REVIEW

Biomarkers and Outcomes in Late-onset Pompe Disease in the Enzyme-replacement Therapy Era

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Abstract: Late-onset Pompe disease (LOPD) is a rare myopathy, genetically inherited in an autosomal recessive pattern, that affects intramuscular glycogen metabolism, due to a deficit in the acid alpha-glucosidase enzyme. The main clinical manifestations encompass proximal muscle motor deficit, as well as diaphragm weakness, which consequently leads to a restrictive respiratory syndrome. Since the approval of enzyme replacement therapy (ERT), the disease prognosis has been markedly improved. Treatment efficiency is usually monitored using generic biomarkers for neuromuscular disease, which lack specificity and are poorly adapted to this particular condition's evolution. This review aims to provide an overview of the various biomarkers that have been used as outcomes in LOPD research, particularly in studies investigating ERT efficiency. Randomized controlled trials (RCTs) generally rely on outcomes such as forced vital capacity (FVC) and the 6-minute walking test (6MWT). Other emerging biomarkers such as urinary glucose tetrasaccharide (Glc4) and microRNAs have been taken into consideration for assessing disease evolution. Additionally, whole-body muscle magnetic resonance imaging (MRI) and diaphragm ultrasound represent emerging tools for disease follow-up. The anti-drug antibodies (anti-rhGAA) developed with the introduction of ERT are of particular interest, given the lack of consensus regarding their impact on treatment efficacy. Despite substantial progress in biomarker research, challenges persist in identifying specific and sensitive outcomes that correlate with clinical outcomes. This review aims to investigate the existing biomarkers and to look into emerging biomarkers in LOPD.

Keywords*: late-onset Pompe disease, biomarkers, metabolic disease, myopathy.*

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INTRODUCTION

Pompe disease is a metabolic myopathy caused by a deficit of glycogen metabolization within cells. It is part of the group of lysosomal storage diseases, as the underlying pathological mechanism is glycogen accumulation primarily in muscle tissue. The genetic mutation responsible for the GAA deficiency is located on chromosome 17, while the most frequent mutation in Caucasian patients is c.-32-13T>G [1].

It is crucial to retain the multi-system involvement of LOPD, as it usually requires an interdisciplinary team of physicians for both diagnosis and monitoring. Besides the skeletal muscle and respiratory impairment, there are several other organ impairments, such as vascular malformations, notably brain aneurysms, cardiac arrhythmias, musculoskeletal changes, osteoporosis, and gastrointestinal and urinary tract alterations [2]. Studies looking into Pompe's pathological aspects showed impairment of smooth muscle alongside skeletal muscle. This was eventually linked to a deterioration of vessel walls, including life-threatening basilar artery and ascending aorta aneurysms, dysphagia, dysphonia, and involvement of the genitourinary tract, as well as the gastrointestinal tract [3].

Before 2006, LOPD was an incurable disease whose treatment was merely palliative. Developing a recombinant human alpha-glucosidase enzymatic replacement therapy (ERT) has become the first specific treatment for this illness. Multiple therapeutic options have been proposed since, and so the need for novel biomarkers to assess the progression of the disease has increased.

Irrespective of the patient's status regarding the treatment or lack thereof, there is a standardized approach for diagnosis and monitoring. A minimum set of assessments for LOPD includes a clinical examination, functional motor studies such as the 6-minute walking test (6MWT) and segmental force testing, followed by an evaluation of respiratory function using spirometry, blood work that indicates muscle loss through elevated creatine kinase (CK) levels, electromyography (EMG), and a general screening for associated organ impairment. Patients undergoing ERT are also tested for anti-drug antibodies (ADA) and urine levels of glucotetrasaccharide (Glc4), a glycogen metabolite. Whole-body muscle MRI (magnetic resonance imaging) is also useful for diagnosis and as an indicator for ERT timing.

Natural history studies of disease progression, as well as randomized controlled studies (RCT) introducing new therapies, have been reporting as primary objectives the motor function using 6MWT and respiratory function by studying the FVC in both seated and supine positions. Some of the real-life published data has also stressed the importance of CK and Glc4 levels, genotype-related complications and fatty infiltration in muscle MRI. The state-of-the-art in monitoring LOPD patients is still lacking consensus on the follow-up parameters that best translate the disease progression in the ERT era.

MOTOR FUNCTION AND CLINICAL BIOMARKERS

Perhaps the easiest tool to start with is the neurological examination and its derived scores. A minimum examination usually includes segmental force testing using MRC scales. However, it is noteworthy that the segmental force testing has essentially little consistency, as it often depends on the examiner. Moreover, previous studies have shown a lack of progression in the MRC sum score (MRC-SS), while the 6MWT was perceived as a more reliable endpoint [4]. Manual muscle testing (MMT) was widely used as a segmental force indicator, and it is based on a 6-point scale (0-5). Gradually, instrumented evaluations using mostly dynamometers were correlated with the MRC grading. However, this remains a poor assessment of the loss of functionality in Pompe disease due to the aforementioned reasons [5,6].

