, there were 9 patients who had stable pulmonary function test and still required NIV before and after they were commenced on ERT. In all remaining cases (17/22) respiratory function remains stable.

The analysis of a subgroup of younger patients (age < 40 years) showed that FVC% was not significantly different at baseline and at follow up 2, 4 and 5 years later ($n = 7$ patients) (all $p = 0.9751$). Supine FVC% for this subgroup was not significantly different at baseline and at follow up at 2, 4 and 5 years. The mean FVC% supine was 41.5 (95% CI 33–50) of predicted normal at baseline. 2 years later it was 35.3 (95% CI 29.3–41.2), after 4 years 38 (95% CI 31.3–44.6) and after 5 years 37 (95% CI 31–43) (all $p = 0.9654$). Similarly, SNIP was not significantly changed in this group ($p = 0.2603$, respectively). The mean SNIP was 29.1 (95% CI 24–34.2) at baseline. After 2 years the mean SNIP was 45.7 (95% CI 37–54), after 4 years 50 (95% CI 42–57.7) and after 5 years 52.6 (95% CI 43.7–61.4), ($p = 0.2603$).

3.5. Creatine kinase (CK)

19/20 patients had elevated level before and after 5 years of ERT. Levels were very heterogeneous, ranging from normal (<180 U/L) up to 7-fold of normal. At baseline the mean CK level was 625 ± 342 U/L,

Table 2
Pulmonary function tests and 6MWT over 5 years follow-up. Data expressed in means ± CI and median (min–max) due to a small sample size.

	baseline		2 years		4 years		5 years	
	mean (±95%CI)	median (min-max)	mean (±95%CI)	median (min-max)	mean (±95%CI)	median (min-max)	mean (±95%CI)	median (min-max)
FVC predicted (%)	55.7 (45–66)	56 (7.9–108)	57.8 (45.8–68.7)	55 (8.9–130)	55.3 (44–66.5)	53.5 (10.9–123)	54.6 (43–66)	56 (11.3–120)
FVC supine (%)	41.8 (33.8–49)	44 (11–67)	46 (34.6–57.8)	42.5 (11 − 110)	48 (36.6–59)	46.5 (11–115)	48.4 (37–59.6)	43 (12 − 110)
SNIP cmH_20	37.5 (30.4–44.5)	32 (15–80)	45 (35.8–54)	48 (12–90)	46 (37.5–54)	45.5 (15–92)	50 (40–60)	50 (11–110)
6MWT (m)	411.5 (338–485)	374.5 (375–524)	266.5 (187–346)	197.5 (90–473)	238.6 (162–315)	181 (30–510)	286.8 (203–370)	296 (120–494)

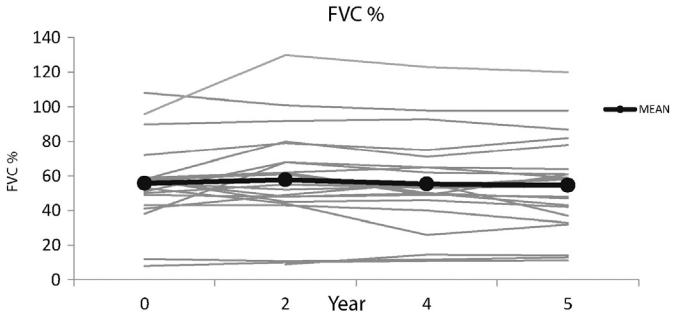

Fig. 1. % FVC at baseline and 2, 4 and 5-year follow-up (p = 0.9815).

