ISSN 2532-1900

ACTA MYOLOGICA

(Myopathies, Cardiomyopathies and Neuromyopathies)

Vol. XXXVII - December 2018

Official Journal of
Mediterranean Society of Myology
and
Associazione Italiana di Miologia

Founders: Giovanni Nigro and Lucia Ines Comi

Three-monthly

G. CONTE ACADEMY - NAPOLI

PACINI EDITORE MEDICINA

Established in 1982 as Cardiomyology

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Acta Myologica publishes 4 issues per year in March, June, September, December. The Journal is available in OPEN ACCESS at: www.actamyologica.it

Acta Myologica is cited in Index Medicus/MEDLINE, Medicine, Excerpta Medica Database (EMBASE), Index Copernicus and monitored for coverage in Chemical Abstracts Service. The Journal is available on PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/journals/1221/).

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Editor in Chief: Luisa Politano

Tribunal Authorization, Napoli N. 3827, January 10, 1989 - Journal registered at “Registro pubblico degli Operatori della Comunicazione” (Pacini Editore srl registration n. 6269 - 29/8/2001).

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Publisher

Pacini Editore

Via A. Gherardesca - 56121 Pisa, Italy

Published by Pacini Editore Srl, Pisa, Italy, December 2018

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ORIGINAL ARTICLES

The role of rehabilitation in the management of late-onset Pompe disease: a narrative review of the level of evidence

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Late-onset Pompe disease (LOPD) is characterized by progressive muscle weakness, respiratory muscle dysfunction, and minor cardiac involvement. Although in LOPD, as in other neuromuscular diseases, controlled low impact sub-maximal aerobic exercise and functional ability exercise can improve general functioning and quality of life, as well as respiratory rehabilitation, the bulk of evidence on that is weak and guidelines are lacking. To date, there is no specific focus on rehabilitation issues in clinical recommendations for the care of patients with Pompe disease, and standard practice predominantly follows general recommendation guidelines for neuromuscular diseases. The Italian Association of Myology, the Italian Association of Pulmonologists, the Italian Society of Neurorehabilitation, and the Italian Society of Physical Medicine and Rehabilitation, have endorsed a project to formulate recommendations on practical, technical, and, whenever possible, disease-specific guidance on rehabilitation procedures in LOPD, with specific reference to the Italian scenario. In this first paper, we review available evidence on the role of rehabilitation in LOPD patients, particularly addressing the unmet needs in the management of motor and respiratory function for these patients.

Key words: endurance and resistance training, late-onset Pompe disease, motor function, rehabilitation, respiratory function

Introduction

Glycogen storage disease type II, also known as Pompe disease, is an autosomal-recessive lysosomal storage disorder caused by the deficiency of the lysosomal acid α-glucosidase, which results in the accumulation of glycogen deposits inside lysosomes within the muscular tissue. Pompe disease manifests clinically across a broad spectrum based on age of onset, progression rate, genetic mutation(s), and disease distribution, and is classified as early- (infantile, classic) or late-onset (non-classic) (1, 2).

Late-onset Pompe disease (LOPD) may present at any age after the second year of life and is characterized by progressive weakness in lower limbs and trunk, with only minor cardiac involvement (3, 4). Respiratory muscle impairment, the primary cause of morbidity and mortality in LOPD patients, is also common and involves both inspiratory and expiratory skeletal muscles (1). To this end, and ahead of the current available enzyme replacement therapy, rehabilitation might be proposed as an effective intervention in improving physical function-

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ing of these patients, however supporting evidence and guidelines to support this are lacking (5).

To date, there are no specific guidelines on rehabilitation issues in clinical recommendations for the care of patients with Pompe disease, and standard practice predominately follows more general recommendations or, where available, guidelines for neuromuscular disease (NMD) (1). This lack of treatment guidelines has led to variable and often limited standards of interventional protocols in clinical practice. Moreover, the low prevalence of Pompe disease has hindered the development of a national or international consensus on the appropriate management of musculoskeletal and respiratory impairment in affected patients (1).

The Italian Association of Myology (AIM), the Italian Association of Hospital Pulmonologists (AIPO), the Italian Society of Neurorehabilitation (SIRN), and the Italian Society of Physical Medicine and Rehabilitation (SIMFER), have endorsed a project to formulate recommendations on practical, technical, and, whenever possible, disease-specific guidance on rehabilitation procedures in LOPD, with specific reference to the Italian scenario. In this first, narrative paper, we review available evidence on the role of rehabilitation in the management of LOPD, and define the bases for standardized protocols for the assessment and rehabilitation of musculoskeletal and respiratory impairments in patients with LOPD.

Methods

A multidisciplinary collaboration of 10 clinicians, members of the AIM, AIPO, SIRN, and SIMFER, was established to review current evidence in the field of rehabilitation in Pompe disease. This open forum agreed that two working groups, one on musculoskeletal rehabilitation and a second on pulmonary rehabilitation, should be developed to evaluate available studies and try to standardize patients' assessment and exercise plan. To date, evidence as to whether rehabilitation is effective in LOPD seems poor and contradictory. Therefore, the authors proposed a literature review based on a search of the EMBASE, CINALH, PubMed, PsychINFO, and Scopus databases, using the following keywords: LOPD and guidelines, rehabilitation and LOPD, training and LOPD, physical activity and LOPD, exercise and LOPD. English language papers published between 2000 and 2017 were considered. Papers were selected for inclusion on the basis of their relevance to the topic, according to Authors' judgment.

The level of the evidence of selected studies was defined according to the Scottish Intercollegiate Guidelines Network (SIGN). In addition, the Grades of Recommendation, Assessment, Development and Evaluation Working Group (GRADE) method was used to evaluate the quality of evidence with respect to each relevant outcome.

The results of the literature research and the outcomes of the SIGN and GRADE evaluation are summarized in Tables 1 and 2.

Rehabilitation of motor function in LOPD: state of the art

Enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase (Myozyme/Lumizyme) was approved in 2006 for the treatment of Pompe disease and positive effects of ERT on skeletal muscle strength,

Table 1. Level of evidence according to the Scottish Intercollegiate Guidelines Network (SIGN) for studies investigating the role of rehabilitation of motor and respiratory functions in patients with late-onset Pompe disease.

Studies (Author, date, reference)	Level of evidence
Borg 1970 (18)	4
Bach et al. 1996 (48)	2+
Bach 1999 (33)	4
Wasserman et al. 1999 (19)	4
Baydur et al. 2001 (49)	2+
Mellies et al. 2001 (50)	2+
Ragette et al. 2002 (51)	2+
Shneerson et al. 2002 (41)	3
Hill et al. 2004 (40)	4
Slonim et al. 2007 (12)	2-
Mellies et al. 2009 (38)	3
Van der Beek et al. 2009 (34)	2+
Vitacca et al. 2009 (39)	4
van den Berg et al. 2010 (27)	2+
van der Ploeg et al. 2010 (11)	1+
Vitacca et al. 2011 (35)	4
Angelini et al. 2012 (6)	2-
de Vries et al. 2012 (7)	2+
Favejee et al. 2012 (5)	3
van der Ploeg et al. 2012 (46)	1+
Ambrosino et al. 2013 (37)	3
Gungor et al. 2013 (8)	2+
Toscano et al. 2013 (10)	1-
Vianello et al. 2013 (47)	2+
Vitacca et al. 2013 (36)	3
Hundsberger et al. 2014 (45)	2-
Bertoldo et al. 2015 (28)	2-
Crescimanno et al. 2015 (20)	2-
Favejee et al. 2015 (17)	2+
Jevnikar et al. 2015 (43)	2-
Schoser et al. 2015 (31)	4
van den Berg et al. 2015 (13)	2+
Aslan et al. 2016 (42)	2-
Jones et al. 2016 (44)	2-
Schoser et al. 2017 (9)	1-

Table 2. Appropriateness of recommendations according to the GRADE method for outcomes addressed in clinical guidelines for the rehabilitation management of motor and respiratory impairments in patients with late-onset Pompe disease.

Clinical Guidelines (Author, date, reference)	Level of evidence for rehabilitation management	GRADE-like recommendations based on level of evidence
Kishnani et al. 2006 (3)	3	• Submaximal, functional, and aerobic exercise may improve muscle function • Gentle daily stretching, orthotic intervention, splinting, seating systems and standing supports may prevent or minimize contracture and deformity
Barba-Romero et al. 2012 (29)	3	• Aerobic exercise may improve motor function
Cupler et al. 2012 (26)	3	• Submaximal aerobic exercise, incorporating functional activities may increase muscle strength • Daily stretching, orthotic devices, appropriate seating position in the wheelchair, and standing supports may prevent or slow the development of muscle contractures and deformities
Boentert et al. 2016 (1)	3	• Chest physiotherapy and MAC may be sufficient only for patients with mild expiratory muscle weakness • MAC techniques should be implemented by trained physiotherapists or respiratory therapists • Air stacking combined with MAC is recommended if cough assistance is indicated and upper airways are patent in cooperative patients • I/E devices are indicated if MAC/air stacking are not feasible or ineffective • HFCWO is indicated if MAC/air stacking are either not feasible or ineffective and I/E cannot be tolerated
Llerena Junior et al. 2016 (30)	2-	• Aerobic and progressive resistance exercise training, incorporated into daily functional activities, with or without ERT, may improve muscle strength and functioning • Orthotic devices and posture correction while the patient is in the wheelchair and support for when the patient stands may prevent joint contractures
Tarnopolsky et al. 2016 (32)	2+	• Tailored endurance exercise and progressive resistance training, with or without ERT, may improve aerobic capacity and normalize muscle strength, motor function, and lean mass

Abbreviations: ERT, enzyme replacement therapy; GRADE, Grades of Recommendation, Assessment, Development and Evaluation Working Group; HFCWO, high frequency chest wall oscillation; I/E, Insufflation/Exsufflation; MAC, manually-assisted cough.

walking distance, respiratory function and survival have been demonstrated in adult patients with LOPD (6-11). Importantly, additional treatments, such as physiotherapy or exercise training, may also benefit patients' fitness and physical functioning, however, evidence of such beneficial outcomes is limited (12, 13). Indeed, the need for guidance and standardization in the use of physiotherapy in clinical practice was highlighted in a Dutch survey of 88 patients with Pompe disease and 31 physiotherapists, which demonstrated a lack of uniformity in the type of physical therapy training programs applied (5). It has also been debated as to whether exercise is beneficial or harmful for patients with myopathic disorders (14-16). To date, only a few studies have investigated the benefits of exercise training in adult patients with Pompe disease. An uncontrolled, prospective study demonstrated that adherence to a high-protein/low-carbohydrate diet and exercise therapy slowed the progressive deterioration of muscle function in LOPD patients (12), and a combination of aerobic, resistance and core stability exercises were shown to be feasible and safe (13) and to improve pain, fatigue and functioning (17) in 23 adult patients with Pompe disease who were receiving ERT and were not dependent on ventilators and/or walking devices.

Adherence to a combination of nutrition and exercise therapy (mean 4.5 ± 2.5 years, range 2-10 years) in 34

patients (aged 25-66 years), ambulatory except for one patient, slowed deterioration of muscle function and improved the natural course of LOPD (12). Progressive worsening of muscle function was significantly slower in 26 patients who were compliant with the treatment regimen whereas progressive impairment of muscle function was reported in 8 noncompliant patients (mean difference between pre- and post-therapy Walton score was -0.29 [95% Confidence Interval (CI) -0.36, -0.19; $p < 0.001$] for compliant patients, and -0.01 [95% CI -0.36, 0.34; $p = 0.95$] for noncompliant patients) (12).

Endurance, core stability and muscle function improved following a 12-week exercise program, which included 36 sessions of standardized aerobic, resistance, and core stability exercises, in 23 adult patients (aged > 17 years) with Pompe disease not dependent on ventilators and/or walking devices and receiving ERT for at least 52 weeks (13). Significant increases in aerobic exercise capacity and distance walked on the 6 minute walking test (6MWT) were demonstrated after training compared with before training (maximum workload capacity 122 *vs* 110 Watt; peak oxygen uptake 75.9% *vs* 69.4% of normal; 6MWT 508 *vs* 492 meters, respectively; all $p < 0.01$). Core stability, and muscle function and strength all improved after 12-weeks' training, with no safety issues reported. Despite being statistically significant, only modest increases were demonstrated in the 6MWT and peak workload capacity, however exercise training appears to be an effective and safe adjuvant therapy for patients with Pompe disease offering added value to treatment with ERT alone (13).

Significant reduction in fatigue ($p = 0.001$) and pain ($p = 0.04$) were also demonstrated after 12-weeks of exercise training in the same cohort, but the motor function and amount of physical activity did not change significantly after training (17). However, these clinical improvements were not correlated with changes in aerobic fitness, muscle strength or core stability.

Proposed protocols for the assessment of musculoskeletal impairments and rehabilitation – The choice of the outcome measure

Evidence supporting the role of musculoskeletal rehabilitation in patients with LOPD remains scant, mainly because it is based on small studies with short follow-up, conducted in a home-based setting. It is therefore difficult to define a standardized protocol for the assessment of motor function for application in the Italian scenario. One of the main related issues, that has to be considered, is the choice of the best clinic-instrumental parameters that are selected as trusted indices to be used to evaluate the efficacy of the motor rehabilitative intervention. However, according to the study conducted in 2015 by Van der Berg and colleagues (13), assessment of motor function should include the following outcome measurements:

Endurance: Aerobic exercise capacity was assessed using an incremental cycle ergometer with progressive increase in exercise intensity until exhaustion (the stepwise load increment was based on the patient's functional capacities within a range of 5-20 Watts/minute), and continuous measurement of patients' heart rates and ventilator parameters using spiroergometry. At exhaustion, the Borg scale (18) evaluated exertional symptoms (scale of 6-20). Measurements of maximum workload capacity and peak oxygen uptake capacity were undertaken, and the ventilatory threshold was assessed using the ventilatory equivalents method (19). Walking distance on the 6MWT was evaluated according to the American Thoracic Society guidelines (20).

Muscle strength: Although muscle force is considered a muscle function parameter with limited relevance in the evaluation of motor performance in myopathic patients, there is no doubt that it can represent a useful index for the construction of motor rehabilitative planning. Maximal voluntary contraction, i.e. maximal isometric segmental muscle strength, the most accepted measurement in exercise laboratories, was measured using a hand-held dynamometry and considered as an index of residual muscle function.