Adult LOPD patients face locomotor disability in more than 80% of cases, having a reduced walking distance by 3-fold compared to healthy controls. Less than 20% of them walk without assistance as the disease progresses [5,7]. The factors that impair functionality are the duration of the disease, age, and ventilation assistance [8]. The French cohort of Pompe patients reported that the loss of mobility results in

30-50% of subjects requiring wheelchairs 10 to 15 years after diagnosis [9].
Given the poor reliability of MMT, the majority of studies have primarily focused on walking performance, with the 6-minute walk test being the most frequently used evaluation. Multiple studies indicated that the distance measured in the 6MWT was between the range of 49.1% to 71.3% of the predicted value. A study conducted on 197 LOPD patients reported a mean walking distance of 58.4% of predicted values [10]. Nevertheless, the performance on this test is directly influenced by cardiorespiratory capacity, and so its specificity is not satisfactory. Hence, the interpretation of the 6MWT is rather difficult, especially if we consider the "floor" effect, that being the inability of this test to measure functionality for non-ambulatory patients. further complicating the clinical interpretation of 6MWT results [11,12].
Functional evaluation, including the 6MWT, is doubtless a better approach for monitoring a slowly progressive myopathy. Other proposed tests included quantitative isometric strength measurement using a dynamometer, hand grip strength, and the 10-meter walking test [4]. Locomotion characteristics such as hip abductor strength and duration of single support were shown to be accurate determinants for locomotor performance, stability, and falls in LOPD [13].

Walking assistance and wheelchair reliance significantly change the patient's approach to everyday life activities and reduce quality of life [14]. Gait impairment is closely linked to motor function, leading to a higher percentage of patients needing assistance as the disease progresses. Published literature indicates that patients who received ERT had a 64% lower likelihood of becoming dependent on a wheelchair [15].

PULMONARY FUNCTION TESTS

In a natural history study, it was reported that more than 70% of patients diagnosed with LOPD develop chronic respiratory failure, even though the average annual decline of the FVC is around 1.5% [16]. However, diaphragmatic involvement and disease evolution are exceedingly variable in both treated and untreated patients, making it essential to assess and monitor respiratory muscle strength on an individual basis. Symptoms during the day that may indicate weakness in respiratory muscles include experiencing difficulty breathing during both physical activity and at rest and generalized fatigability. During sleep, patients complain of difficulty breathing while lying flat (orthopnea), frequent disruptions in sleep, waking up with headaches, feeling excessively sleepy during the day, and experiencing persistent tiredness. Alveolar hypoventilation tends to happen more often when lying on the back and during REM sleep, when muscle activity naturally decreases. When coughing is impaired, patients are at a higher risk of inhaling food or liquids into the lungs, leading to complications like mucus blockage and respiratory infections. Recurrent respiratory infections or slow recovery from such illnesses might indicate weakness in respiratory muscles even before clinically evident respiratory symptoms emerge [17].

The restrictive respiratory syndrome is determined by the diaphragm deficit, and so it is crucial to correctly perform the pulmonary functional tests by measuring the FVC in both seated and supine positions. A postural difference of more than 25% in FVC is pathognomonic for diagnosing the diaphragmatic motor deficit [18]. Besides FVC, pulmonary functional tests allow the earlier detection of inspiratory muscles deficit by measuring a reduced maximum inspiratory pressure and also a decreased sniff nasal inspiratory pressure. Another useful biomarker is cough effectiveness, as it correlates with pulmonary infections risk, and it can be measured through peak cough flow [19].

A sleep breathing disorder is often found in LOPD patients and is crucial for determining the necessity of non-invasive ventilation (NIV). Polysomnography (PSG)

detects respiratory events and stages of sleep. It, combined with CO_2 monitoring, offers the best sensitivity for identifying sleep breathing disorders, enabling earlier diagnosis of nocturnal hypoventilation by recognizing hypercapnia associated with REM sleep. Consequently, a baseline sleep evaluation at diagnosis should encompass a PSG [17].

Early introduction of NIV yields a more favorable respiratory outcome. The non-invasive ventilation criteria involve having symptoms of a sleep breathing disorder or notable inspiratory muscle weakness along with at least one of the following: daytime hypercapnia (defined as the arterial blood $PaCO_2 \geq 45$ mmHg), nocturnal hypercapnia (defined as the arterial blood $PaCO_2/tcCO_2 > 50$ mmHg), at least five consecutive minutes of nocturnal oxygen desaturation (SaO2) < 90%, FVC < 50% predicted, or MIP < 60 cm H2O. Prolonged invasive ventilation through tracheostomy is indicated if NIV is not suitable, if there's no improvement in gas exchange with NIV, or if access to the airway is restricted.

