Long-term effects of enzyme replacement therapy in an elderly cohort of late-onset Pompe disease

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Received 12 August 2021; received in revised form 6 January 2022; accepted 7 January 2022
Available online xxx

Abstract

Enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase (rhGAA) in late-onset Pompe disease (LOPD) shows beneficial effects in the first years often followed by a decline. We aimed to examine long-term ERT effects in an elderly LOPD cohort. Patients with age at diagnosis/start of ERT $\geq$50 years and ERT duration $\geq$ seven years were included. Outcome parameters were MRC sum-score, 6 Minute Walk Test 6MWT, Quick Motor Function Test QMFT, forced vital capacity FVC sitting/supine, CK levels and rhGAA IgG antibody titers. We retrospectively analysed six patients with a median age at diagnosis/start of ERT of 63 years (range 52–69), and a median ERT duration of eight years (range 7–12). 6MWT improved in 4/6, and 2/6 each showed an improvement or stabilization in muscle strength and FVC supine. In contrast, FVC showed a decline in all patients in a sitting position, and QMFT worsened in 5/6. CK levels decreased in all patients. Antibody titers were not associated with treatment effects. Highest titers were present in best responders who were female, still ambulatory and without ventilatory support at follow-up. ERT effects were very heterogeneous and showed best results in 6MWT, followed by muscle strength in manual testing and FVC supine.

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Keywords: Enzyme replacement therapy; Late-onset; Long-term; Pompe disease; Antibody titers; CK creatine kinase.

1. Introduction

Pompe disease is an autosomal recessive lysosomal glycogen storage disorder in which a deficiency of alpha-1,4 glucosidase (GAA) leads to a preferential accumulation of glycogen in muscle cells. Late-onset Pompe disease (LOPD) manifests in late childhood, adolescence or adulthood with a predominant proximal muscle weakness (limb girdle phenotype) and respiratory dysfunction.

The diagnosis is based on the measurement of GAA activity (dried blood spot test, DBS) or analysis of GAA activity in skeletal muscle tissue, fibroblasts or lymphocytes, skeletal muscle biopsy, muscle MRI, and as gold standard on the molecular genetic analysis. A high variety of pathogenic variants in the GAA gene have been identified in the last few years correlating partly with the clinical phenotype [1], of which the c.32–13T>G variant is the most common one in LOPD [2,3]. Pathogenic GAA variants can be classified as potentially mild, potentially less severe and very severe. Despite various diagnostic methods, the time span between first clinical symptoms and correct diagnosis is often more than six years [4]. This is particularly devastating in the view of an available therapy.

Since 2006, LOPD patients can be treated by enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase (rhGAA, Alglucosidase Alfa). Initial studies had shown evidence of clinical improvement already in the first year of treatment [5–7]. The clinical course in the subsequent years of ERT is variable, but marked overall by stabilization or mild improvement until the second to third year [8–11]. In the recent years, more experience has been

\cite{doi:10.1016/j.nmd.2022.01.001}

Please cite this article as: M. Winkler, C. von Landenberg, K. Kuchenbecker et al., Long-term effects of enzyme replacement therapy in an elderly cohort of late-onset Pompe disease, Neuromuscular Disorders, \cite{doi:10.1016/j.nmd.2022.01.001}
gained with long-term effects of ERT in LOPD patients, although data from elderly patients are particularly rare.

In 2017, a five-year prospective study on the clinical outcome of 102 LOPD patients under ERT was published [12]. The authors showed a benefit in muscle strength, pulmonary function and daily life activities compared to untreated LOPD patients with best ERT results present after two to three years. These results were confirmed by a long-term outcome study after ten years of treatment in 30 adult Pompe patients from the Netherlands and France. Here, 93% profited in the first years, followed by a secondary decline after three to five years [13]. These results were in line with recent long-term data on ERT in LOPD from Spain, Taiwan, Italy and Germany (STIG study) also showing initial improvement followed by a secondary decline [14]. Some smaller LOPD cohorts showed comparable long-term results [2]. Not surprising, the positive effects of ERT refer to overall morbidity but also to survival [15]. It is not yet known whether long-term treatment benefits in LOPD are influenced by the age at onset of ERT. The median ages of patients from first long-term ERT data analyses were 45 years up to 46.5 years by start of ERT [5,7]. Our analyses focus on long-term results of ERT in elderly LOPD patients that were diagnosed in adulthood.