Muscle Function: Muscle function assessment comprised three timed tests (10 meter running, climbing four steps, and rising from supine to standing position), plus the Quick Motor Function Test (QMFT) (21). The QMFT was designed specifically for and validated in patients with Pompe disease, and consists of 16 specific motor skills related to daily activities scored on a 5-point scale (0 "cannot perform" to 4 "can perform with no effort"); a total score, expressed as a percentage of the maximum score, is obtained by adding the scores of all items.

The Rasch-built Pompe-specific Activity (R-PAct) scale was validated to specifically quantify the effects of Pompe disease on patient's ability in activities of daily life and social participation (22). This 18-item scale demonstrated good discriminative ability and external construct validity. Furthermore this assessment tool was recently used in a 5-year prospective study, which aimed to evaluate the long-term benefit of ERT in 102 adult patients with Pompe disease (23).

In addition, an assessment of how musculoskeletal impairments and rehabilitation affect quality of life (QoL) should be undertaken. The Individualized Neuromuscular Quality of Life (INQoL) questionnaire (24), which consists of 45 questions within 10 sections, was designed specifically for NMD and validated for the Italian population (25).

The above assessment methods are in line with guidelines on the diagnosis and management of patients with Pompe disease issued in 2006 by the American College of Medical Genetics (ACMG) (3). Musculoskeletal functional rehabilitation recommendations from the ACMG include: monitoring of cardiorespiratory status; screening for osteopenia/osteoporosis; assessment of musculoskeletal impairments, functional deficits, levels of disability and social participation; enhancement of muscle function; prevention of secondary musculoskeletal impairments; functioning optimization with adaptation and assistive technology; patient and family education about the natural course of Pompe disease and recommendations for intervention (3). With regards to the enhancement of muscle function, the ACMG recommended that guidelines from other progressive muscle diseases were to be followed, including: sub-maximal, functional and aerobic exercise; avoidance of excessive resistive and eccentric exercise; avoidance of overwork weakness; and avoidance of disuse atrophy (3).

The proposal for an International Classification of Functioning, Disability and Health (ICF)-based approach (3), although dated now back to 2006, offers an internationally agreed standard for describing and monitoring functioning, has been endorsed by the World Health Organization, and offers a framework for the identification of the categories of functional damage, structural damage, and limitation of activities of daily living (ADL) and of social participation. In Italy, according to the 2013 national health plan, the ICF has become mandatory to allow access to both physiotherapy and aids. It is therefore important to publish data based on an ICF checklist of items, which recognizes issues specifically related to people affected by LOPD.

The choice of the protocol

In 2012, the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) convened a consensus committee to create consensus-based treatment and management recommendations for the treatment of LOPD (26). Participants clarified that overall management of musculoskeletal issues in LOPD patients should preserve motor function, prevent secondary complications, maximize benefits of ERT, promote overall health, and improve QoL. Importantly, the AANEM recognized that there were no established guidelines for muscle strengthening or therapeutic exercise for patients with LOPD (26). Although a small number of studies have shown that sub-maximal aerobic exercise may increase muscle function and strength, further studies with larger sample sizes are needed. Moreover, the AANEM recommended the implementation of general precautions regarding strengthening exercises, that are followed for other degenerative muscle diseases, also be applied to LOPD (26). Furthermore, due to the risk of cardiopulmonary compromise in LOPD, it was recommended that LOPD patients were evaluated by a pulmonologist prior to starting an exercise regimen (26).

The frequency and intensity of treatments, from a functional and rehabilitative point of view, was also addressed by the AANEM. It was recommended that therapeutic exercise should start slowly with incremental increases from mild to moderate intensity in order to achieve aerobic levels approximately 60-70% of maximal effort; rest periods should be allowed for and the patients should aim for a frequency of 3-5 treatment days per week (26). A stretching regimen, performed as part of the daily routine, should be implemented.

Patients with Pompe disease may be affected by low bone mineral density (BMD), putting them at risk of fragility fractures. Indeed, 31 out of 46 patients (67%) had BMD Z-score < -1 SD, with the decrease in bone density present in both the infantile and late-onset forms of Pompe disease (27). Moreover, low BMD was correlated with decreased proximal muscle strength. A recent study also identified an increased risk of asymptomatic and atraumatic vertebral fractures in patients with LOPD walking without assistance and not ventilated, who did not have a significant impairment of bone mass (28). The AANEM recommended that patients with LOPD undergo annual screening with dual-energy X-ray absorptiometry (DXA) and fall risk assessment (26).

In 2012, clinical guidelines for LOPD published by the Spanish Society of Internal Medicine, Spanish Society of Neurology, and the Spanish Society of Pneumology and Thoracic Surgery, concluded that nutritional intervention and aerobic exercise can improve motor function in patients with LOPD, albeit with a low level of evidence (29).

More recently, the 2016 Brazilian guidelines for the diagnosis, treatment and clinical monitoring of patients with juvenile and adult Pompe disease were based on the ICF criteria (30). Recommendations for the management of the musculoskeletal impairments in Pompe disease, included: enrolling the patient in the International Pompe Registry; physical examination; physical/occupational therapy; management of contractures; vitamins and minerals supplementation (30).

Other groups have addressed the issue of motor rehabilitation in LOPD. The 208th European Neuromuscular Centre international workshop agreed on a minimal dataset of outcome measures for adult patients with Pompe disease (31). These included; muscle strength (manual muscle testing using the Medical Research Council grading scale, hand-held dynamometry, quantitative muscle testing), muscle function (6MWT, four timed tests including walking 10 meters, climbing four steps, stand-

ing up from the supine position, and standing up from a chair), pulmonary function (forced vital capacity [FVC] standing and sitting, maximal inspiratory pressure [MIP], maximal expiratory pressure [MEP], ventilation status), patient reported outcomes (Rasch-built Pompe-specific activity scale, fatigue severity scale), and other information (treatment and survival status) (31). It was envisaged that the minimal dataset will allow for data sharing purposes in order to address specific research questions.

Evidence-based guidelines on the diagnosis and management of Pompe disease from a Canadian expert panel identified seven management guidelines and made six recommendations (based on best clinical practices but with insufficient data to draw guidelines) (32). Recommendations related to the assessment of musculoskeletal impairments and rehabilitation included the following two statements: "Patients with LOPD should be encouraged to perform both resistance and cardiovascular exercise to improve general conditioning and quality of life. Interventions should be tailored to individual abilities" and, "Periodic quality of life assessments and/or motor function tests, which can include questionnaires, should be part of the routine management of patients with LOPD" (32).

Rehabilitation of respiratory function in LOPD: state of the art

In LOPD, morbidity and mortality due to progressive respiratory muscle weakness are a major concern and management of respiratory function should include a multidisciplinary approach of neurologists, pulmonologists, and intensive care specialists (1). Clinical presentation of respiratory muscle function impairments in LOPD patients includes restrictive ventilation (hypo-expanding thorax), ineffective cough, alteration of blood gases (from hypoventilation), impaired respiratory muscle strength, alteration of the respiratory pattern (relationship between respiratory rate and current volume), alteration of sleep pattern, and dyspnea in ADL.

The choice of the outcome measure

International guidelines for the management of respiratory function in LOPD patients are well-grounded and defined, and indicate, among other recommendations, essential respiratory function tests to be performed from the onset of the disease to advanced phases (1), including: pulmonary function tests, peak cough flow (PCF), strength of the respiratory muscles, competence of the glottis in the cough, measurement of oxygen saturation (SaO_2) at night, blood gas analysis, and transcutaneous monitoring of paO_2 and $paCO_2$ (Table 3).

These evaluations must be performed initially and repeated over time. Other aspects to be investigated concern stress tolerance, including: incremental tests (with evaluation of desaturation and level of dyspnea); endurance tests (on a treadmill, with evaluation of desaturation and level of dyspnea); study of sleep quality (through standardized scales); 6MWT (with evaluation of desaturation and level of dyspnea); and, evaluation of ADL (from the point of view of both motor and respiratory function).

The pulsed arterial saturation and day time are important to diagnose the development of respiratory failure and to define the timing of the initiation of mechanical ventilation (33). Furthermore, the measurement of oxyhemoglobin saturation is a useful and non-invasive element for monitoring the presence of catarrhal space over time (1).

It is crucial that respiratory function is measured over time since the evolution of respiratory symptoms is highly variable, as demonstrated by a study of 16 untreated patients with LOPD in which only one third of patients presented a rapid respiratory decline over a mean follow-up of 16 years (34). Depending on the rate of disease progression, the authors recommended regular monitoring of LOPD patients every 6-12 months. Consequently, the need to repeat tests of respiratory function over time is extremely variable between patients, which contribute to the heterogeneity of existing approaches in the management of Pompe disease, as confirmed by the results of two surveys recently conducted in Italy (35, 36).

The choice of the protocol

Patients should undergo regular evaluation by a pulmonologist who should initiate respiratory aids as needed so that potentially catastrophic situations during acute chest colds can be avoided (37, 38). Indeed, early diagnosis, aggressive treatment and close follow-up after an acute event are imperative to avoid further deterioration towards acute respiratory failure and hospitalization (1).

Dedicated approaches to pulmonologists' intervention in the management of LOPD have been published by Italian researchers (37). The cornerstones of the respiratory rehabilitative intervention are represented by the treatment of nocturnal hypoventilation and the management of secretions (39). Bronchial disruption must be suggested for preventive purposes and becomes imperative in cases where there is a catarrhal obstruction, which can be detected by auscultation and by clinical signs and symptoms. The main objectives of this therapy are to promote airway clearance, the prevention and treatment of respiratory atelectasis and infections, and the maintenance of a normal ventilation/perfusion ratio. These peripheral disruption interventions act with the purpose of

Table 3. Essential respiratory function tests for the management of respiratory function in patients with late-onset Pompe disease.

Respiratory function test	Description
Pulmonary function tests	Slow vital capacity and FVC both in a sitting and supine position where a restrictive ventilator pattern is usually diagnosed [vital capacity values < 50% predicted (49)] or inspiratory vital capacity values [< 60% predictive of sleep-disordered breathing and < 40% predictive of sleep-related hypoventilation (50, 51)] (1)
Peak cough flow	Measurement of air flow generated during the cough evaluates the effectiveness of the mechanism of cough [a value < 160 L/min reflects inadequate airway clearance (48)]
Strength of the respiratory muscles	MIP, MEP, and sniff nasal inspiratory pressure are indicators of diaphragm weakness and are therefore indications for NIV or poor ability to generate cough (1)
Competence of the glottis in the cough	Calculated using the passive maximum intake inspiratory capacity, which is the maximum capacity of the lung to be passively inflated through air boluses delivered by a fan or an Ambu flask (1)
Measurement of SaO_2 at night	Measurement of SaO_2 at night using cardiorespiratory monitoring or polysomnography. Sleep studies are useful to monitor nocturnal hypoventilation (and therefore the need for NIV) by measurement of nocturnal oximetry, use of a CO_2 transdermal tension meter as well as a complete sleep study using polysomnography (1)
Blood gas analysis	Measurement of oxygen and carbon dioxide levels in an arterial blood sample to monitor the adequacy of oxygenation and ventilation. This is the 'gold standard' for the assessment of hypoventilation
Transcutaneous monitoring of paO_2 and $paCO_2$	Provides information on both the CO_2 status and O_2 delivery to the tissues

Abbreviations: FVC, forced vital capacity; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; NIV, non-invasive ventilation; paCO2, partial pressure of carbon dioxide; paO2, partial pressure of oxygen; SaO2, oxygen saturation.

increasing the air flow at the peripheral level and with it promote the recovery of secretions in the upper airways ("Flutter" PEP Mask, autogenous drainage, ELTGOL [total slow expiration, performed at glottis open and in lateral decubitus]).

Cough assistance becomes necessary when PCF values below 270 L/min are reached, and techniques used include manually assisted coughing, air stacking, insufflation/exsufflation, and high frequency chest wall oscillation (1). Selective assistance to the inspiratory phase can be obtained by means of hyperinsufflation (air stacking with fan or an Ambu flask), selective expiratory assistance by manual compression of the rib cage and abdomen (abdominal thrust), and global cough assistance with air-stacking plus abdominal thrust or a specific instrument called an in-exsufflator, which acts by delivering, in rapid succession, a positive pressure of insufflation and a negative expiratory pressure.

Mechanical ventilation is achieved using either non-invasive ventilation (NIV) or invasive ventilation. The indications of the guidelines for NIV are less conservative than in the past, with the intent of recruiting patients earlier to encourage gradual adaptation to these procedures (1). Patients who develop hypercapnic acute respiratory failure should be referred to a specialized center for assessment of long term mechanical ventilation. NIV increases survival, prevents nocturnal hypoventilation, improves nighttime saturation, sleep-related respiratory disorders, and gas exchange, improves QoL, avoids or postpones tracheotomy, and relieves symptoms (40, 41).

Home mechanical ventilation is to be considered when the patient has daytime hypercapnia ($paCO_2 > 45$ mmHg) or orthopnea or symptoms of nocturnal hypoventilation (morning headache, daytime hypersomnolence, disturbed sleep with frequent awakenings) in association with at least one of the following symptoms: vital capacity < 50% of theoretical, MIP/MEP < 60% of theoretical, nocturnal oxygen desaturation (SaO_2 < 88% for more than 5 consecutive minutes), and close exacerbations (1).

Published evidence supports the effectiveness of respiratory muscle training in increasing MIP (42, 43) and improving the strength of both inspiratory and expiratory muscles (the latter being important for the cough function) (44). Inspiratory muscle training for 8-weeks was shown to have a significant ($p = 0.01$ *vs* baseline) positive effect on MIP in 8 patients with LOPD who were receiving ERT (42). This finding was confirmed in 8 patients with LOPD treated with ERT who completed 24 months of respiratory muscle training and showed significant increases in MIP over a period of 24 months ($p < 0.05$ at 3, 6, 9, 12, and 24 months *vs* baseline) (43). Evaluation of MEP over the 24-month treatment period also demonstrated significant increases in MEP from baseline at 3, 6, and 9 months

Table 4. Outcome measures for respiratory function assessment in patients with late-onset Pompe disease based on disease stage. Reproduced with permission from Ambrosino et al. (37).

Disease stage	Outcome measures
Stage 1	• Improvement/stabilization of vital capacity (% expected) and respiratory muscle strength tests (MIP/MEP)
Stage 2	• Improvement/stabilization of vital capacity (% predicted) and respiratory muscle strength tests (MIP/MEP) • Reduction of stress dyspnea • Increased CPEF (manual/mechanical assistance) • Reduction in number, frequency and duration of pulmonary infections (bronchopneumonia episodes or atelectasis proved with radiologic examination) • Reduction of exacerbations that require antibiotics • Sleep quality improvement • Life quality improvement
Stage 3	• Reduction in number, frequency and duration of pulmonary infections and bronchoaspirations • Reduced ventilation hours (< 8/day) • Change of the type of ventilation assistance (from controlled to assisted) • Tracheostomy removal • Improved ability in common daily activities after MV • Sleep quality improvement • Life quality improvement

Abbreviations: CPEF, cough peak expiratory flow; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; MV, mechanical ventilation.