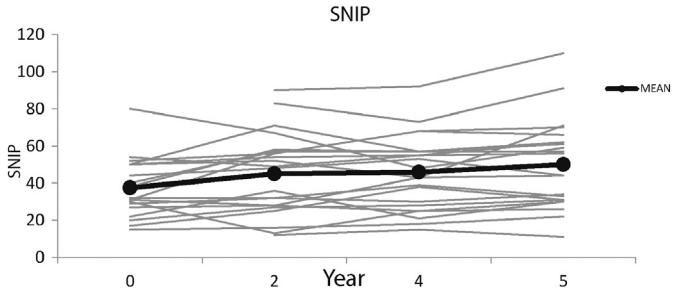

Fig. 3. Sniff Nasal Inspiratory Pressure (SNIP) at baseline and 2, 4 and 5 years of follow-up ($p = 0.2893$).

at 2 years 425 ± 193 U/L, at 4 years 344 ± 179 U/L (95% CI −475 to −87.1), at 5 years 314 ± 171 U/L (95% CI -512.3 to −110.1). Overall there was a significant mean decrease of 49.7% in CK serum levels ($p = 0002$). CK level has shown a gradual decline during ERT in 20 patients, however due to patients' low muscle mass it was difficult to interpret results.

No significant correlations with other factors, such as age, therapy duration or physical activity, were found.

3.6. SF-36 questionnaires

SF-36 physical and SF-36 mental baseline results of our patients group were 15 (35 ± 19) and 3.5 (46.5 95% CI 28.5–64.5) points below the 1998 U.S. general population norms (M = 50, 95% CI 40–60) and did not change on a group or individual basis from baseline to month 60 of ERT ($p = 0.8627$ and $p = 0.8571$, respectively).

3.7. Anti-GAA antibody measurement and safety of the drug

Anti-rh-GAA IgG antibody titre values were available for 20 patients, resulting negative in 4 cases and elevated in 16 (ranging from 1:800 to 1:25,699, median 1:1600). Patient number 13 (Table 1), despite very high antibody titre repeated on two occasions, showed stable predicted % FVC over five years and did not require NIV or mobility aids. None of four patients who had negative antibody titre reported (patient 4, 5, 6 and 16) required NIV. Number 4 patient showed a slight increase in % predicted FVC from 59% at baseline to 64% at 5-year follow-up. Number 5 patient had a decrease in % FVC predicted from 108% at baseline to 98% at 5-year follow-up. In patients number 6 and 16 we noticed a stable %predicted FVC after ERT treatment over 5 years.

3.8. Discontinuation of ERT

Four patients (Number 3, 10, 18 and 22) discontinued ERT at their own request (fatigue, continuous decline despite ERT and incompliance) after 4, 3.5, 4 and 5 years of ERT, respectively. One of these patients (Table 1: number 18) died from pneumonia 18 months post cessation of ERT at the age of 65. His pulmonary function tests had been relatively stable at 0.5 year cessation and at this point he refused further testing. Patient number 22 was restarted on ERT 20 months post cessation. She reported a significant reduction in standing balance and increased falls. Her physical decline was not any faster compared to while she was on ERT. All patients had positive antibodies titre (Table 1). These four patients did not show any improvement in pulmonary function tests, in particularly FVC % predicted, when started on ERT and they remained stable post cessation. No change in ventilator setting was required.

In general alglucosidase alpha was well tolerated. Adverse events on ERT during 5 years were mild if any. We noted two deaths, one described above and another one of a patient at age 71 years who had been on ERT for 7 years (Table 1: patient 2).

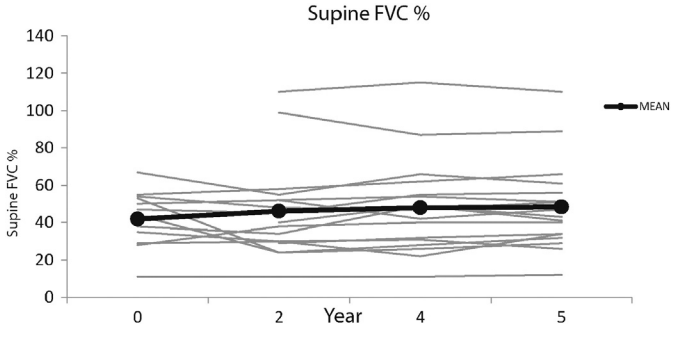

Fig. 2. Supine % FVC at baseline and 2, 4 and 5-year follow-up ($p = 0.8680$).