2. Material and methods

We performed a detailed retrospective analysis of elderly LOPD patients diagnosed, treated and regularly examined at our Neuromuscular Diseases Section (University Hospital of Bonn, Germany). Patients had infusion visits every other week and regular biannual to annual follow-up visits.

Inclusion criteria for the selection of the patients were i) age at diagnosis after 50 years; ii) ERT with 20mg/kg Alglucosidase Alfa intravenously every other week; iii) duration of ERT at least seven years; iv) start of ERT at time of diagnosis; v) ability to walk at the beginning of the observation period; and vi) follow-up visits every six to twelve months.

Diagnosis of LOPD was based on the biochemical analysis of the alpha-1,4 glucosidase activity in skeletal muscle tissue, lymphocytes and/or DBS as well as the presence of two heterozygous pathogenic GAA variants in all patients. All GAA variants of our patients had been described before and are listed as pathogenic variants in the respective database (please refer to www.pompevariantdatabase.nl). We categorized our patients’ GAA variants according to their pathogenicity (see for Table 1). Full individual genetic data are available from the authors on demand and could not be listed here for data protection issues.

Six patients qualified for the study (three female/three male). A diagnostic open skeletal muscle biopsy had been performed in five patients.

Based on the retrospective nature of the study on routinely analysed clinical data, the local ethics committee of the University of Bonn decided that no further consent was required (decision number 241/21).

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

2.1. Clinical outcome parameters

We performed the 6 Minute Walk Test (6MWT) and presented the values in absolute numbers and in % predicted to compare patients’ results to normal values (sex, age, weight, height). For the comparative analysis, we decided to focus on 6MWT, % predicted [16].

To analyze the muscle strength we applied manual muscle testing using the Medical Research Council (MRC) grading scale. The following muscle groups were tested to calculate the MRC sum-score (range 0–95): neck flexors, bilateral shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip extensors, hip adductors, hip abductors, knee flexors, and knee extensors. The individual score was subsequently converted to a percentage of the maximum possible score [17].

The Quick Motor Function Test (QMFT) was used as a muscle function test that comprises different items especially designed for Pompe patients [18]. The test consists of 16 items, each with four possible subpoints (range 0–4) and a maximum score of 64. We analyzed the absolute and relative values, the latter given as a percentage of the maximum possible score.

The forced vital capacity (FVC) was measured by using a hand-held spirometer in upright (sitting) and supine position. FVC values were given as absolute numbers and % predicted [19].

2.2. Diagnostic and laboratory parameters

Analysed laboratory parameters were CK levels in blood and IgG antibody titers against rhGAA. The CK levels were measured routinely in our diagnostic laboratory. The rhGAA antibody titers were tested yearly via enzyme-linked immunosorbent assay (ELISA) in Sanofi Genzyme Clinical Speciality Laboratory, One Mountain Road, Framingham, USA until 2019, subsequently in LabCorp, Service provider for Health Care Diagnostic Service, USA; on behalf of Sanofi Genzyme Europe B.V.. IgG antibody titers against rhGAA were categorized into three groups according to the literature [20]: low (0–< 1:1.250), intermediate (1:1.250–<1:31.250), high (≥ 1:31.250).

Open skeletal muscle biopsies in five patients were performed as standard surgical procedures at baseline for diagnostic issues and underwent standard histological analyses (H&E, modified Gomori trichrome, ATPase (pH 4.2, 4.6 and 9.4), reduced nicotinamide adenine dinucleotide (NADH), periodic acid Schiff (PAS), oil-red-O, acid phosphatase, Congo red, myoadenylate deaminase, phosphofructokinase, succinic dehydrogenase (SDH), cytochrome-c-oxidase (COX) and myophosphorylase stainings). PAS, acid phosphatase and H&E stainings were analysed with respect to PAS- and acid phosphatase-positive vacuoles. There was no quantification of
the glycogen load performed in skeletal muscle tissue. The glycogen accumulation was evaluated by three independent observers who were blinded for patients’ identities and had diagnostic experience in assessing histopathological muscle biopsy findings. The patients’ glycogen accumulations were compared to normal muscle biopsy results, and the severeness was graded into (1) normal findings, (2) moderate, and (3) severe glycogen deposits.