(all $p < 0.05$) but not at 12 and 24 months; FVC remained stable throughout the study period (43). In addition, Jones et al. showed improved inspiratory and expiratory muscle strength in 8 adults with LOPD receiving ERT following a 12-week respiratory muscle training program, with positive changes largely persistent after 3-months detraining (44). The authors concluded that respiratory muscle training offers a potential adjunctive treatment for respiratory weakness in patients with LOPD.

However, a survey on attitudes and practices in Italy for the management of NMD found that rehabilitative approaches used in clinical practice include mainly mechanical ventilation (96.5%) and bronchial disruption (84.2%), while respiratory muscle training was used in only 36.6% of cases (36).

Ambrosino and colleagues divided the possible outcome measures for patients with LOPD into three groups based on the level of progression and disease severity (Table 4) (37). This is a clinical-functional classification, and it would be of interest to link with the ICF criteria. Studies on the use of ERT in LOPD use FVC, MEP and MIP as the main outcome measures (6, 7, 10, 11, 45, 46), however these endpoints are characterized by high variability, which reduces the reliability of such spirometric data. An alternative endpoint can be represented by the number of hours of mechanical ventilation required (47).

Lastly, considerations on the palliative setting should be performed, which are in line with position papers referring to the Italian setting (35, 39). For end-stage LOPD patients, the wishes of the patient should be respected, decisions must be based on a shared process, and medical treatment must be proportionate; if the patient chooses to not use mechanical ventilation, they must receive adequate palliative care.

Unmet needs in the rehabilitation of LOPD patients and future perspectives

On the bases of the criticisms discussed in the first paper produced by this multidisciplinary group, a number of unmet needs have been identified in the rehabilitation of motor and respiratory function of LOPD patients (Table 5).

The next step of the working group will be to investigate which functioning categories, according to the ICF, are most impaired in patients with LOPD, and to propose a practical approach to address specific management strategies, including physical activity, therapeutic exercise programs for global and specific motor function impairments, patients' education for healthy lifestyle, enhancement strategies to improve social participation, and QoL.

Acknowledgements

Carmelo Chisari has received reimbursements for participation in advisory boards and for educational activities from Sanofi Genzyme and Merz Italy.

Table 5. Unmet needs in the rehabilitation of motor and respiratory function of LOPD patients.

Unmet needs
Lack of definitions for adapted physical activity
Clinical and functional heterogeneity of LOPD patients
Poor identification of patients to whom protocols can be applied
Lack of guidelines based on well-grounded evidence
Poor identification of impairment and disability, also according to ICF classification
Lack of consensus on outcomes for clinical studies
Lack of different protocols for different clusters of patients
Modification of rehabilitation procedures on the basis of ERT
Evaluation of the influence of nutrition/supplementation on rehabilitation outcomes
Evaluation of the influence of the severity of pulmonary function impairment on rehabilitation programs for motor impairments
Lack of evidence on the safety of the specific rehabilitation procedures during the course of LOPD
Lack of definition of the rehabilitation approach according to current regulations – for instance, in Italy and in many European countries, ICF classification is required

Abbreviations: ERT, enzyme replacement therapy; ICF, International Classification of Functioning, Disability and Health; LOPD, late-onset Pompe disease.

Tiziana Enrica Giovanna Mongini has received reimbursements for participation in advisory boards from Sanofi Genzyme.

Antonio Toscano has received reimbursements for participation in advisory boards and for speaking at congresses from Sanofi Genzyme, Amicus and CSL Behring.

Gabriele Siciliano has received honoraria for lecturing from CSL Behring and Sanofi Genzyme and travel grants from Grifols, Sanofi Genzyme and CSL Behring.

Giovanni Iolascon, Michele Vitacca, Elena Carraro, Pietro Fiore, Sonia Messina, and Valeria A. Sansone declare no conflicts of interest.

This project was realized with the unconditional support provided by Sanofi Genzyme Italy.

Medical writing was performed by Luca Giacomelli, PhD, and Melanie Gatt, PhD, independent medical writers, on behalf of Springer Healthcare.

Coordinator: First Class S.r.l. Meetings and Conferences.

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Acta Myologica • 2018; XXXVII: p. 252-256

Metabolic impairments in patients with myotonic dystrophy type 2

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Objectives: metabolic syndrome (MetS) increases risk of cardiovascular diseases and diabetes mellitus type 2. Aim of this study was to investigate frequency and features of MetS in a large cohort of patients with DM2.

Materials & methods: this cross-sectional study included 47 DM2 patients. Patients were matched with 94 healthy controls (HCs) for gender and age. MetS was diagnosed according to the new worldwide consensus criteria from 2009.

Results: mean age of DM2 patients was 52 ± 11 years, 15 (32%) were males, and mean disease duration was 15 ± 14 years. MetS was present in 53% of DM2 patients and 46% of HCs ($p > 0.05$). All components of the MetS appeared with the similar frequency in DM2 and HCs, respectively: hypertension 64 *vs* 52%, central obesity 62 *vs* 74%, hypertriglyceridemia 49 *vs* 39%, hyperglycemia 42 *vs* 33% and low HDL cholesterol 30 *vs* 42% ($p > 0.05$). DM2 patients were more commonly on lipid lowering therapy compared to HCs (12 *vs* 3%, $p = 0.05$). Fifteen (32%) patients with DM2 and only one (1%) subject from control group had diabetes mellitus ($p < 0.01$). Insulin resistance was found in thirty (65%) patients with DM2. Presence of MetS was not associated with patient's gender, age, severity nor duration of the disease ($p > 0.05$).

Conclusions: more than half of DM2 subjects met the criteria for the MetS. We suppose that treatment of metabolic disturbances may reduce cardiovascular complications and improve quality of life in patients with DM2, which is progressive and still incurable disorder.

Key words: myotonic dystrophy type 2, metabolic syndrome, obesity

Introduction

Myotonic dystrophy type 2 (DM2) is an autosomal dominant, slowly progressive, multi-systemic disease, caused by CCTG repeat expansion in intron 1 of the *CNBP* gene that codes protein called CCHC-type zinc finger nucleic acid binding protein (1). Metabolic syndrome (MetS) is a cluster of metabolic and hemodynamic disturbances that appear together, and multiply risk of cardiovascular diseases and diabetes mellitus type 2 (2).

Patients with neuromuscular diseases (NMD) have a higher frequency of cardiovascular and metabolic impairments in comparison to general population, which is probably caused by muscle weakness, fatique and reduced mobility (3, 4). MetS was found in 55% of 11 patients with different slowly progressive NMD, and all components of MetS were more frequent in NMD patients in comparison to healty controls (HCs) (3). Also, total energy expenditure was significantly lower in patient group.

We have previosly reported a high frequency of metabolic disorders in myotonic dystrophy type 1 (DM1), but only 17% of these patients fulfilled criteria for the diagnosis of MetS (5). Nevertheless, in the study by Shieh et al. different MetS criteria were applied and frequency of MetS in DM1 was 41% (6).

There are no studies that specifically examined frequency of MetS in DM2 patients. It seems that DM2 patients have more severe metabolic impairments compared to DM1 (1,7). Some authors suggested that diabetes mellitus type 2 and arterial hypertension are more freqeunt in DM2 than in DM1 (8, 9). Also, hypertrygliceridemia and hypercholesterolemia are probably more common in DM2 than in DM1 (7). There are even some sugesstions that DM2 patients, unlike DM1 patients, may frequently have atherosclerosis and coronary heart disease (1).

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Aim of this study was to investigate frequency and features of the MetS in a large cohort of patients with DM2.

Methods

Forty seven DM2 patients (mean age 51.9 ± 11.1 years, 31.9% males) were recruited consecutively from the Outpatient and Inpatient Units of the Neurology Clinic, Clinical Centre of Serbia, University of Belgrade, from January 1, 2015 to December 31, 2015. All patients had multi-systemic features of DM2 and no other comorbidities. DM2 patients were matched for gender and age with 94 HCs (mean age 51.9 ± 11.1 years, 31.9% males). Control group comprised of patients' healthy family members and staff of the Neurology Clinic, Clinical Centre of Serbia, University of Belgrade. Study was approved by the Ethics Committee of the School of Medicine, University of Belgrade, and written informed consent was obtained from all subjects participating in the study.

Clinical and electrophysiological diagnosis of DM2 was confirmed by standard PCR and repeat primed-PCR assessing the presence of increased CCTG repeats in the *CNBP* gene (10). Severity of muscle weakness was assessed by the Medical Research Council scale, ranging from 0 to 5 (0 = no muscle contraction, 5 = normal muscle strenght) (11). Manual muscle testing of all patients was performed by two neurologists (V.R.S. and S.P.). Following muscles were tested bilaterally: shoulder abductors and adductors, elbow flexors and extensors, wrist flexors and extensors, finger flexors and extensors, thumb opponens, hip flexors, extensors, abductors and adductors, knee flexors and extensors, ankle plantar and dorsal flexors. We added strength of the weakest muscle of the proximal and distal muscle groups of upper and lower limbs with maximum score being 20 (12).

MetS was defined according to the joint statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, the National Heart, Lung, and Blood Institute, the American Heart Association, the World Heart Organization (WHO), the International Atherosclerosis Society, and the International Association for the Study of Obesity (13). MetS was diagnosed when at least three of five criteria were present:

1. increased waist circumference (≥ 94 cm for men and ≥ 80 cm for women);
2. increased serum triglyceride level (≥ 1.7 mmol/L) or use of lipid lowering agents;
3. reduced serum level of high-density lipoprotein (HDL) cholesterol (< 1.0 mmol/L for men and < 1.3 mmol/L for women);
4. elevated blood pressure (≥ systolic 130 mmHg and/or diastolic ≥ 85 mmHg), diagnosis of hypertension, or use of antihypertensive drugs;
5. increased serum fasting glucose level (≥ 5.6 mmol/L) or use of antidiabetic drugs.

Fasting serum levels of total, HDL and LDL cholesterol, as well as of triglycerides and glucose, were measured by standard laboratory methods. Fasting plasma insulin concentration was measured using radioimmunoassay (RIA), and normal values according to our laboratory are 5-25 mIU/L. HOMA (Homeostatis Model Assessment) index of insulin resistance (IR) was calculated according to the following formula: glycemia (mmol/L) x insulin (mU/L)/22.5 (14). IR was defined if HOMA index was higher than 2.6 (15).

Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2). Nutritional status was assessed using the WHO guidelines: underweighted if BMI < 18.5 kg/m^2, well nourished if BMI 18.5-25.0 kg/m^2, overweighted if BMI 25-30 kg/m^2, and obese if BMI > 30 kg/m^2 (16).

Normality of data was tested by the Kolmogorov-Smirnov test. For comparison between two groups (DM2 patients *vs* HCs and DM2 patients with certain metabolic impairment *vs* DM2 patients without certain metabolic impairment), chi-square test, Mann-Whitney U-test, and Student t-test were used. In all statistical analyses, significant testing was two-sided, with p level set up at 0.05 (statistically significant) and 0.01 (highly statistically significant).

Results

A total number of 47 DM2 patients were included (Table 1). MetS was present in 25 (53.2%) DM2 patients and 43 (45.7%) HCs (p > 0.05). Mean number of MetS components was similar in both groups (2.4 ± 1.4 *vs* 2.4 ± 1.3; p > 0.05). Frequency of the individual components of MetS is shown in Figure 1, while metabolic and

Table 1. Sociodemographic and clinical data of DM2 patients (n = 47).

Sociodemographic and clinical data	DM2 patients
Gender (% males)	31.9
Age (x ± SD, years)	51.9 ± 11.1
Education (x ± SD, years)	11.3 ± 3.1
Age at onset (x ± SD, years)	37.2 ± 11.1
Disease duration (x ± SD, years)	15.0 ± 13.7
Muscle weakness (MRC)	
upper limb - proximal	4.3 ± 0.6
upper limb - distal	4.4 ± 0.7
lower limb - proximal	3.9 ± 0.7
lower limb - distal	4.5 ± 0.7
total	17.0 ± 2.1

x: mean value; SD: standard deviation; MRC: Medical Research Council.

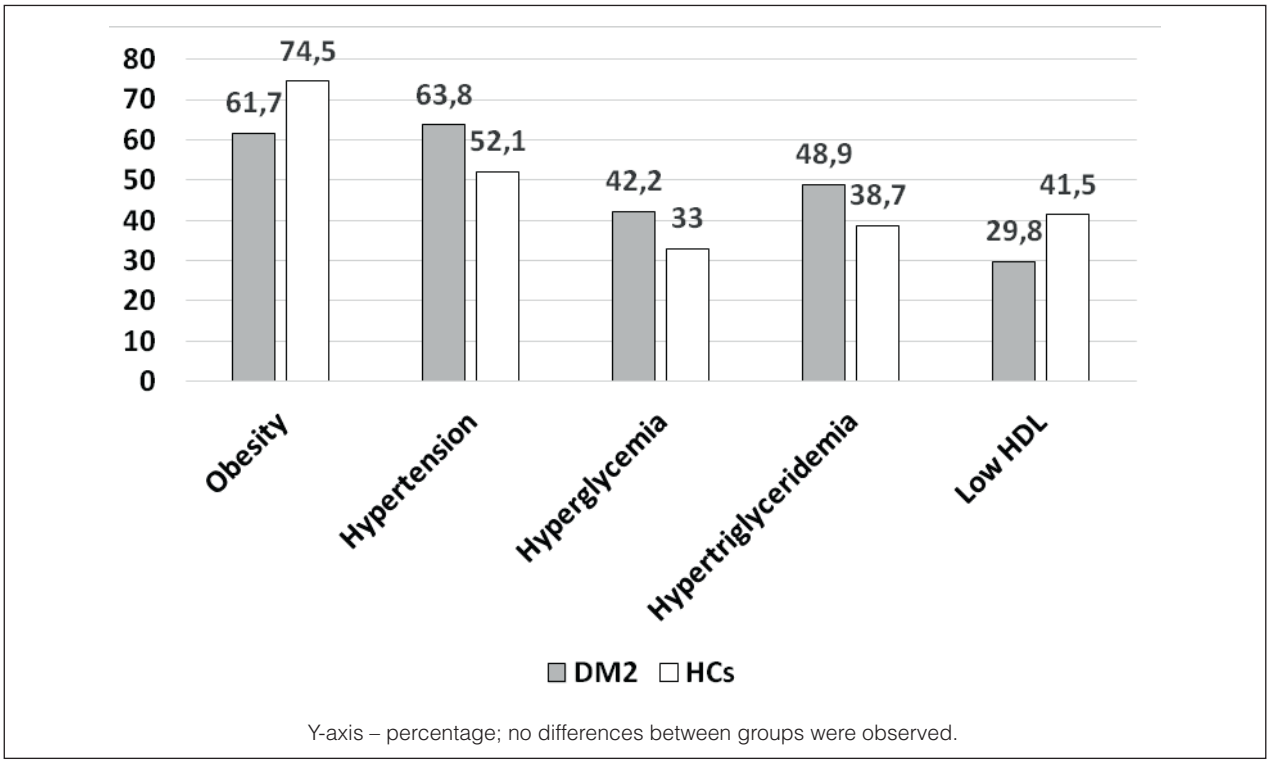

Figure 1. Frequency of MetS and its components in DM2 patients (n = 47) and HCs (n = 94).

hemodynamic parameters are presented in Table 2. Arterial hypertension was present in 63.8% of DM2 patients and 52.1% of HCs ($p > 0.05$). Visceral obesity was present in 61.7% of DM2 patients and 74.5% of HCs ($p > 0.05$). Mean BMI in DM2 patients was 25.2 ± 3.6 kg/m^2: 15% were undernourished, 51% of them were well-nourished, 27% were overweighted, and 7% obese.