4. Discussion

This study is the longest observational period in LOPD reported so far (5 years). As it is a small cohort it is not possible to analyse factors that may potentially affect the response to therapy i.e., gene mutation, GAA enzyme levels or severity of the diseases etc.

While a study by van der Ploeg (2010) included walking and non-ventilated patients [6], the patients included in this retrospective observational study had a wide spread of disease spectrum including severely affected patients that required NIV (9/22) or were confined to wheelchairs (3/22) [17, 19]. This more severely afflicted cohort necessitated careful clinical assessment measures and it was not always possible to get all the relevant tests performed. We observed that during ERT the number of patients requiring overnight NIV increased. It is important to note that while most patients continued to remain stable in terms of mobility and respiratory function, we identified some patients who showed no response to therapy and continued to decline in various parameters. It is however difficult to conclude if their decline would have been faster had they not received ERT. Four patients in this cohort have discontinued ERT as the patients felt no benefits of treatment. In a very pragmatic way one can say that patients who do not stabilise or improve on ERT are non-responders to ERT.

There has been published work suggesting that ERT efficacy in advanced patients appeared to be lower than in milder patients [30]. Based on the experience of this cohort even severely affected patients with this condition should be given a trial of therapy to see the response. If they continue to decline then one has to consider the benefit of ERT. Some patients in this cohort who had advanced disease at the baseline remained stable while others with advanced disease showed no response.

It was shown that 10–15 years after diagnosis of LOPD 30–50% of the patients are wheelchair-bound or ventilator dependent [2, 4, 5] Our study confirms this. Furthermore, patients with a wheelchair and/or respiratory support have a shorter life expectancy at any age compared to patients without wheelchair and respiratory support [3]. Age, wheelchair and ventilator dependency, and level of handicap appear to be the main indicators of lower life expectancy [3]. Respiratory impairment is

the most important prognostic factor in patients with LOPD and it is the leading cause of death in Pompe disease [1, 3]. Two reported mortalities in this cohort were due to respiratory complications and both the patients were wheel chair dependent and required NIV.

There is no clear correlation between severity of limb-girdle and respiratory involvement; one-third of adult patients require ventilator support while they are still able to walk [31] similarly to our cohort of patients (27.2%; 6/22). In LOPD some patients develop diaphragmatic weakness, and experience sleep disturbances and/or recurrent respiratory tract infections [31] that were also observed in our study.

In published reports pulmonary function measured by vital capacity declines by a mean of 1.6–4.6% per year [32, 33] and assisted ventilation is commenced 15.1–19.4 years after the first symptoms of disease i.e., skeletal muscle weakness with loss of ambulation, development of respiratory failure and scoliosis from truncal weakness [5, 34]. Additionally, diaphragm weakness has previously been shown to occur in 38% of untreated patients with Pompe disease with a decrease of Vital Capacity (VC) in sitting and supine positions of 0.9 and 1.2% points per year [29], respectively. Monitoring of the pulmonary function is therefore essential in LOPD in order to evaluate the need for mechanical ventilation. Various pulmonary function tests have been used to assess it, FVC % upright and supine being the most commonly used.

Our study demonstrated general stabilization in respiratory function in the cohort irrespectively of their age. In general no significant decline can be considered as a positive response to the therapy. A gradual decline in FVC% predicted in four cases and FVC% supine in two cases was noted. Only one patient (number 17) showed a decline in both pulmonary function tests. We did not document the FVC % improvement in the first year as it was shown beforehand [6, 16, 17, 24] but we documented a noticeable overall upward trend in FVC% supine and SNIP results measured over time (Figs. 2, 3).

We did not find SNIP parameter very useful in monitoring pulmonary function due to varied fluctuation in-between visits most likely due to technical reasons. FVC supine and upright was more informative over a longer period.