2.3. Statistical analysis

According to the advice of the “Institut für Medizinische Biometrie, Informatik und Epidemiologie” of the University Hospital Bonn we did not perform mathematical statistical analyses but analysed our data by descriptive statistical methods due to very small patient numbers. Longitudinal data were thus analyzed by describing each individual’s clinical course.

We present the values of MRC sum-score, QMFT sum-score, 6MWT % predicted and FVC % predicted of each patient in scatter plots. For each score we chose the annual follow-up data, even if more data were evaluated (e.g. biannual) to ensure continuous comparability. The graphical course was categorized in eight groups which described the clinical response over time [13]: 1=improvement throughout the observation, 2=initial improvement followed by stabilization, 3=initial improvement followed by decline, 4=stable throughout the observation, 5=initial stabilization followed by decline, 6=decline throughout the observation, 7=initial decline followed by stabilization or improvement, 8=unclassified. Three independent observers were blinded for the identification of the patients, assessed the clinical data and classified the courses to the eight groups. Observers were clinicians with experience in the treatment of LOPD patients. The grading was defined as unambiguous if all observers came to the same conclusion.

In addition to the description of the clinical course, we compared baseline data (at beginning of ERT) with last outcome parameters (after a median of eight years of treatment). Here three categories were selected for the classification. An (1) improvement was defined as > 2% from baseline, (2) stabilization as +/- 2% from baseline and (3) decline as < 2% below baseline at follow-up.

3. Results

3.1. Cohort characteristics

Six patients were included, three males and three females. All patients had the common c.−32–13T>G variant of the
GAA gene. In all patients ERT was started immediately after diagnosis. The median age at diagnosis and start of ERT was 63 years (range 52–69 years). Median duration between first symptoms and diagnosis was 7.5 years (range 5–26 years). The median ERT duration and time span to follow-up was eight years (range 7–12 years). No patient died and no patient had any infusions-associated reaction (IAR) during the observation period.

At baseline, all patients were able to walk unassisted and showed the typical clinical phenotype with proximal muscle weakness. Two of six patients already needed a non-invasive ventilation at baseline due to obstructive sleep apnea syndrome (pat. 1 and 2). Two further patients had mild respiratory symptoms (pat. 3 and 5), and two had no respiratory limitations at baseline (pat. 4 and 6).

Our patients had no further co-morbidities at the time of ERT initiation which could affect the lung function or the ability to walk.

More detailed patients’ characteristics at baseline are listed in Table 1.

3.2. Diagnostic and laboratory parameters

Data of baseline enzymatic activity of alpha-1,4 glucosidase and CK levels are presented in Table 1, while individual data courses over time are shown in Fig. 1. In brief, all patients showed decreased baseline enzymatic activity of alpha-1,4 glucosidase and mildly to moderately increased CK levels at baseline with a decrease of CK levels under treatment. IgG antibodies against rhGAA were not present in all of our patients at baseline. Five of six patients developed IgG antibodies against rhGAA under ERT, and females showed highest antibody titers in our cohort.

Diagnostic skeletal muscle biopsies were performed in five of six patients (pat. 1–5) before ERT start. All muscle biopsies demonstrated lysosomal glycogen accumulation typical for Pompe disease. All showed PAS- and acid phosphatase-positive vacuoles; two (pat. 1 and 2) to a mild extent, and three (pat. 3–5) showed severe changes, in which pat. 4 had the most severe findings (Fig. 2). There was no muscle biopsy with normal histopathological findings.

3.3. Clinical parameters

3.3.1. Ability to walk

At the beginning of ERT, all patients were able to walk unaided. Over time, four patients (pat. 1, 2, 3, 5) became dependent on walking aids after one, four, three and four years of ERT, respectively. Pat. 1, 2 and 5 used a walker, whereas pat. 3 preferred to use canes. Two patients (pat. 2 and 5) subsequently became wheelchair-dependent eight and 12 years after start of ERT, one of them (pat. 2) due to a hypertensive pontine hemorrhage. Two females were still able to walk unassisted at the end of the clinical observation period (pat. 4 and 6).