Hypertriglyceridemia was present in 48.9% of patients with DM2 and 38.7% of HCs ($p > 0.05$). Low HDL cholesterol level was found in 29.8% of DM2 patients and 41.5% of HCs ($p > 0.05$). However, DM2 patients were more frequently on lipid lowering therapy (11.6% *vs* 3.2%, $p = 0.05$).

Majority of DM2 patients had IR (63.8%). Diabetes melitus type 2 was present in 31.9% and glucose intolerance in 3.4% of patients. Patients with DM2 were more likely to be on oral hypoglycemic medications and/or insulin therapy than HCs (27.6% *vs* 1.1%, $p < 0.01$).

There were no significant differences in the frequency of MetS, hypertension, dyslipidaemia and hyperglycemia between men and women with DM2 ($p > 0.05$). On the other hand, central obesity was more common in women than in men 75.0% *vs* 33.3% ($p < 0.01$). DM2 patients with hypertension were more likely to be older than normotensive ones (55.7 ± 9.0 years *vs* 45.2 ± 11.5 years, $p < 0.01$). Frequency of MetS and its components showed

Table 2. Metabolic and hemodynamic parameters in DM2 patients and HCs.

Parameter	DM2 patients (n = 47)	HCs (n = 94)
Waist circumference (x ± SD, cm)	91.4 ± 11.1	92.6 ± 12.5
Systolic blood pressure (x ± SD, mmHg)	128.6 ± 16.6	125.4 ± 16.7
Diastolic blood pressure (x ± SD, mmHg)*	82.2 ± 8.8	78.8 ± 10.1
Glycemia (x ± SD, mmol/l)	5.9 ± 3.0	5.4 ± 0.7
Triglycerides (x ± SD, mmol/l)	1.8 ± 0.9	1.6 ± 1.5
Total cholesterol (x ± SD, mmol/l) **	6.2 ± 1.5	5.3 ± 1.1
HDL (x ± SD, mmol/l) **	1.6 ± 0.5	1.3 ± 0.6
LDL (x ± SD, mmol/l) **	3.9 ± 1.3	3.0 ± 0.9

x: mean value; SD: standard deviation; HDL: high-density lipoprotein, LDL: low-density lipoprotein; * $p < 0.05$, ** $p < 0.01$.

no correlation with patients' age ($p > 0.05$). In addition, there was no correlation of muscle strength, disease duration and level of education with the frequency of MetS and its components ($p > 0.05$).

Discussion

Half of our DM2 patients had MetS. In patients with DM1 MetS prevalence ranged from 17% to 41% depending on the criteria (5, 6). Higher frequency of MetS in patients with DM2 compared to DM1 may be due to the later onset and later diagnosis of DM2 (9, 12, 17). Similarly, frequency of the MetS in a general population depends on the age of population studied (18). Azizi et al found that frequency of MetS increased from < 5.6% in participants aged 30-39 years to 17.5% in participants aged 60-69 years (19). MetS was present in 42% of subjects older than 70 (20). MetS was also common (45.7%) in our HCs with mean age of 51 years. This is in accordance with the findings of Djokic et al. who found MetS in 28% of patients aged 40-49 and 43% of patients aged 50-59 at the primary health care institutions in Serbia (21).

Majority of studies found that arterial hypertension is the most frequent component of the MetS in general population 22). Similarly, arterial hypertension was the most common component of the MetS (64%) in our DM2 cohort. Furthermore, DM2 patients had a higher mean diastolic pressure compared to the control group (82.2 ± 8.8 mmHg *vs* 78.8 ± 10.1 mmHg, $p < 0.05$). On the other hand, arterial hypertension was present in only 18% of DM1 patients, and even arterial hypotension is common in this disease (5). Difference in the prevalence of arterial hypertension in DM1 versus DM2 patients may be due to the different molecular genetic mechanisms of these two conditions (23).

Visceral obesity was the second most common component of the MetS in our cohort (61.7%). Literature data on visceral obesity in DM2 patients are very limited. Visceral obesity and IR are considered to be the key components of the MetS (2). In our cohort, 34% of DM2 patients were overweighted or obese. Tieleman et al. found that mean BMI was similar in patients with DM2 and DM1 (24). On the other hand, DM1 patients were found to have a higher BMI, longer waist circumference and higher percentage of fat compared to age matched controls (4, 25). Although obesity can occur as a consequence of the physical inactivity due to the muscle weakness, Gagnon et al. found obese DM1 patients even among those with mild muscle weakness (26). This suggest the importance of investigating other risk factors for obesity, such as socio-economic status, and lifestyle habits including eating high-calorie food rich in fat and carbohydrates (26).

Serum triglycerides were elevated in approximately 50% of our DM2 patients, and mean serum total cholesterol and LDL cholesterol were significantly higher in DM2 than in HCs. It is of note that levels of good cholesterol, i.e. HDL, were also higher in DM2. In a small cohort of 20 DM2 patients, Heatwole et al. reported hypercholesterolemia in 63% and hypertriglyceridemia in 26% percent of DM2 patients (7). In our previous study dyslipidaemia was the most common component of the MetS in patients with DM1 - 67% of patients had elevated triglycerides, while 35% had low HDL level (5). Around 12% of DM2 patients were on cholesterol lowering therapy which is of a practical importance because statins may worsen muscle weakness. It is well known that DM2 can be diagnosed in some patients with myalgias after introduction of the cholesterol lowering therapy (27).

IR was diagnosed in 64% and diabetes melitus type 2 in 32% of our DM2 patients. Savkur et al. demonstrated that aberrant regulation of the alternative splicing of insulin receptor is associated with insulin resistance in DM (28). Impaired insulin secretion, reduction of lean body mass and increased serum leptin levels are other mechanisms associated with IR (25, 29). Renna and colleagues recently reported that DM skeletal muscle exhibits alterations of post-receptor signalling (including basal phosphorylation levels of Akt/PKB, p70S6K, GSK3β and ERK1/2), regardless of the alteration of insulin receptor splicing (30). Frequency of diabetes in our DM2 patients was higher than previously reported frequency in DM1. This suggests that eventual mechanisms that protect DM1 patients from development of diabetes, may not be present in DM2.

Although prevalence of MetS increases with age in a general population, in our DM2 cohort this correlation was not observed. On the other hand, we found association between arterial hypertension and aging which is similar to the findings from a general population (31). Disease duration and muscle weakness showed no correlation with MetS. This suggests that muscle weakness is not the key factor for development of MetS in DM2.

The main limitations of our study are lack of data regarding insulin levels and HOMA index in HC group, and lack of data on testosteron level in DM2 subjects since testosteron may have influence on visceral obesity and insulin resistance in DM1 and DM2 (32, 33). On the other hand, it is of note that none of our patients received testosteron therapy.

Conclusions

MetS was common in DM2 patients but not more frequent than in HCs. Regular screening for metabolic and hemodynamic disturbances in DM2 would enable early diagnosis and therapy. We suppose that treatment of metabolic disturbances may reduce cardiovascular complications and improve quality of life in patients with DM2, which is progressive and still incurable disorder.

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Immune-mediated necrotizing myopathy due to statins exposure

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Statin-induced necrotizing autoimmune myopathy (IMNM) is an autoimmune disorder induced by anti-3-hydroxy-3-methylglutaryl-coenzyme-A reductase (anti-HMGCR) antibodies. We performed a retrospective clinical, histological, and radiological evaluation of 5 patients with a 3-year therapeutic follow-up. All patients used statins and then experienced proximal weakness that persisted after drug cessation. Muscle biopsies revealed a primary necrotizing myopathy without inflammatory infiltrates. All patients required immunomodulant combination therapy to achieve clinical remission. Magnetic resonance imaging (MRI) showed the presence of edema in the medial gastrocnemius, posterior and central loggia of the thigh, posterior loggia of the arm, and the infraspinatus and subscapularis muscles, as well as extensive inflammation of the subcutaneous tissues and muscolaris fasciae. Serum analysis, muscle biopsy, and MRI are fundamental for IMNM diagnosis and follow-up. The growing use of statins in the general population raises the importance of acquaintance with this disease in clinical practice.

Key words: HMGCR autoantibodies, muscular MRI, necrotizing myopathy

Introduction

Inflammatory myopathies constitute a heterogeneous group of disorders targeting skeletal muscle. Different inflammatory myopathies vary with regards to prognosis and response to pharmacological therapy. Immune-mediated necrotizing myopathy (IMNM) is a recently recognized category of idiopathic inflammatory myopathy. The autoimmune nature of IMNM is suggested by its frequent association with two specific autoantibodies: 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and signal recognition particle (SRP) (1). Among patients using statins, the estimated IMNM incidence rate is 2-3 per 100,000 patients, with increased risk among patients over 50 years of age (2, 3).

Histological characteristics of IMNM include the presence of necrotic fibers without inflammatory cell infiltrates. The underlying pathogenesis remains unclear, but statins appear to play a major role. Statins can trigger the expression of anti-HMGCR antibodies. This induces muscle synthesis of HMGCR enzyme, which is normally poorly expressed in mature muscle cells, potentially maintaining inflammatory activity even after statin discontinuation (4-6). First-line treatment of IMNM involves steroids, which is generally effective although steroid treatment usually must be administered in combination with other immunosuppressive agents (9, 10).

Over the last decade, muscle magnetic resonance imaging (MRI) has become a very useful tool in the diagnosis and follow-up of patients with myopathies. Muscle MRI provides information regarding skeletal muscle structure and function, such as the presence of edema and/or fatty infiltration, and it is a good technique for monitoring disease progression (7). To date, only one study has analyzed the muscle involvement pattern in patients with IMNM, reporting widespread muscle involvement and a trend towards atrophy and fatty replacement (8). The predominantly involved muscles are the lateral obturators, glutei, and the thigh medial and posterior compartment (8).

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The widespread use of statins in the general population increases the importance of being familiar with IMNM in daily clinical practice. In the present study, we aimed to describe the clinical and histological characteristics of 5 patients affected with IMNM, as well as their post-treatment outcomes, and to illustrate a new MRI pattern for IMNM recognition that may be helpful in early diagnosis.

Methods

Patients. This study included 5 patients belonging to a database approved by the local Ethical Committee. They were diagnosed with IMNM and followed at our Institute from 2014 to 2017. Inclusion criteria were exposure to statins, progressively increasing CK serum activity despite therapy discontinuation, clinical presentation involving subacute onset of severe proximal hyposthenia, necrotizing pattern at muscle biopsy, and serum positivity for anti-HMGCR antibodies.

Each patient was clinically evaluated at the onset of symptoms, as well as during treatment to assess the response to therapy. All patients underwent anti-HMGCR antibody screening tests, EMG, neoplastic screening, muscle biopsy, and muscle MRI.

Diagnostic Imaging. Muscle MRI images of the legs and right arm were acquired using Turbo Spin Echo (TSE) sequences T1, fat sensitive, and Short tau-inversion-recovery (STIR) T2-weighted, fluid sensitive, on a Philips Achieva 1.5T MRI system. Axial images were contiguously acquired throughout the pelvic girdle, thigh and leg to allow for evaluation of the full extent of each muscle. In the arm study, images partially include shoulder girdle.

MRI scanning was performed before therapy in 4 of the 5 patients, and after treatment in all 5 patients. Each muscle was graded according to the degree of fatty substitution apparent on T1WI sequences using the scale proposed by Mercuri et al. (11) Similarly, muscle edema was graded based on the T2-STIR sequences using a 4-point scale (none = 0, mild = 1, moderate = 2, severe = 3) (12). We also assessed the presence of both soft-tissue and perifascicular edema.

Muscle Biopsy and Serum Analysis. After all patients signed the specific informed consent, skeletal muscle biopsy was performed. Muscle biopsy samples were prepared and analyzed using standard light microscopy techniques (13). Serum concentration of anti-HMGCR antibodies was screened for the presence of by the ELISA method using a commercial kit (QUANTA Lite® HMGCR ELISA; Inova Diagnostics, San Diego, Ca, USA) on a Quantalyser® 160 instrument (Inova Diagnostics, San Diego, Ca, USA) as previously described (14).

Treatment. All patients underwent immunosuppression with a combination of multiple drugs (Table 1).