Another important element of respiratory care for LOPD patients is ensuring effective secretion clearance and cough assistance. Inspiratory and respiratory muscle training improves endurance, strength, and respiratory function.

LABORATORY TESTS AND BIOLOGICAL BIOMARKERS

Blood work that usually reveals pathological titers in LOPD includes CK, hepatic enzymes, and cardiac troponin. CK titer is mildly increased, but it can also be within the normal range. Elevation of plasma cardiac troponin T levels, as well as elevated alanine aminotransferase and aspartate aminotransferase levels, are found, however, without specificity in disease monitoring [19].

A promising biomarker is represented by the urinary Glc4, which was investigated as an objective in evaluating the effectiveness of ERT [20]. Higher levels of Glc4 appear to be present in individuals with infantile-onset Pompe disease (IOPD), pointing to a possible link with the age at which symptoms first appear. It has also been suggested that Glc4 dosage could serve as a confirmatory diagnostic test following a positive dried blood spot screening. While elevated levels of Glc4 may be diagnostically useful, it's crucial to understand that these increases are not exclusive to Pompe disease. Elevated titers are also seen in urinary tract infections, certain neoplasms, pancreatitis, or rhabdomyolysis. This lack of specificity requires careful interpretation of results within the broader clinical context [21].

There is an ongoing debate regarding the reference intervals for Glc4, given that some patients might have normal Glc4 excretion earlier in the disease progression. Glc4 was initially applied to monitor the response to treatment; however, the lack of specificity rendered non-satisfactory results [20]. On the other hand, emerging biomarkers such as microRNAs have been proposed for monitoring ERT efficiency [22]. To date, there is no large-scale consensus regarding the optimal blood biomarker to assess disease progression.

Muscle biopsy may be performed for the purpose of diagnosis, although with the recent availability of NGS panels, genetic studies have become increasingly more used for diagnosis. Pompe disease is characterized by intramuscular glycogen deposits, revealed by vacuolar myopathy at the muscle biopsy. Additionally, there is an increased acid phosphatase reactivity. Previously published research investigated the interest in using muscle biopsy for monitoring disease progression. Early treatment initiation has been associated with a reduction in vacuolated fibers and a decrease in PAS-positive material observed in muscle biopsies [23,24].

MUSCLE MRI

Muscle MRI shows promise as a diagnostic tool and follow-up for LOPD. The most common MRI pattern involves consistent axial muscle engagement, particularly affecting muscles that extend the trunk. These anomalies may manifest even before symptoms appear, potentially prompting clinical suspicion [25,26]. The particular correlation between paravertebral muscle atrophy and abdominal muscle weakness is specific to Pompe disease. Although paravertebral muscle deficit is seen in other inherited and acquired myopathies, axial weakness is a key component in PD. Muscle MRI identifies more subtle modifications earlier on in the evolution. One of the first muscles to be affected is the adductor magnus muscle, followed by the posterior thigh muscles. As PD progresses, studies reported knee extensor group involvement, frequently sparing the rectus femoris, sartorius, and gracilis muscles, thus revealing an impairment pattern that helps differentiate it from muscular dystrophies. Abnormalities in diaphragmatic MRI can also be identified, despite normal pulmonary function tests [27].

MRI studies might be helpful in determining the optimal timing to initiate treatment, especially since fat replacement within the muscle tissue can precede clinical weakness. Seemingly, mild muscle involvement yields a more favorable response to ERT. One study reported that the psoas muscle fat fraction, determined by MRI, has stabilized as a consequence of ERT. Nevertheless, more advanced MRI sequences like water/fat (Dixon) were in contradiction with this initial research and reported an increased fat fraction despite treatment [28,29].

CONCLUSIONS

In spite of ongoing efforts to increase specificity and sensibility for outcomes measuring Pompe disease evolution and ERT efficiency, further refinement of outcome measures is warranted. Ambulation and functional tests that are meant to assess locomotion are limited in their performances; however, the 6MWT is still preferred as a primary endpoint in RCTs and observational studies. Pulmonary functional tests, especially FVC, are essential for understanding respiratory impairment in these patients, directly correlated to diaphragm deficit. Nevertheless, it is less useful for patients under invasive ventilation and severe respiratory failure. There are ongoing studies that look at blood and urine biomarkers, as well as muscle MRI; however, the accessibility of these techniques is currently limited.

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