3.3.2. 6 Minute walk test (6MWT, absolute numbers and % predicted)

Initially, patients reached between 60 m and 475 m; median 281 m (% predicted: 13% to 98%; median 49%). By last follow up (after eight to 12 years of ERT) patients walked 0–509 m in 6MWT; median 307 m (0–115%; median 58.5%). Individual changes in 6MWT (% predicted) from baseline versus last measured outcome were +5%, +9.7%, +6%, and +17% (pat. 1, 3, 4, and 6 after 7, 12, 8, and 7 years of ERT, respectively). Individual courses in % predicted are imaged in Fig. 1. Two patients (pat. 2 and 5) showed a decline over time with a final loss of ambulation. In detail, pat. 2 lost ambulation at the age of 68 years due to a pontine hemorrhage of unclear cause resulting in a moderate paraparesis of the legs 8 years after ERT initiation. Pat. 5 refused to perform the 6MWT because she was afraid of falling, and finally lost ambulation at the age of 80 years, 12 years after ERT start. Seven years after ERT start, she temporarily felt more safe while walking and could perform the 6MWT, which might be due to the initiation of NIV that year. However, this assumed relation is hypothetic, and again 6MWT data could not be collected in the subsequent five years. Two patients (pat. 4 and 6) showed an improvement, one patient (pat. 1) a decline followed by improvement, and one patient (pat. 3) an improvement followed by decline.

3.3.3. MRC sum-score (% of maximum)

The muscle strength of all six patients was evaluated annually by MRC sum-scores. At the start of ERT the score ranged from 68.4% to 87.4%; median 82.15%. By last follow up (median after eight years of ERT) the patients reached scores between 68.4% and 83.2%; median 71.6%. Individual changes in MRC sum-score (% of maximum) from baseline versus last measured outcome were −4.2%, 0%, −4.3%, −11.6%, −4.2%, and +4.1% (pat. 1, 2, 3, 4, 5, 6 after 7, 9, 12, 8, 12, and 7 years of ERT, respectively). The courses over time were individually different and are shown in detail in Fig. 1. Two patients (pat. 2 and 5) are categorized as stabilization over time, one patient (pat. 1) as stabilization followed by decline; one patient (pat. 3) as improvement followed by decline, one patient (pat. 4) as decline followed by stabilization and further one patient (pat. 6) as improvement followed by stabilization. No patient had a continuous decline.

3.3.4. QMFT sum-score (% of maximum)

The QMFT was performed by all patients. However, the test was not applied in all patients on an annual base. QMFT was first applied in two patients after three and five years of ERT, respectively (pat. 2 and 5). In four of six patients, the score was evaluated before ERT start with values ranging from 43.8% to 68.8%; median 57.1%. Last outcome in these four patients (median after 7.5 years) showed values between 12.5% and 70.3%; median 40.6%. Individual changes in QMFT (% of maximum) from baseline versus last measured outcome were −27.7%, −31.3%, −12.5%, −15.7%, −12.5%, and +7.8% (pat. 1, 2, 3, 4, 5, 6 after 7, 9, 12, 8, 12,
Fig. 1. Response to ERT over time; clinical course of A: 6MWT, FVC sitting and FVC supine in % predicted (values see left y-axis; black) and rhGAA IgG antibody titers (AB IgG, see right y-axis, log scale; red). None of our patients had rhGAA antibodies at baseline. Pat. 4 did not develop rhGAA antibodies in the first year of treatment. In pat. 2, there were no antibodies detectable over 9 years of observation. In pat. 4, antibody titers were not examined in year 8. rhGAA IgG antibody titers of 0 are not shown. B: MRC sum-score and QMFT in % of maximum (values see left y-axis; black) and CK levels in U/l (see right y-axis; blue). C: color-matched analyzes of clinical courses under ERT over a median of 8 years.
and 7 years of ERT, respectively). The score course was analysed in all six patients (for details see Fig. 1). Four of six patients showed a decline, one patient (pat. 5) a stabilization followed by decline and one patient (pat. 6) an improvement.