Results

Demographics and Clinical Features. All patients showed moderate to severe proximal and trunk weakness and myalgia. Only Patient 2 showed occasional dysphagia. The patients exhibited extremely high CK levels with

Table 1. Clinical features, instrumental examination, and drug treatments of patients with statin-related IMNM.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age and sex	67, W	65, M	59, M	76, M	63, W
Statin (years of use)	Simvastatin, atorvastatin (6 years)	Atorvastatin (5 years)	Atorvastatin (3 years)	Atorvastatin (10 years)	Atorvastatin (5 years)
EMG findings	Myopathic, fibrillation, repetitive discharges	Myopathic, fibrillation	Myopathic, fibrillation potentials, repetitive discharges	Myopathic, fibrillation	Myopathic, fibrillation, repetitive discharges
Autoantibodies	Negative	Negative	Negative	Negative	Negative
Treatment	PRD, AZA, IVIg, MTX, MFN	PRD, AZA, IVIg	PRD, AZA	PRD, IVIg, MTX, MFN	PRD, MTX, IVIg
Treatment period before recovery	2 years	1 year	1 year	2 years	2 years
Clinical follow-up	Normalization of muscle strength	Improvement of muscle strength	Normalization of muscle strength	Normalization of muscle strength	Improvement of muscle strength

The autoantibodies tested were: ANA, ENA, ANCA, Mi2, Ku, PM-Scl, 100 and 75, Jo1, SRP, PL 7, 12, EJ, and OJ.
PRD, prednisone; AZA, azathioprine; IVIG, intravenous immunoglobulin; MTX, methotrexate; MFN, mycophenolate mofetil.

peak values between 5900 and 9584 U/L. All patients had a medical history of long-term statin use (3 to 18 years). Patient 1 had history of hypertension, diabetes mellitus and dyslipidaemia. EMG revealed a myopathic pattern with fibrillation activity and bizarre high frequency discharges in all patients. All patients started steroid treatment for at least 6 months, after the ineffectiveness of this therapy we performed three cycles of IVIg one month apart from one another. If patients still had symptoms or signs of myopathy, we had another immunosuppressive therapy.

Table 1 shows the patients' detailed clinical features, instrumental examinations, and treatments.

Histology. Muscle examination showed several, scattered necrotic fibers in all specimens, without evidence of inflammatory infiltrates or perifascicular atrophy (Fig. 1). All samples exhibited fiber size variability. Cell typing revealed a scattered presence of CD4+ or CD8+ cells, with no clear distribution pattern. Human leukocyte antigen type I (HLA I) autoantibodiess positivity was detected only in necrotic fibers except for patient 3 muscle biopsy which showed membrane positivity also in some scattered non-necrotic fibers (Fig. 1). Anti-membrane attack complex autoantibodies negative in all muscle biopsies.

Serum Antibody Analysis. Anti-HMGCR autoantibodiess were markedly positive in all patients at the time of diagnosis before treatment. After one month of treatment and at six months after CK normalization, anti-HMGCR autoantibodiess were still detectable in all patients,.

MRI Imaging. Pre-treatment MRI showed several grades of edema at the level of the central and posterior loggia of the thigh and pelvic girdle, and a mild trend towards atrophy and fatty replacement. The most affected

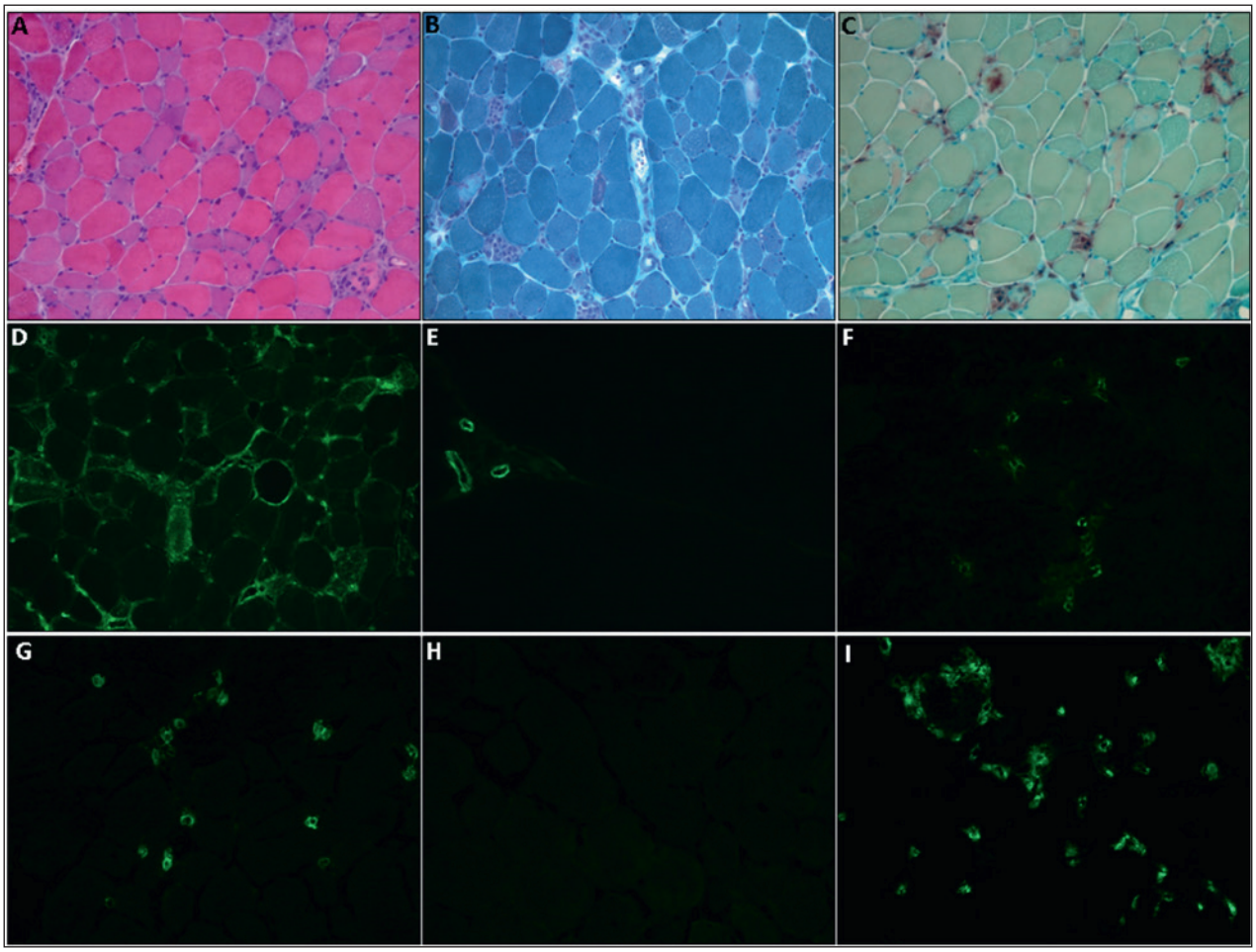

Figure 1. Histological findings with hematoxylin-eosin staining (A) and Gomori trichrome (B) and histochemical findings with acid phosphatase (C) revealing necrotic muscle fibers without any cellular infiltrates. Immunohistochemical evidence of HLA autoantibodies positivity present only in necrotic fibers. MHC class I are expressed only in the cytoplasm of necrotic muscle fibers (D,E). We observed also a variable prevalence of CD8+ cells (F) or CD4+ cells (G,H,I) with no clear distribution pattern.

muscles were the adductor muscles, particularly the adductor brevis, semimembranosus and long head biceps, as well as the obturators and gluteus maximum. At the leg level, the most affected muscle was the medial gastrocnemius whereas at the arm level triceps and deltoid, followed by infraspinatus and subscapularis, were mostly involved. Figure 2 shows the different grades of fatty replacement and levels of edema documented in the thighs, legs, and arms in pre-treatment MRI scans. Inflammation of the subcutaneous tissue and the muscular fascia was

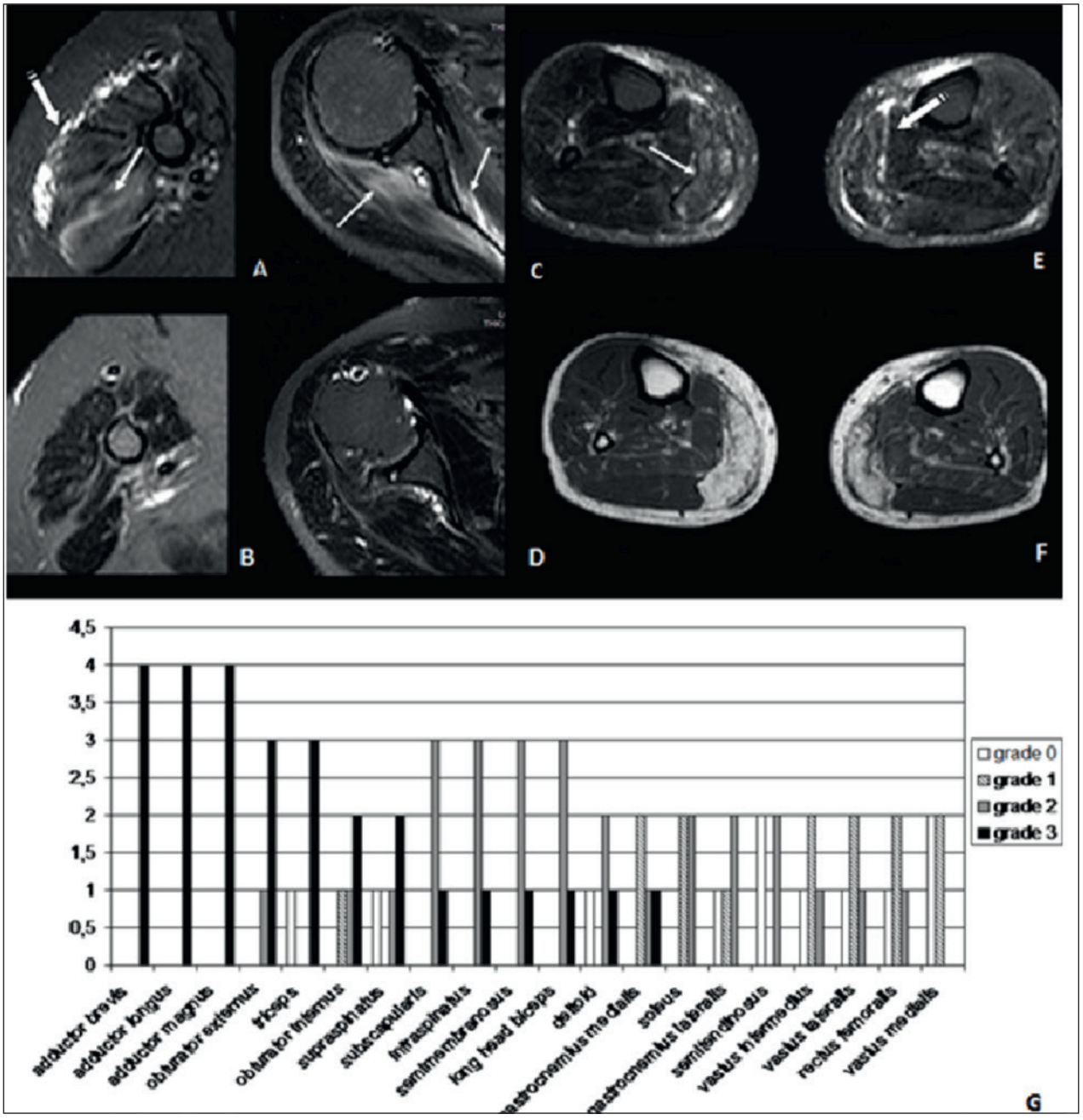

Figure 2. Figure A-C-E. Pre-treatment axial T2 STIR images show edema respectively at the level of the deltoid, subscapularis and infraspinatus muscles and the medial heads of the gastrocnemius muscles bilaterally. Edema is seen at the level of the subcutaneus tissue and the fascia (dotted arrows). Figure B-D-F. Post-treatment axial T2 STIR images document complete resolution of the oedema in the corresponding compartments. Figure G: Extension of oedema of individual muscles on pre treatment MRI, assessed on axial T2WI STIR images. A 4-point scale graduation represent an average of the individual score of the 5 patients for each muscle.

observed in 3 of 4 patients. Post-treatment MRI revealed complete resolution of edema. The grades of fatty replacement remained largely unchanged.

Discussion

Statin use has consistently increased in the last twenty years and has led to more frequent toxic neuromuscular complications, usually self-limiting after drug discontinuation. Quite often, however, statin use induces an autoimmune reaction and causes the development of an aggressive IMNM. The disease is quite rare and, also due to the lack of a validated commercial diagnostic kit, often still under-diagnosed.

In our study all patients had taken atorvastatin. However, due to the small number of examined patients, further studies are certainly needed to validate this association. The 5 patients affected with statin-induced IMNM with serum positivity for anti-HMGCR antibodies and typical pattern of severe necrotizing muscular biopsy, showing acute weakness of trunk flexor and limb girdle muscles, whereas the bulbar muscles were generally spared. Axial involvement is not common among other types of inflammatory myopathies (15). Indeed, in statin-induced myopathies, MRI imaging reportedly shows involvement of the dorsal muscle groups of both the thighs (8, 16). Compared to others toxic and drug-induced myopathies, MRI imaging in our IMNM patients revealed more extensive edema and a trend towards fatty muscle replacement. These findings are in agreement with the recent literature (8). Moreover, extending the study to leg, arm and shoulder girdle, we found a new pattern recognition involving also the medial gastrocnemius and, at the arm level, triceps and deltoid followed by infraspinatus and subscapularis. We also observed inflammation of the subcutaneous tissues and of muscolaris fasciae of both arms and legs, which has not been previously reported to our knowledge (Fig. 2). These findings suggest that patients affected with IMNM may exhibit a wider systemic inflammatory response that is not limited to skeletal muscles.

These new MRI findings allow to improve the differential diagnosis between IMNM myopathy and other inflammatory myopathies and to distinguish IMNM from the toxic myopathy related to statin intake. This distinction is important because toxic myopathy usually improves and then resolves following interruption of statin intake; conversely, IMNM myopathy progressively worsens even after statin suspension and causes a very severe, sometimes hardly or non-reversible, damage to the muscles. Ultimately, muscle MRI may be a useful tool to monitor the evolution of muscle disease over time, but it can be used also as a fist-line screening to identify the most affected muscle and therefore increase the diagnostic accuracy of the skeletal muscle biopsy.

Appropriate therapeutic control is tricky in this type of inflammatory myopathy. Each patient required several immunosuppressive treatments before achieving clinical control. Indeed, all patients were initially treated with IV steroids, followed by high-dose oral steroid therapy, with concomitant superimposition of additional immunosuppressive drugs. Moreover all patients achieved normalization of CK levels and improved muscle strength, albeit with different drug associations and time intervals until response. In two patients, tapering attempts were followed by immediate increase of CK levels that required drug restoration.

In our present study, we aimed to provide a systematic comprehension of this currently under-diagnosed disease in terms of both diagnostic tools and therapeutic options, and to help define the MRI pattern for IMNM recognition to improve its diagnosis.