The age-related differences in supine FVC % were noticed previously [35] with a significant decline in supine FVC % in the elderly. Patients are more likely to improve if they are younger, independent of artificial ventilation, have a better FVC in upright position, and have less severe muscle weakness at the start of treatment. This suggests that starting ERT early in the disease course may be beneficial [35]. Our study did not confirm these findings however, the low number ($n = 7$) of younger patients (below the age of 40), mean that this result should be interpreted with caution.

Our observations suggested that ERT did not enhance the muscle endurance significantly as shown in the 6MWT results. This might be due to different therapy response of different muscle fibre types and connective tissue involvement, to a different body composition and volume of distribution of enzymes, or to a variable mannose receptor density in different muscles [19]. Individual responses are likely to be affected by some of these parameters, but they cannot explain differences observed at group level.

At present, there has been no clear understanding of the mechanisms involved in the variable clinical presentation and outcome in the LOPD population. Van der Ploeg [2] suggested that the respiratory and musculoskeletal symptoms in LOPD do not emerge until GAA activity remains above 30% of average normal activity measured on skin fibroblasts [2] and not in leucocytes. In our study due to a small cohort we failed to confirm a correlation between the enzyme concentration in leucocytes and the age of diagnosis or onset of symptoms (Table 1). 11 out of 17 patients with known genotype carry the c.-32-13T>G mutation. The correlation between genotype and phenotype is most likely blurred by being a small cohort and other modifying factors described previously [36, 37, 38]. Kroos et al. [38] reported that the presence of at least one mutation allows the production of a protein with some residual activity that leads to the late-onset forms. However, another study showed that no common mutation is associated with <1%GAA activity in LOPD patients [39]. The documented clinical variability in patients with similar genotypes [36] still makes genotype–phenotype correlation an unresolved issue. Modifying genetic factors are expected to influence the final clinical presentation of the disease [38]. Importantly, we also observed that the height of *anti* rh-GAA titre was independent of the GAA genotype. In our group of patients' *anti*-GAA antibody titre was as high as 1:25,699. However, it is difficult to draw conclusions in terms of its impact on ERT response.

Limitations of the study also include the absence of a control (non-treated group), some missing baseline pulmonary function tests, anti rh-GAA antibody titre and 6MWT. In our cohort there were patients who have shown a gradual decline despite the ERT. That possibly explains the increase in patients requiring ventilator support and more wheel chair dependency by the end of five years in spite of overall pulmonary functions and 6MWT staying stable in the cohort.

As shown before, the efficacy of ERT in patients with LOPD who have developed progressive respiratory failure is not entirely clear. Studies that documented some positive findings had limitations including absence of a control group, short duration of follow-up and heterogeneity of patients [18, 24, 25, 40]. Additionally, the use of ventilator support, which itself has a positive effect on pulmonary function in those patients might have altered the patients' clinical status and thus affected the therapy assessment results [41].

Many of those studies followed up patients for 1 year [24], 3 years [17, 18, 42] and 4 years [19]. All publications pointed out the difficulties to obtain consistent outcome measures in LOPD patients due to heterogeneous presentation of the disease and natural course and the high variability of individual response. It is important to determine whether administration of ERT is effective, or whether therapy is only effective for a short duration.

5. Conclusion

ERT treatment with alglucosidase alpha was associated with no significant change in pulmonary function tests and 6MWT at the end of a 5-year therapy. In the light that the natural progression of the conditions shows gradual decline, the overall stability of pulmonary function tests and mobility can be considered as positive response to the therapy. However, some patients have shown a degree of decline in their pulmonary function tests and motor functions as they most likely have not benefited from the treatment and are non-responders. Overall ERT with alglucosidase alpha may delay *de novo* ventilation in LOPD patients. ERT needs further awareness to better enlighten differences in response to ERT and to recognise the non-responders. Further trials are required to look at the confounding factors that will have impact on outcomes in patients receiving ERT.

Conflict of interest

KS received travel grants from Genzyme, Shire and Amicus. No conflict of interest for this publication.

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