3.3.5. FVC sitting and supine (% predicted)

At the time of diagnosis and before initiation of ERT, two of six patients (pat. 1 and 2) needed a non-invasive ventilation (NIV). They both had been diagnosed with obstructive sleep apnea syndrome. Over time, two further patients required a
NIV therapy (pat. 3 and 5). Pat. 3 was diagnosed with a sleep-related breathing disorder due to diaphragmatic paresis four months after ERT initiation at the age of 66 years, and pat. 5 was in need of NIV during the night six years after ERT start at the age of 74 years. Pat. 4 and 6 had a sufficient respiratory function until the end of the study observation period (8- and 7-years follow-up) with no need of NIV.

Before start of ERT all patients showed a FVC sitting (% predicted) between 53.6% and 95.1%; median 75.2%. After a median of eight years of ERT the values ranged from 34.6% to 66.3%; median 60.0%. Individual changes in FVC sitting (% predicted) from baseline to the end of the observation period were $-3.2\%$, $-6.4\%$, $-11.6\%$, $-28.8\%$, $-51.6\%$, and $-17.7\%$ (pat. 1, 2, 3, 4, 5, 6 after 7, 9, 12, 8, 12, and 7 years of ERT, respectively). Three of six patients showed a decline over the whole observation time. One patient (pat. 2) had an initial improvement followed by decline, and two patients (pat. 3 and 6) a stabilization followed by decline. Detailed courses are imaged in Fig. 1.

At baseline before start of ERT FVC supine (% predicted) of all six patients ranged from 31.2% to 83.1%; median 55.6%. After a median ERT duration of eight years, the median FVC supine declined to 47.5% (range 33% to 59.3%). Individual changes in FVC supine (% predicted) from baseline versus last measured outcome were $-5.4\%$, $+1.8\%$, $-3.4\%$, $-21.5\%$, $-48.2\%$, and $+18.3\%$ (pat. 1, 2, 3, 4, 5, 6 after 7, 9, 12, 8, 12, and 7 years of ERT, respectively). Similar to FVC sitting, the courses of FVC supine were heterogeneous, however not exactly consistent with results in the sitting position (see Fig. 1). In analogy to FVC sitting results, three patients showed a decline over time (pat. 1, 4 and 5) and one further patient (pat. 3) had a stabilization followed by decline. In contrast to FVC sitting results, one patient (pat. 2) showed a stabilization in supine position, while an improvement followed by decline was observed in FVC sitting, and one further patient (pat. 6) experienced an improvement in FVC supine, while showing a stabilization followed by a decline in FVC sitting.

In five of our six patients, the comparison of FVC sitting and supine (% predicted) at baseline versus last follow-up showed that the differences between both parameters decreased over time, meaning that the curves converged. In detail, the differences accounted for $-8.2\%$, $-8.2\%$, $-7.3\%$, $-3.1\%$ and $-36\%$ in pat. 2, 3, 4, 5 and 6. Only in pat. 1, both parameters diverged over time with an increase of the difference between FVC sitting and supine of $+2.2\%$.

There was no association between the diagnostic and laboratory parameters (CK levels, baseline enzyme activity of alpha-1,4-glucosidase, IgG antibody titers against rhAGG or histopathological findings) and the clinical outcome (6MWT, MRC sum-score, QMFT and FVC sitting/supine).

Two patients (pat. 4 and 6) stand out as very good responders, due to the best clinical results under treatment after 8 and 7 years of ERT, respectively (best values in 6MWT, FVC sitting and supine without NIV and one of the three best values in MRC sum-score and QMFT). Intriguingly, both patients showed the highest IgG antibody titers against rhAGG after three and four years of ERT, and pat. 4 had demonstrated the most severe histopathological findings in muscle biopsy.