Acknowledgements

The authors wish to thank the “Associazione Amici del Centro Dino Ferrari, University of Milan”; The Biobank of Skeletal Muscle, Peripheral Nerve, DNA and Cell Cultures, which is a member of the Telethon Network of Genetic Biobanks, funded by Telethon Italy; and the Eurobiobank Network. Representatives of these networks gave their consent to be named.

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Novel desmoplakin mutations in familial Carvajal syndrome

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Desmoplakin is encoded by *DSP* gene, whose altered function leads to skin and hair abnormalities, and heart diseases. The whole triad of these traits characterizes the Carvajal syndrome (CS).

CS is an autosomal recessive genetic disorder, mapping on chromosome 6q24 and caused by mutations in DSP gene.

We report a patient with CS caused by two novel mutations in *DSP* gene, inherited from his parents, both asymptomatic. The same phenotype was present in his younger sister who showed skin abnormality and woolly hairs. The segregation analysis of the known loci in DSP gene performed by genetic testing, was able to established the trans position of the two mutations (c.6986T > C and c.7123G > C) in the patient and his sister. The first mutation has been inherited from the mother, the other one from the father. The resulting compound heterozygous mutation in the siblings, is likely the cause of the disease.

Key words: Carvajal syndrome, cardiocutaneous phenotype, desmoplakin mutations

Desmosomes are cell junctional complexes involved in the regulation of homeostasis. They participate in signalling cascades and provide resistance against mechanical stress, maintaining the strength and rigidity of the cells (1). This property is especially important for cardiac and epidermal tissues, where adjacent cell contacts largely contribute to correct functioning (2). A crucial component of desmosomes is desmoplakin encoded by the DSP gene (3), whose mutations have been associated to the Carvajal syndrome (CS). This syndrome is an autosomal recessive genetic disorder characterized by the following manifestations: woolly hair, striate palmoplantar keratoderma and left ventricular dilated cardiomyopathy (DCM). Additional phenotypic signs include dental abnormalities and leukonychia (4, 5). The symptoms of the disease usually manifest over time. Woolly hair is present from birth, while palmoplantar keratoderma develops after infancy. Left ventricular dilatation is usually asymptomatic at an early age and cannot be diagnosed without instrumental cardiologic examination. Complaints of chest pain appear in 8-12 years. DCM in CS progresses rapidly, leading to heart failure or sudden death in adolescence. CS is caused by homozygous or compound heterozygous mutations in the DSP gene often occurring in hot spots of the gene, exons 23 and 24 (6, 7).

In this article, we report a familial CS with two affected sibs sharing a compound heterozygous mutation in DSP gene, inherited from asymptomatic parents.

Case presentation

The proband is a 11-year-old boy presenting focal keratoderma, specifically affecting the palmoplantar epidermis, woolly hair, and DCM (Fig. 1), consistent with a diagnosis of Carvajal Syndrome. Woolly hair has been observed from birth, while the keratoderma appeared during the second year of life. The manifestation of the DCM occurred at the age of 10 years with complaints of dyspnea and weakness. The cardiomyopathy was confirmed by cardiological examination including electrocardiography (ECG) echocardiography (Echo) and computed tomography (CT). A year later, despite the pharmacological treatment, the physicians observed a significant and rapid evolution of biatrial and biventricular dilatation with severe systolic dysfunction of both ventricles. The patient was hospitalized to receive optimal therapy for severe heart failure and then, at the age of 11 years, he underwent successful orthotopic heart transplantation.

The 5-year-old sister of the proband had similar clinical manifestations as skin abnormality and woolly hairs, appearing from birth (Fig. 1d-e). She was asymptomatic

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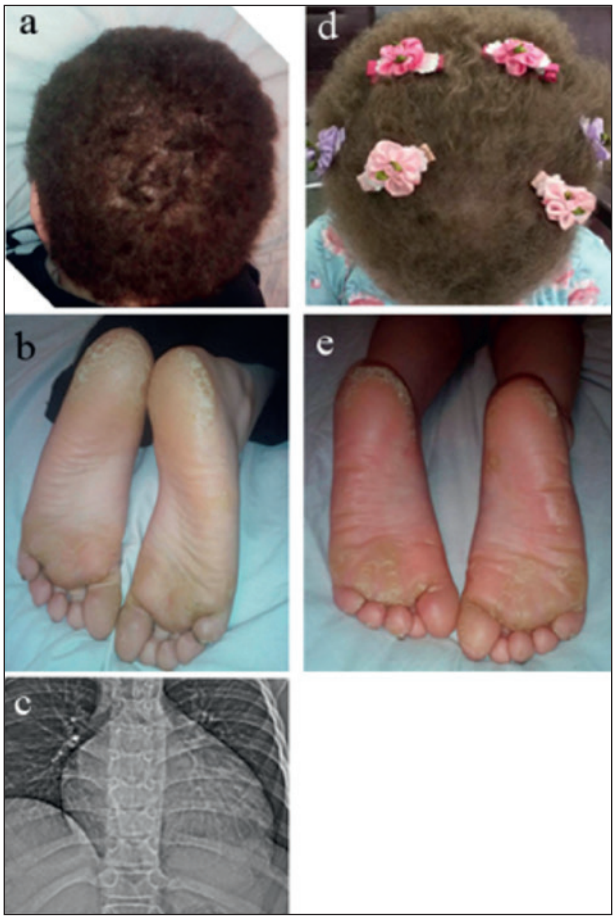

Figure 1. Clinical findings. Manifestations of the Carvajal syndrome in the proband (a, b) and his sister (d, e.); CT scan of the patient's chest with signs of cardiomegaly (c).

and showed no signs of cardiac involvement. Since the age of 4 she underwent a comprehensive cardiac examination every 6 months, without any pathological result. ECG, Holter, and Echo investigation.

Genetics study

The NGS analysis was performed in the Laboratory of molecular pathology "Genomed Ltd." (Moscow). using a panel for inherited heart diseases. Two heterozygous mutations in exon 24 of the DSP gene were found – c.7123G > C and c.6986T > C (NM_004415.2), not represented in the gnomAD database, so they can be defined as truly rare variants.

The first mutation – c.7123G > C – leads to the amino acid substitution p.Gly2375Arg (rs376923069). Only GO-ESP database reports 0.0001 (1/13006) frequency for this mutation; however the G > A change in the same position, that leads to Gly > Arg substitution too, has a 0.00006 (2/31394) frequency, according to gnomAD. c.7123G > C was found in one individual with DCM, woolly hair, keratoderma and classified as variant of uncertain significance (VUS) in the "Actionable exomic incidental findings in 6503 participants", and it was previously described by Alcalai et al. (2003) in a case study of a patient with familial autosomal recessive arrhythmogenic right ventricular dysplasia, woolly hair and a pemphigous-like skin disorder (8).

The second mutation – c.6986T > C – that results in p.Leu2329Pro substitution, is not reported neither in the population databases nor mentioned in published reports.

To follow the segregation of the variants, we performed the segregation analysis in the whole family. The research was carried out according to the Declaration of Helsinki Principles. The parents gave written consent for genetic research after being informed. Their DNA was extracted from buccal epithelium by the phenol-chloroform method. The 24th exon of DSP gene was amplified and sequenced using the Sanger method. The mother was found to be carrier of the c.6986T > C mutation, the father carrier of the 7123G > C mutation (Fig. 2). Both parents of the proband are asymptomatic.

The analisys of segregation in the family established the trans position for c.7123G > C and c.6986T > C variants. As a consequence, the structure of the DSP gene is disrupted on both chromosomes 6 in the proband and his sister.

The combination of these variants forms a compound heterozygous conditon having a high probability to be the cause of the disease. Currently, the ClinVar database includes more than 108 pathogenic and 78 likely pathogenic variations in the DSP gene that are associated with

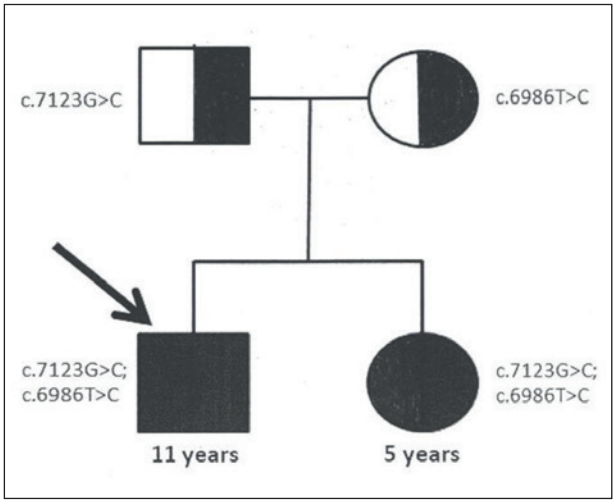

Figure 2. Results of the segregation analysis. The arrow indicates the proband; ■ = affected; ◑ = carrier.

Table 1. Different predictors' scores provided by Condel.

Predictor	The substitution	
	p.Gly2375Arg	p.Leu2329Pro
SIFT 1→0	0.08	0.0
PPH2 0→1	1.0	1.0
MA 0→4	3.63	3.31
FATHMM +8→-5	-2.23	-2.64
Condel 0→1	0.73	0.68
Condel label	Deleterious	Deleterious

The direction of the arrows shows the way of increasing pathogenicity strength of the variants.

defects in epidermal tissue (skin, hair) in a wide range of phenotypes, frequently with heart abnormalities (ClinVar 2018).

The evaluation of the c.7123G > C and c.6986T > C variants according to the algorithm ACMG/AMP (9) is likely pathogenic. The following criteria have been assigned: PM1 (variant is located in a mutational hot spot), PM2 (absent fromcontrols/extremely low frequency), PM3 (detected in trans with a pathogenic variant), PP1 (co-segregation with disease), PP4 (patient's phenotype is highly specific for a disease), PP3 (results of in silico analysis). Computational methods predict a deleterious impact of the mutations on the protein functions (Table 1). The authors of the ACMG/AMP guideline recommend to use a PP1 as stronger evidence under specific conditions. Based on the facts outlined above, we consider the segregation of the two mutations in this family as a strong evidence of pathogenicity.

Discussion

An unfavorable prognosis of the Carvajal syndrome is associated with early onset of the disease, high risk of sudden cardiac death and a rapidly developing heart failure consequent to the progressive dilatation of the heart chambers (10). Heart failure develops in patients with CS in early childhood, and an extreme dilatation of the left ventricle is detected in more than 90% of patients in their early teens.

Although the young sister of the proband does not show cardiac involvement, considering her young age an onset of the heart disease in future cannot be excluded.

In this regard, international experts recommend to perform in children presenting palmoplantar keratoderma and woolly hair a comprehensive cardiological evaluation by ECG, Holter monitoring and Echo as soon as possible in order to detect possible cardiac anomalies and children at risk of premature death. An early diagnosis may be lifesaving in these patients (10).

Conclusions

We describe two cases of CS syndrome due to novel mutations in the DSP gene. The pathogenicity of the two variants – c.6986T > C and c.7123G > C – was assumed by the clear segregation of the disease with mutations in exon 24 of the DSP gene, and by the presence in the family of heterozygous unaffected parents who generated two children with Carvajal's syndrome, sharing mutations in a compound heterozygous condition.

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CASE REPORTS

Heart transplantation in a patient with Myotonic Dystrophy type 1 and end-stage dilated cardiomyopathy: a short term follow-up

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Myotonic dystrophy type 1 (DM1) or Steinert's disease is the most common muscular dystrophy in adult life with an estimated prevalence of 1:8000. Cardiac involvement, including arrhythmias and conduction disorders, contributes significantly to the morbidity and mortality of the disease. Mild ventricular dysfunction has also been reported associated with conduction disorders, but severe ventricular systolic dysfunction is not a frequent feature and usually occurs late in the course of the disease. Heart transplantation is currently considered the ultimate gold standard surgical approach in the treatment of refractory heart failure in general population. To date, considering the shortage of donors that limit the achievement of a greater number of heart transplants and the reluctance of the cardiac surgeons to transplant patients with dystrophic cardiomyopathy, little is known about the number of patients with DM1 transplanted and their outcome. We report the case of a 44 year old patient with Steinert disease who showed an early onset ventricular dysfunction refractory to optimal medical and cardiac resincronization therapy, and underwent to successful heart transplantation. At our knowledge, this is the second heart transplantation performed in a patient affected by Steinert disease after the one reported by Conraads et al in 2002.

Key words: myotonic dystrophy type 1, heart transplantation, dilated cardiomyopathy

Introduction

Steinert's disease or Myotonic Dystrophy type 1 (DM1) is an autosomal dominant multisystemic disorder characterized by myotonia, muscle and facial weakness, cataracts, cognitive, endocrine and gastrointestinal involvement. Cardiac involvement affects the conduction system in about 80% of cases and usually follows the onset of myopathy (1). One third of patients with DM1 may have sudden cardiac death, likely due to the onset of malignant ventricular arrhythmias, so the early identification and treatment of the cardiac impairment is the main key to prevent this tragic event. Advanced degrees of conduction abnormalities and arrhythmias are indicated as significant predictors of mortality in patients with DM1 (2, 3). Myocardial contractility is less commonly impaired and heart failure (HF) may occur late in the course of the disease as the final stage of the cardiomyopathy (4, 5). Despite cardiac involvement, DM1 patients are usually asymptomatic, probably due to the limited level of activity and consequently reduced cardiac demand (3). Heart transplantation (HT) is currently considered the ultimate gold-standard surgical approach in the treatment of refractory heart failure (RHF), a situation in which the patients present with great functional limitation and high mortality rate (6). Thus, HT should be taken into account for patients in III and IV NYHA class, who need recurrent hospitalizations, and present with a poor prognosis despite the therapeutic optimization.

To date, because of the shortage of donors and the high operative risk related to muscle impairment and respiratory failure in patients with DM1, heart transplanta-

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tion is not considered an appropriate option in these patients (7).

We report the second case of a successful heart transplantation in patient with Myotonic Dystrophy type 1 who showed an early ventricular dysfunction, despite the employ of optimal medical and cardiac resynchronization therapy.

Case report

A 44-year-old man, affected by Steinert disease and regularly followed at the Cardiomyology and Medical Genetics of the University Hospital "Luigi Vanvitelli" since the time of his diagnosis (2003), recently needed frequent hospitalisations for exacerbations of signs and symptoms of congestive heart failure, occurred in 2016.