Disconcordant courses were also present in individual clinical outcome parameters. When comparing the individual clinical parameters, the MRC sum-score and the QMFT showed similar courses over time, but none of them showed complete concordance. The same applies to the comparison of the MRC sum-score and 6MWT with one exception (pat. 3), while only pat. 6 showed a well matched course of QMFT and 6MWT. Moreover, when comparing FVC and QMFT, MRC sum-score and 6MWT, only partially identical courses over time were displayed. FVC sitting and supine courses were completely consistent in four of six patients. The other two patients (pat. 2 and 6) showed a better response to ERT in FVC supine than FVC sitting, meaning stabilization (FVC supine) versus improvement followed by decline (FVC sitting) and improvement versus stabilization followed by decline.

4. Discussion

ERT showed a benefit of variable extent in our six older adult LOPD patients over a median of eight treatment years ranging up to 12 years. Four of six patients improved in 6MWT over time. The two patients without improvement in 6MWT became wheelchair-dependent (pat. 2 and 5), in which pat. 2 lost the ability to walk due to a pontine hemorrhage. Two of six of our patients showed a stabilization in muscle strength and one of six patients had an improvement in QMFT over time. However, the remaining five of six patients decreased in their motor functionality, which must be interpreted as a loss of abilities in their activities of daily living. Interestingly, the FVC sitting showed a decline in all patients, while a stabilization/improvement could be reached in two of six patients in the supine position. Unfortunately, age-matched comparative long-term natural history data on motor and respiratory functions is lacking in LOPD, especially in the elderly.

QMFT and MRC sum-score are useful tests in LOPD but not sensitive enough to detect subtle differences

To our knowledge, this is the first long-term evaluation of QMFT in adult LOPD patients which is a helpful tool to record the clinical outcome under ERT, mainly because the QMFT maps everyday functions and therefore reflects the functional impairment relevant in daily life. QMFT was used in a long-term observation of patients with childhood-onset Pompe disease, in which an improvement could be detected, while the same cohort showed a stabilization in MRC and an improvement in 6MWT [21]. We found corresponding results between QMFT and MRC sum-score, which is a more established testing tool to evaluate the effects of ERT in LOPD [8,10,12,13]. Physiologically, functional muscle strength decreases with increasing age. Age-adjusted reference values are often available to compensate for this effect in test tools. These are unfortunately missing for both, MRC sum-score and QMFT. This lack of age-related
reference values could contribute to the decline seen in both tests in our patients. Moreover, the clinical course curve of both tests (see Fig. 1) runs without relevant fluctuations. This is surprising in view of the otherwise individual and variable courses. It is possible that at least the MRC sum-score in LOPD is not sensitive enough to detect subtle differences in muscle function. Despite the worsening of skeletal muscle function over time, our elderly patients performed quite well in manual muscle testing (MRC sum-score, 19 muscle groups tested) when comparative data in untreated LOPD over an average of 1.6 years is considered that showed mean declines of approximately 1.3% per year (MRC sum-score, 25 muscles groups tested [22]). However, untreated control data is highly limited.

Best results were obtained in 6MWT and FVC supine, which could be due to a better effect of ERT on the diaphragm

In our cohort, the best responses in general were detected in the 6MWT and FVC. The response in 6MWT is consistent with the long-time data over nine years of treatment in six LOPD patients published in the STIG study [14]. By comparison, untreated LOPD patients do not show any 6MWT improvement over time. Instead, an annual decline of 3 m could be demonstrated over an observation period of 18 months [23,24]. The course in FVC sitting published in the STIG study was also similar to our cohort with best responses in patients with a high baseline level. As demonstrated in ERT treatment studies on LOPD finally leading to approval of the therapy, ERT is able to achieve an improved outcome in motor and respiratory functions of LOPD patients when compared to untreated patients and natural history data. Natural history studies on LOPD showed mean annual declines of 1.7% and 4.6% in FVC upright (%) predicted), and these findings were consistent with a 2.2% decline that occurred over 78 weeks in the placebo group of a randomized study of Alglucosidase Alfa in LOPD [23,25,26]. FVC supine (%) predicted) had shown a mean decline of 1.2% and 1.3% per year, however the observation time was only 1.6 years on average [22,27]. In total, currently available data on untreated adults and children demonstrate an annual decline of FVC between 1 and 5.5% [22,23,25–28]. Unfortunately, there is no long-term data on untreated elderly adult LOPD patients over decades which impedes a direct comparison of our results to historic data. Nevertheless, our patients’ respiratory performance over time appears to be favorable at least in two out of 6 patients irrespective of the lack of comparative data. In our cohort, the effects of ERT on respiratory function however varied between the measurement of FVC sitting and supine. While all patients showed a decreased FVC sitting over time, FVC supine improved or stabilized in those two out of 6 patients. Moreover, in all but one patient the differences between FVC sitting and supine decreased over time while the respective curves converged. One hypothesis explaining this intriguing observation could be a better effect of ERT on the diaphragm muscles. By use of dynamic 3D MRI scan the most severe diaphragm weakness could be shown in Pompe patients with the worst respiratory function [29]. Thus, the diaphragm is most relevant for the pulmonary function in LOPD, and its function is well reflected by testing respiratory parameters in the supine position.

Unfortunately, no FVC supine measurements were performed in the STIG cohort, therefore, future studies will have to tell whether the better response in FVC supine compared to FVC sitting is a reproducible observation in LOPD under ERT [14].

6MWT and FVC tests are simply to apply, but each of them only represents a specific area of muscle function. However, Pompe disease is a multisystemic disease, that affects various muscle groups and organ systems, even though the disease is particularly pronounced in proximal skeletal muscles. The function of arm/shoulder muscles e.g. is not taken into account in the 6MWT or FVC testing, but a respective affection can represent a significant limitation in everyday life for the patient. Therefore, it is important to evaluate the effect of ERT according to various tests not only including the 6MWT and FVC, but also the MRC sum-score and, above all, the QMFT to obtain a holistic impression of the patients presentation, even if all tests show specific limitations.

Neither the severity of histopathological changes nor enzyme activity at baseline could be used as predictive markers for ERT response

Corresponding to the literature, > 50% of our patients showed an initial benefit in the first years of ERT followed by a variable response or secondary decline over the next years. The reasons for this effect are not fully understood and subject of current discussions. Kupers et al., 2017, discussed a multifactorial process behind this clinical course [12]. The idea is that the elimination of glycogen from patient muscle, which is a mechanic barrier in muscle structure, leads to a smoother muscle function due to a better structure [30]. After the first years of treatment the known increase in autophagy and a decreased number of satellite cells in combination with the older age of the patients could dominate the ongoing processes, and a decrease in benefit might be the consequence [12,31,32]. Interestingly, we could not find an association between the baseline histopathological findings and the clinical outcome.

In our patients, we performed diagnostic muscle biopsies before treatment; therefore, no statement can be made about the possible histopathological modifications under treatment. From the clinical point of view however, the good/stable responses in elderly LOPD stress the importance of immediate ERT initiation, even if the skeletal muscle tissue is already severely damaged.

It could be assumed that enzyme activity in muscle tissue with most severe histopathological findings is also the lowest. However, this could not be shown in our small cohort. Besides, one might have expected that a lower enzyme activity at baseline is associated with a worse clinical outcome. Interestingly, this was not confirmed either. Therefore, neither the muscle biopsy findings nor the enzyme activity can be
used to assess the clinical severity of the disease or to predict the clinical response to ERT in elderly LOPD. Thus, clinical parameters that have so far been used to evaluate the indication (e.g. start/stop criteria) and course of the therapy seem to be more meaningful.

**Females showed better response to ERT than males**

In analogy to the observation of van der Ploeg et al., 2010 [5] in LOPD and Meijen et al., 2017 [21] in childhood-onset Pompe disease (median age at onset 11.9 years) our female patients showed a better outcome of ERT than the males. In general, it is not yet clear whether this is a specific finding or a random gender distribution effect in the respective cohorts.