The diagnosis of DM1, based on family history (father and one brother affected) and presence of typical clinical features (myotonic phenomenon, mild distal skeletal muscle atrophy, cataract, gastrointestinal disturbances, endocrine deficiency), was subsequently confirmed by molecular testing, that showed a pathological expansion (500 CTG triplets). In 2005, a bicameral pacemaker (PM) was implanted because evidence of first degree (PR interval ≥ 255 ms) plus second-degree type 2 atrio-ventricular block (8-12), and concomitant episodes of paroxysmal atrial fibrillation (AF), a frequent finding in this population (13-22, 23-28). The implant was followed by an improvement of symptoms and quality of life. In 2013, the PM was uploaded to a cardioverter defibrillator (ICD), because of the detection of not sustained ventricular tachycardia (NSVT) in pacemaker stored electrograms. According to our protocol the uploading is usually performed to prevent the risk of sudden cardiac death, frequently observed in these patients as in others muscular dystrophies (29-32). At six-months follow-up, the epicardial CRT did not induce symptom relief, nor improvement of the ejection fraction (Fig. 1) or reduction of the arrhythmic risk.

Three years later during a routine cardiological check, signs of congestive heart failure (CHF) were detected. Transthoracic echocardiography showed a dilated cardiomyopathy, with a left ventricular end-diastolic diameter (LVEDD) of 7.4 cm and an ejection fraction, calculated by the Simpson and Teichholz method, of 25%. Pharmacological treatment was changed to achieve symptom remission. Six months later the patient was hospitalised for a new episode of HF [fatigue, muscle weakness, dyspnea, ortophnea, edema and palpitations, New York Heart Association (NYHA) class III]. At the control, blood pressure (BP) was 107/57 mmHg and heart rate (HR) 70/bpm, crackles at the basal field of lungs and pretibial edema were detected. Chest X-ray confirmed cardiac dilation and pulmonary congestion. In the following 12 months, despite the optimization of the medical therapy, the patient experienced two further episodes of acute heart failure.The therapy was changed again and included a more aggressive loop diuretic therapy, β-blockers, spironolactone and ACE inhibitors (33). As no relief in symptoms of heart failure was obtained, the patient underwent – after the acquisition of informed consent – cardiac resynchronization therapy (34-36) using an epicardial approach because of angiographic evidence of right subclavian vein occlusion (37). As six-months later, no symptom relief was reported by the patient, nor an improvement in the ejection fraction detected on the echocardiogram, the patient was addressed to heart transplantation that was performed in June 2018. At the time of transplant preevaluation, the patient showed a mild muscular impairment and no respiratory involvement.

Follow-up

The intraoperative course did not reveal any complication; the postoperative course was prolonged due to transient severe respiratory failure requiring antibiotic therapy and mechanical ventilation. The invasive ventilation was withdrawn 3 days after surgery and antibiotic therapy prolonged for 20 days. As post-operative immunosuppression, the patient received cyclosporine A and everolimus. Subsequently, oral prednisone was added to maintain immunosuppression. At one month follow-up the patient showed a successful functional rehabilitation with a good performance status. Neither evidence of graft dysfunction nor progression of muscular impairment was detected after 1 and 3 months, respectively. The cardiological post-operative follow-up included evaluation of patient's clinical status and echocardiography. At 3 months follow-up, no symptoms of heart failure (e.g. breathlessness, ankle swelling and fatigue) nor clinical signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) were found and the patient's exercise tolerance was slightly improved. Transthoracic echocardiography showed normal heart size (left ventricular end-diastolic diameter – LVEDD – was 4, 2 cm) and systolic function (EF and FS were 64% and 37%, respectively) (Fig. 2). The observed enlargement of the left atrium is a normal post-transplantation finding.

Discussion

Cardiac complications – as conduction system anomalies and arrhythmias – in patients suffering from Myotonic Dystrophy type 1 have been frequently described in the literature. Conversely dilated cardiomyopahy in general and end-stage cardiomyopathy in particular is

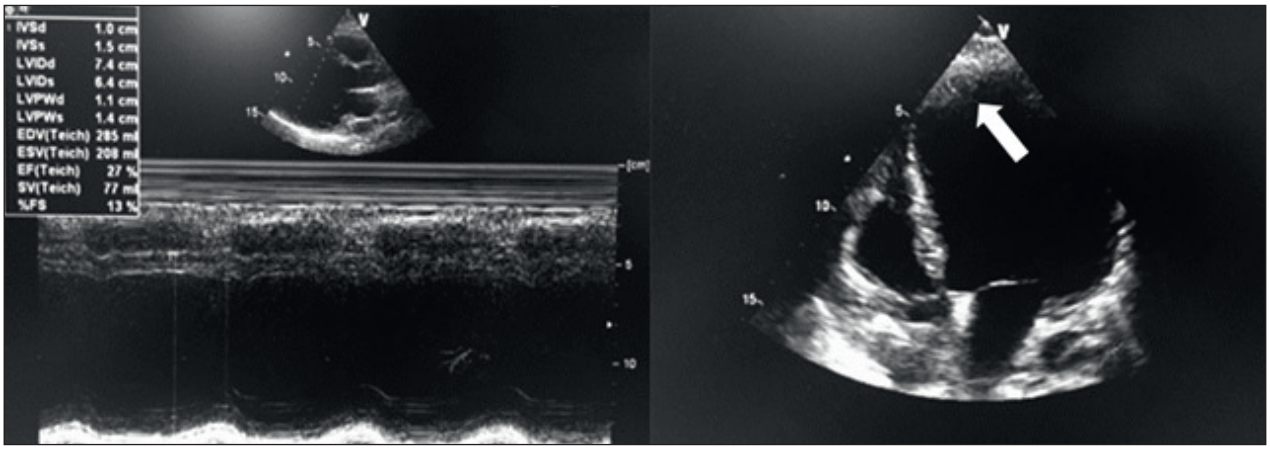

Figure 1. Echocardiographic findings six months after ICD-CRT implantation. Epicardial resynchronization therapy did not induce improvement in left ventricular ejection fraction and reverse remodeling. Please note a blood clot (thrombus) in the left apical ventricle (white arrow).

uncommon (8). The clinical recognition of congestive heart failure in muscular diseases has some more difficulties, as fatigue is often inherent to muscle weakness while exercise tolerance can be impaired by the muscle disease itself. In the classic clinical picture of myotonic dystrophy, skeletal muscle impairment appears years before the onset of cardiac symptoms. Nevertheless, in some cases, cardiomyopathy may represent the initial and unique manifestation of the inherited myopathy (4, 5), as it happened in our patient, in which a marked discrepancy between skeletal muscle and cardiac involvement was observed. In fact, while myopathy was mild and slowly progressive, cardiomyopathy displayed a rapid and severe course requiring HT about 15 years after the diagnosis.

The early onset of heart failure in this patient could be related to the electromechanical delay caused by the intra- and inter-ventricular asynchrony induced by the chronic right apical pacing that causes an uncoordination in the heart contraction which in turn accelerates the progression of the heart failure, as previously reported (37).

Heart transplantation is an elective treatment in patients with ischemic disease and refractary end-stage HF; it is usually accepted that this procedure significantly increases survival, exercise capacity and quality of life compared with conventional treatment (6). However controlled trials are not available.

Inherited myopathies in patients with endstage cardiomyopathies have always been considered a relative controindication for HT (39) because of the perioperative risk secondary to respiratory muscle weakness. Furthermore, a possible progression of the underlying myopathy due to immunosuppressive therapy, is a potential side effect with unknown consequences on the quality of life and prognosis. However, previous papers showed that clinical outcomes of cardiac transplantation in Duchenne/Becker patients with end-stage dystrophinopathic cardiomyopathy seem to be similar to a matched cohort of patients undergoing transplantation for idiopathic dilated cardiomyopathy (40-43). In particular, Cripe et al. (42) reported the case of a 14-year-old patient with intermediate Duchenne Muscular Dystrophy (IDMD), preserved pulmonary function and severe dilated cardiomyopathy who underwent successful cardiac transplantation and survived four years later. Rees et al. (43) described heart transplantation in 3 patients with DMD with a mean duration of follow-up of 40 months. All patients tolerated immunosuppression, had no complications in post-operative intubation and were able to be rehabilitated.

In our experience (40) on 4 patients with end-stage dystrophinopathic cardiomyopathy (3 Becker patients and 1 with X-linked dilated cardiomyopathy), the outcomes were without complications both in the post-operative follow-up and in the long-term follow-up.

These experiences suggest that cardiac transplantation can be successfully performed in patients with muscular dystrophy in general and in patients with Steinert disease, who present a severe cardiomyopathy, provided that they have a preserved pulmonary function and a mild muscle impairment. However, reports on clinical outcomes of cardiac transplantation in patients with muscular dystrophies or extended follow-up periods are still rare and are advisable. At our knowledge, this the second case of heart transplantation, described in literature, in a patient with Steinert disease, after that reported by Conraads et al., in 2002 (44), with satisfactory short-term results.

This case report reinforces the increasing opinon that patients with muscular disorders should have the opportunity to access cardiac transplantation because of under-

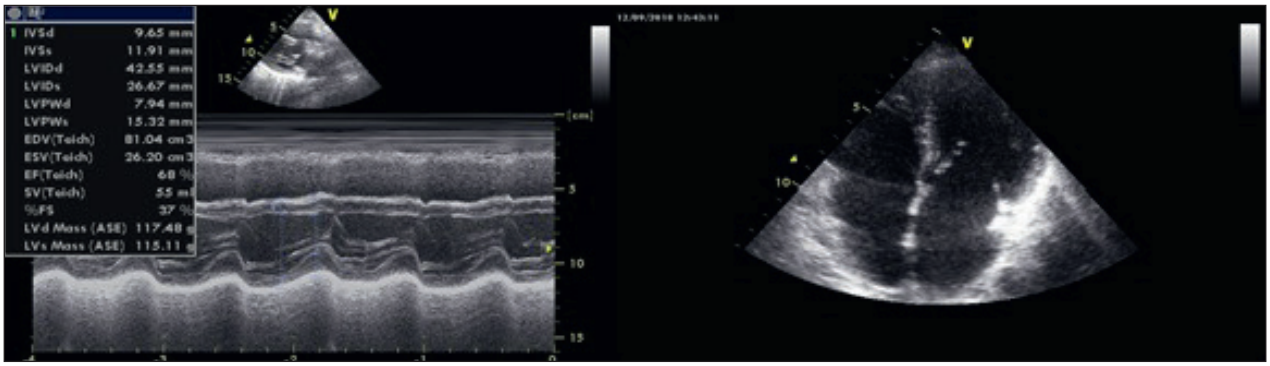

Figure 2. Echocardiographic findings 3-month after heart transplantation. The apical view and M-mode scan of the left ventricle derived from two-dimensional parasternal long axis view show a normal ventricular cavitary diameters and ventricular systolic function.

lying myopathy, as long as there is a careful selection of patients especially with regard to muscle and respiratory function.

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Therapeutic approach with Ataluren in Duchenne symptomatic carriers with nonsense mutations in dystrophin gene. Results of a 9-month follow-up in a case report

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Duchenne muscular Dystrophy (DMD) is a X-linked degenerative disorder affecting skeletal muscles and myocardium caused by mutations in the dystrophin gene, mainly deletions and duplications. Point-mutations account for 13% and stop codon mutations are even more unfrequent. A drug treatment for patients with DMD caused by stop codon gene mutations and still ambulant, has become recently available, based on the clear demonstration of its efficacy in slowing the course of the disease. The drug is able to read through the stop codon; furthermore it has the advantage of an oral administration and a better patient's compliance. We report a case of a still ambulant 27 year-old DMD symptomatic carrier with a stop-codon mutation in exon 53 (c.7792C > T; p.Gln2598Stop), who started the treatment with Ataluren at a dosage of 2,250 mg/die, reporting a prompt subjective improvement in muscle strength. Unfortunately two months after, the patient discontinued taking the drug for a traumatic femur fracture requiring surgical repair and prolonged rehabilitation. With the resumption of the drug intake in February 2018, the patient reported almost immediately an improvement in motor skills, including the possibility of recovering walking, first with support and then unsupported. These results seem even more encouraging, as Duchenne patients hardly recover the ability to walk following a fracture at this age and extend the possibility to treat with ataluren also the symptomatic Duchenne carriers who have nonsense dystrophin gene mutations. Furthermore the case here reported supports the concept that symptomatic DMD female carriers must enjoy the same therapeutic opportunities offered to males.

Key words: Duchenne dystrophy, symptomatic DMD carriers, Ataluren

Introduction

Duchenne muscular dystrophy (DMD) is the most frequent muscle disorder in childhood, characterized by progressive muscle wasting and weakness, leading to the loss of ambulation usually about the age of 12 years. It is caused by mutations in the dystrophin gene, encoding the protein dystrophin. The most part of mutations are deletions (75%) followed by duplications (15-20%) and point mutations (5-10%). Among the latter the nonsense mutations are even rarer. Dystrophyn plays a critical role in maintaining the sarcolemmal stability during muscle contraction.

Treatment options for DMD have been widely explored over the past 30 years. Steroids are considered standard care for DMD patients and have demonstrated evident benefits to patients by increasing muscle strength, reducing muscle fibrosis and inflammation (1). However several side-effects have been reported in the literature, whose severity often depends on the type of steroid used (prednisone *vs* deflazacort) (2). Several approaches aimed at restoring dystrophin expression have been recently reported with promising results. They include gene replacement through the use of viral and nonviral approaches (3, 4), overexpression of utrophin that was proposed to act as a surrogate to compensate for the lack of dystrophin (5) with promising results also in humans (6), and strategies to ameliorate symptoms by increasing muscle strength, reducing muscle fibrosis, and decreasing inflammation. Although promising, these strategies can only improve the quality of life of patients and delay the disease progression.

In recent years, great emphasis has been placed on the discovery of pharmacological approaches able to restore normal, full-length dystrophin and potentially

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reverse the course of the disease. Read-through (RT) of nonsense mutations, thank to its ability to bypass the premature stop codon and to act on virtually any region of the dystrophin gene, independently of the location in which the mutation resides, is one of these approaches.

The ability of certain antibiotics to suppress PTCs in eukaryotic cells has been known since the early 1990s (7).

In 2003 our work group reported the results (8) with gentamicine treatment in 4 Duchenne patients, with point mutations resulting in premature stop codons, still ambulant or in wheelchair stage for less than 4 months. Skeletal muscle changes were monitored by dynamic tests and Creatine Kinase (CK) values; at the beginning and end of treatment, cardiac and respiratory status were evaluated by electrocardiography, echocardiography, acoustic densitometry and vital capacity. Three out of four patients, who had the most permissive UGA as stop codon, showed positive results; in one patient – the youngest among them – there was a dramatic re-expression of dystrophin by both immuno-histochemistry and Western blot.