Out of our six patients, there were two females with a notably good response (pat. 4 and 6). Pat. 4 showed an especially good motor response despite the most severe histopathological findings. She had an improvement in 6MWT and was not dependent on walking aids after nine years of ERT. Also, in MRC sum-score a stabilization after initial decline could be achieved, though the QMFT showed a decline. While there was a continuous decline in FVC sitting and supine, she had the best baseline values at start and some of the best without NIV after 8 years of ERT in absolute terms. The other good responder is pat. 6. She improved in 6MWT, QMFT and initially in MRC sum-score with a secondary stabilization. She was also able to walk without aids after 7 years of ERT. The respiratory function showed an initial increase in FVC sitting with a secondary decline, while she improved over time in FVC supine and also did not require NIV. Both patients had mildly increased CK levels at the beginning of ERT (618 U/l and 277 U/l) and normal values after 7 to 8 years. These both notably good responders in our cohort had the highest antibody titers with intermediate and high values and the lowest weight-adapted biweekly dose of ERT, but there was no association between the temporal course of antibody values and clinical outcome.

**Alglucosidase ALFA antibody titers, GAA variant and CK levels had no impact on response to ERT**

We could not detect an influence of the Alglucosidase Alfa antibody titers on clinical outcome parameters, as it is known from infantile-onset Pompe disease where high values of IgG antibody titers against rhGAA may lead to a worse response and to an immune reaction to treatment [33,34]. De Vries et al. 2017 analysed the rhGAA IgG antibody titers in LOPD patients (median age at start of ERT 52 years) in response to treatment and the number of IAR [35]. They concluded that patients with higher antibody titers had slightly more IAR, but there was no correlation between antibody titers and ERT effects. The absence of neutralization of rhGAA by IgG antibodies could be explained with decreased antibody titers over time under ERT and the presence of non-inhibitory IgGs [36]. In our cohort, the previously described reduction of rhGAA antibody titers over time and the missing correlation to ERT effects could also be shown, but it is nevertheless noticeable that the highest titers in our cohort were found in the best responders. This is one further indicator for the missing influence of IgG antibody titers against rhGAA to treatment response in adult LOPD. All our patients received an ERT dosage of 20 mg/kg body weight every other week. The weight-adapted dosage given every other week did not show an association with the antibody titers; however, the two best responders received the lowest biweekly dose. Intriguingly, one male did not develop antibodies over nine years of observation.

The common GAA variant c.32–13T>G and one potentially less severe or very severe pathogenic variant in the second allele were detected in all patients with no association between genotype and clinical data. Similarly, there was no association between baseline enzyme activity and histopathological findings present before treatment with clinical outcome under ERT.

CK levels started with mildly increased values and decreased until the end of observation to normal or nearly normal levels in all patients. This could however be explained by a reduction of muscle mass in the clinical course and is also reported in other adult muscle diseases [37].

**Our elderly cohort showed a similar response to ert as younger published cohorts**

The age at symptom onset in our cohort (median 49 years) and the age at start of ERT (median 63 years) were higher than in previously reported cohorts (e.g. age at start of ERT median 49 years [13] or 52 years [12] and age at symptom onset median 33 years [12]). Interestingly, despite the higher ages and longer disease courses ERT responses were comparable to those previously described in younger patient cohorts. From this, it can be concluded that the treatment in older LOPD patients is equally indicated and justified.

5. Conclusion

This retrospective observational study showed that long-term ERT in our small cohort of older adult LOPD patients led to highly variable but satisfactory outcomes in motor and respiratory function especially in the supine position reflecting diaphragm muscle strength. While the 6MWT improved in the majority of our patients and muscle strength stabilized or improved in two of six patients, motor function tests worsened in all but one patient. Also, respiratory function tests showed a decline in all patients in a sitting position, whereas equal tests in a supine position resulted in an improvement in two patients over time.

Treatment effects were not influenced by the presence of IgG rhGAA antibodies, GAA genotype, baseline skeletal muscle biopsy findings or enzyme activity. These data compared to previously published (nearly 10 years) younger ERT-treated LOPD patients indicate a justification and likely benefit of ERT also in the elderly. Our data underline that the various diagnostic parameters (enzyme activity at baseline,
Declaration of Competing Interest

Katharina Kuchenbecker does not have any conflict of interest to declare. Maren Winkler and Christina von Landenberg received travel funds from Sanofi Genzyme. Maren Winkler, Christina von Landenberg, and Jens Reimann serve as co-investigators for Amicus Therapeutics. Cornelia Kornblum received travel funds, speaker honoraria and served as paid advisory board member and/or principal investigator for Sanofi Genzyme and Amicus Therapeutics.

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