Recently a new drug, derivative of aminoglycosides has been developed by PTC Therapeutics. It is *Ataluren*, a novel, orally administered small-molecule compound approved within the European Union, Iceland, Liechtenstein, Norway, Israel and South Korea under the trade name Translarna™ to treat patients with DMD still ambulant, aged 5 years and older. Ataluren interacts with the ribosome, which is the component of the cell that decodes the mRNA molecule and manufactures proteins, enabling it to read through premature nonsense stop signals on mRNA and allow the cell to produce a full-length, functional protein.

In 2017, a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial (9) was carried out at 54 sites in 18 countries located in North America, Europe, the Asia-Pacific region, and Latin America. Boys aged 7-16 years with nonsense mutation DMD (nmDMD) and a baseline 6-minute walk distance (6MWD) of 150m or more and 80% or less of the predicted normal value for age and height were randomly assigned (1:1), to receive ataluren orally three times daily (40 mg/kg per day) or matching placebo. Randomisation was stratified by age (< 9 years *vs* ≥ 9 years), duration of previous corticosteroid use (6 months to < 12 months *vs* ≥ 12 months), and baseline 6MWD (< 350 m *vs* ≥ 350 m). The primary endpoint was change in 6MWD from baseline to week 48.

The results showed that 6MWD values did not differ significantly between patients in the ataluren group compared to the placebo group. However, a significant effect of ataluren was observed in the subgroup of patients with a baseline 6MWD between 300 and 400 m. Furthermore patients in the ataluren group had a less decline in physical function compared with patients in the placebo group, as measured by the timed function tests after 48 weeks of treatment, though only the four-stair descend was statistically significant.

These results encouraged clinicians to extend the treatment with Ataluren also in DMD non ambulant patients. Ebrahimi-Fakhari et al. (10) reported their experience in 4 non-ambulatory nmDMD patients, routinely investigated by cardiac function, pulmonary function tests and muscle strength. Mean age at loss of ambulation was 10.1 ± 0.5 years, mean age when initiating Ataluren treatment 14.1 ± 1.4 years. They compared changes in left ventricular fractional shortening, forced volume vital capacity and BMI from two defined time periods (18-26-month period prior to and after Ataluren start). They concluded that serial echocardiography, pulmonary lung function tests, and assessment of muscle strength indicated mild attenuation of disease progression after initiation of Ataluren treatment in all DMD patients.

DMD female carriers are usually asymptomatic. However, 2.5-7.8% of them may present muscle symptoms and/or cardiomyopathy, due to the reduced synthesis of dystrophin. Several pathogenic mechanisms have been suggested to explain the onset of symptoms in female carriers, the most frequent among them is a skewed X- chromosome inactivation with percentages of silencing the X-chromosome carrying the wild allele from 85 to 100%.

We decided to assess the response to the treatment with Ataluren in a symptomatic DMD female carrier sharing a non sense mutation in dystrophin gene.

Case report

We report the case of a DMD manifesting carrier aged 26 years and still ambulant, who received ataluren in the last 9 months. She came at our observation when she was 12 years old. The mother reported a first medical evaluation when she was 18 months old for delay in motor milestones and very high CK levels (11.000 U/L *vs* 195 U/L). Muscle biopsy revealed a mosaic pattern of dystrophin with marked reduction/absence of dystrophin in most fibers, alternating with others with normal protein expression. PCR molecular analysis did not reveal any deletion/duplication, confirmed by MLPA testing. The X-chromosome inactivation analysis was not informative. The patient was treated with deflazacort and anti-oxidant drugs (Vitamin C, Vitamine E, Ubiquinone) since the age of 12, with a slight deterioration in muscle strength; at the age of 25 year she was still able to walk, but she had lost the ability to to get up from the floor at the age of 10 years. In 2016 the NGS analysis identified the causative mutation of the disease in a stop-codon (p.Gln2598Stop) at exon 53 of the dystrophin gene, so in October 2017, at

Table 1. Results of dynamic test pre and post-treatment with Ataluren.

NSAA	6MWT	PUL
Baseline	100 meters	55/80
Baseline (after fracture)	Unable to walk	52/80
12 weeks of treatment	76 meters (with support)	55/80
24 weeks of treatment	52 meters (without support)	56/80
36 weeks of treatment	55 meters (without support)	56/80

the age of 25 years 6 months, she was elected for treatment with Ataluren, at a dosage of 2250 mg/die, soon reporting a subjective well-being and a strength improvement. In December 2017 the patient, due a traumatic femur breaking surgically corrected, was forced to discontinue the drug for 2 months. With the resumption of the drug intake in February 2018, the patient reported almost immediately an improvement in motor skills, including the possibility of recovering walking, first with support and then unsupported. The results of the dynamic tests – North Star (NSAA), 6 Minute Walking Test (6MWT) and Power – Upper-Limbs (PUL), performed before treatment and at three-month intervals are shown in Table 1. No change in cardiac function and respiratory tests was appreciated between baseline and 9 month evaluation.

Discussion

Data here reported – though preliminary and limited to only one patient – suggest that treatment with Ataluren can be of benefit in older patients with DMD and should be extended to symptomatic DMD female carriers to. In our case, the results are even more encouraging as Duchenne patients hardly recover the ability to walk after a fracture at this age. In fact, after 36 weeks of treatment, our patient recovered the motor skill before the accident, and refers a greater autonomy in daily life, confirmed by the INQoL test. These results also extend the possibility to treat with ataluren the symptomatic Duchenne female carriers, presenting nonsense dystrophin gene mutations.

Acknowledgements

Biological samples used in this report are from the Naples Human Mutation Genetic Biobank (NHMGB) that is partner of the Telethon Network of Genetic Biobanks (TNGB) and Eurobiobank (EBB).

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NEWS FROM AROUND THE WORLD

AIM

The XIX congress of the Italian Association of Myology will be held in Bergamo from 5 to 9 June 2019, organised by dr. Angela Berardinelli. The Congress will be preceded by a satellite Symposium entitled: *Physical exercise: pros and cons for taking care of myopathic patients*. Those interested will find the program and the registration form at the end of the "forthcoming meetings" section. All the readers are welcome.

MSM

The 14th Meeting of the Mediterranean Society of Myology (MSM) will be held in 2020.

WMS

The 24th annual congress of the World Muscle Society will be held in the heart of Copenhagen in the old Tivoli Garden Concert Hall and adjoining buildings. Join WMS for the networking reception to be held on Tuesday 1st October in the theatre, Det Ny Teater, located a 5-minute walk from Tivoli gardens. This will follow the long tradition of WMS to facilitate networking and catch up on the latest developments in myology around the world during this 4-day meeting.

Contributions about new advances across the neuromuscular field are very welcome. The main thematic topics that will be addressed in the plenary sessions will be:

1. Metabolic disturbances in neuromuscular diseases.
2. Extra-muscular manifestations in neuromuscular diseases.
3. Advances in the treatment of neuromuscular disorders.

Early bird registration is before Wednesday 8th May 2019 (midnight GMT).

As usual, the meeting will be preceded by a teaching course, which will be held in Copenhagen on September 30th and October 1st 2019.

FORTHCOMING MEETINGS

2019

February 5-6

Biospecimen Reasearch Symposium. Berlin, Germany. Information: website: *www.isber.org*

March 6-8

Advances in skeletal muscle biology in health and disease, University of Florida, Gainesville, FL, US. Information: website: *http://myology.institute.ufl.edu/conferences/muscle-biology-conference*

March 25-28

Myology 2019 AFM-Téléthon Scientific Congress in Myology, Bordeaux, France. Information: website: *http://www.afm-telethon.com*

April 4-5

11th Annual Neuromuscular Translational Research Conference, Newcastle, UK. Information: website: *http://www.ucl.ac.uk/cnmd/events*

May 4-10

American Academy of Neurology, 71st Annual Meeting, Philadelphia,PA, US. Information: website: *https://www.aan.com/conferences-community/upcoming-conference-dates*

May 7-10

ISBER 2019. Shangai, China. Information: website: *www.isber.org*

May 8-11

Heart Rhythm 40th Annual Scientific Sessions (HRS). Chicago, IL. Information: website: *http://www.hrssessions.org*

May 15-17

Annual Meeting of the French Society for Extracellular Matrix Biology. Reims, France. Information: *www.univ-reims.eu; comnco@comnconews.com*

June 6

Physical exercise: pros and cons for taking care of myopathic patients. Satellite Symposiun of the 19th Annual Meeting of the Italian Association of Myology. Pavia, Italy. Information: website: *www.fclassevents.com*

June 6-8

19th annual Meeting of the Italian Association of Myology - Bergamo, Italy. Information: website: *www.fclassevents.com*

June 15-18

The European Human Genetics Conference 2019. Gothenburg, Sweden. Information: *conference@eshg.org*

June 29 - July 2

European Academy of Neurology, 5th Congress, Oslo, Norway.Information: website: *https://www.ean.org/oslo2019/5th-Congress-of-the-European-Academy-of-Neurology-Oslo-2019.3649.0.html*

September 24-28

24th Congress of World Muscle Society. Copenhagen, Denmark. Information: website: *www.worldmusclesociety.org*

October 22-26

ASHG Annual Meeting. Toronto, Canada. Information: website: *www.ashg.org*

October 24-27

Asia Pacific Heart Rhythm Society (APHRS). Bangkok, Thailand. Information: website: *http://www.aphrs.org*

November 13-15

Third International Conference on Genomic Medicine (GeneMed-2019) in Baltimore, USA Information: website: *http://unitedscientificgroup.com/conferences/genemed*

2020

April 25 - May 1

American Academy of Neurology, 72nd Annual Meeting. Toronto, Ontario, Canada.
Information: website: *https://www.aan.com/conferences-community/upcoming-conference-dates/*

June 6-9

The European Human Genetics Conference 2020, Berlin, Germany. Information: *conference@eshg.org*

September 30 - October 4

25th Congress of World Muscle Society. Toronto, Canada. Information: website: www.*worldmusclesociety.org*

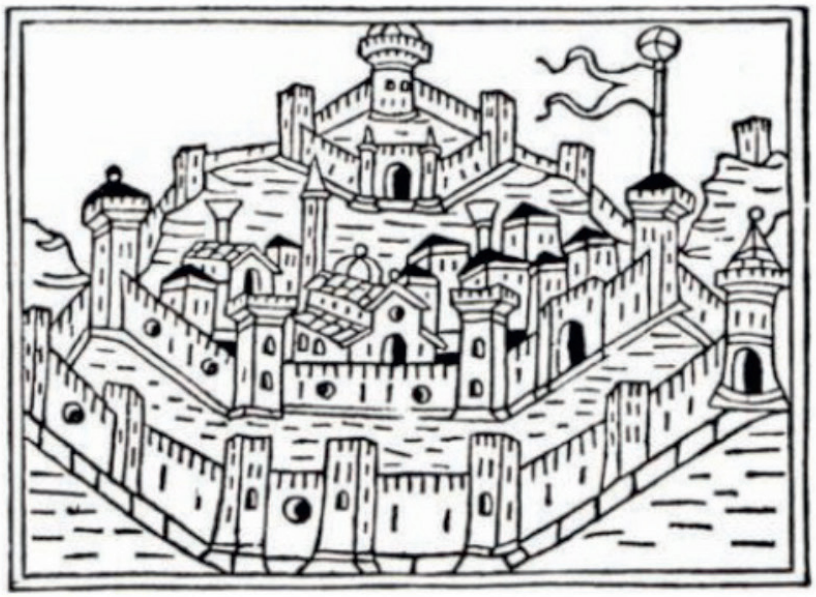
October 27-31

ASHG Annual Meeting. San Diego, CA,USA. Information: website: *www.ashg.org*

2021

September 21-25

26th Congress of World Muscle Society. Prague, Czech Republic, Information: website: *www.worldmusclesociety.org*



SAVE *the* DATE

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ITALIAN ASSOCIATION OF MYOLOGY

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5-8 Giugno 2019

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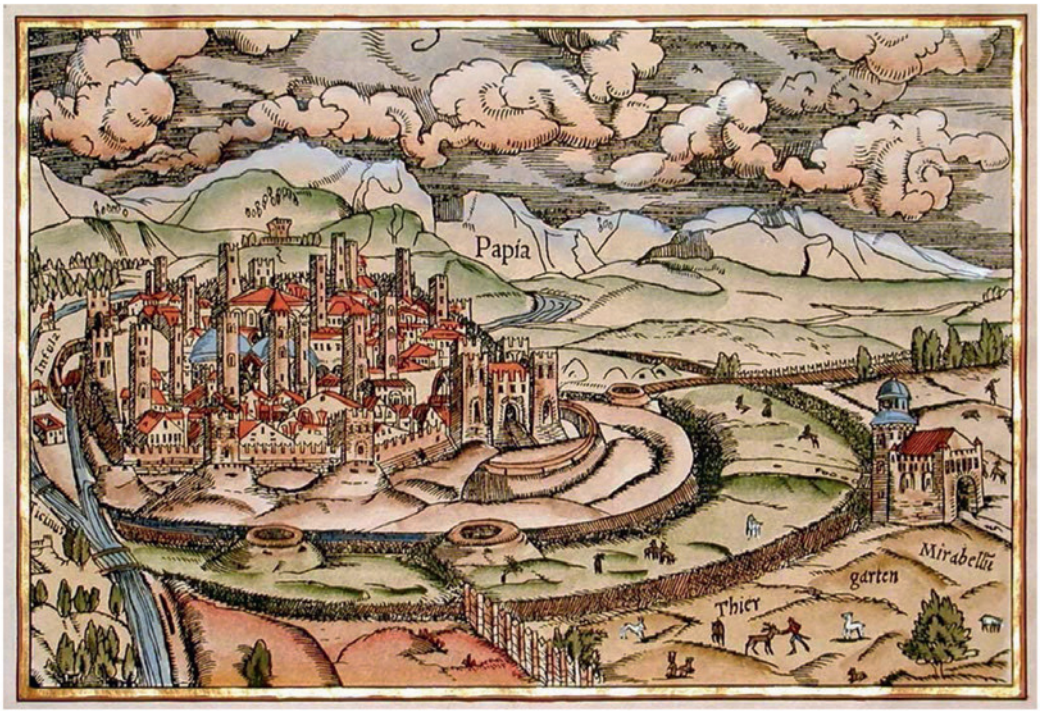

Physical exercise: pros and cons for taking care of myopathic patients

ASSOCIAZIONE ITALIANA MIOLOGIA
ITALIAN ASSOCIATION OF MYOLOGY

Pavia, 5 Giugno 2019

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