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Management of motor rehabilitation in individuals with muscular dystrophies.  
1st Consensus Conference report from UILDM - Italian Muscular Dystrophy Association (Rome, January 25-26, 2019)

Maria Elena Lombardo¹, Elena Carraro², Cristina Sancricca²,³, Michela Armando¹, Michela Catteruccia¹, Elena Mazzone⁴, Giulia Ricci⁷, Ferdinando Salamino⁶, Filippo Maria Santorelli⁹, Massimiliano Filosto¹⁰ on behalf of UILDM (Italian Muscular Dystrophy Association) and Italian Consensus Conference Group on motor rehabilitation in muscular dystrophy

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Muscular dystrophy (MD) is a group of neuromuscular diseases characterized by progressive muscle weakness due to various mutations in several genes involved in muscle structure and function. The age at onset, evolution and severity of the different forms of MD can vary and there is often impairment of motor function and activities of daily living. Although there have been important scientific advances with regard to pharmacological therapies for many forms of MD, rehabilitation management remains central to ensuring the patient’s psychophysical well-being. Here we report the results of an Italian consensus conference promoted by UILDM (Unione Italiana Lotta alla Distrofia Muscolare, the Italian Muscular
Dystrophy Association) in order to establish general indications and agreed protocols for motor rehabilitation of the different forms of MD.

Key words: muscular dystrophy, rehabilitation, exercise

**Introduction**

Muscular dystrophy (MD) is a collective term referring to a group of inherited neuromuscular diseases characterized by progressive muscle weakness due to various mutations in several genes involved in muscle structure and function.

Although the age at onset, evolution, and severity of the disease can vary, several features are common to all the forms of MD, namely progressive weakness, often accompanied by muscle contractures, spinal deformity, and an increased risk of bone fragility and fractures. Most of these conditions are associated with cardiac and respiratory involvement, and different forms of intellectual disability can also be present in some of them. For this reason, MD requires multidisciplinary management.

Even though recent years have seen considerable progress in the molecular characterization and diagnosis of MD, no effective treatment is yet available for the majority of forms, and general management and rehabilitation continue to have a key role in maintaining an acceptable functional status in affected patients.

The multidisciplinary management of MD should be aimed at preserving motor function, preventing secondary complications, promoting overall health, and improving patients’ autonomy and quality of life (QoL).

With regard to the aim of preserving motor function, physical exercise and management of contractures are two areas that deserve careful consideration.

The role of physical exercise in MD is still highly controversial. Some argue that it should be considered potentially harmful due to the poor regenerative ability of muscle in MD, and the possibility of wasting due to overwork in response to external stimuli/stresses. On this basis, physical exercise has traditionally been discouraged in MD. On the other hand, the beneficial effects of physical activity per se could potentially help to maintain function and prevent non-use atrophy in MD patients. Since it remains unclear how best to balance the drawbacks and benefits of physical exercise, we believe that there is now a fundamental need for more precise indications, based on the F.I.T.T. (frequency, intensity, time and type) model of physical exercise, in order to ensure optimal management of these patients.

Very recently, a paper was published describing a multidisciplinary rehabilitation approach involving physical activity and therapeutic exercise in late-onset Pompe disease, a severe metabolic myopathy for infant forms, while late onset cases span from asymptomatic (high CK) to relatively severe cases with respiratory insufficiency. The authors proposed operational protocols based on physical activity and on therapeutic exercise and respiratory rehabilitation.

Joint contractures and/or deformities are frequent in several forms of MD; they are a consequence of muscle degeneration, muscle fibrosis, and reduced mobility, which together cause significant muscle imbalance. Careful management of rehabilitation interventions specifically aimed at preventing contractures is fundamental to maintaining motor function and preserving patient autonomy.

To date, internationally validated guidelines on rehabilitation are available only for Duchenne muscular dystrophy (DMD), and it is unclear whether they can be applied to other forms of MD.

In view of the aforementioned considerations, we performed a systematic and comprehensive analysis of the biomedical literature related to neuromuscular rehabilitation in MD with the aim of drawing up a consensus document on recommendations for clinical practice. This document was commissioned by UILDM (Unione Italiana Lotta alla Distrofia Muscolare, the Italian Muscular Dystrophy Association), which represents and supports patients suffering from neuromuscular diseases.

**Methods**

The purpose of this study was to obtain consensus statements from an expert panel (the ‘Jury’), after presentation and discussion of relevant literature data.

We used the consensus conference methodology, which is an excellent means of reaching conclusions and formulating crucial statements in the field of health care. It is recommended for addressing clinical issues on which available good quality evidence is limited.

The consensus conference was carried out according to the US National Institutes of Health Consensus Development Program and the Methodological Handbook of the Italian National Guideline System. The project was coordinated by a scientific board (the “Board”) made up of nine experts: multidisciplinary clinicians (3 neurologists, 2 child neurologists, 2 physiatrists, 1 physiotherapist) plus a supervisor specialized in consensus conference methodology. In the first step, the Board generated research questions in accordance with the P.I.C.O. (i.e., Population, Intervention, Comparison, Outcome) model, used in the field of evidence-based medicine. Nine topics were covered, in order to provide recommendations on the most important aspects of motor rehabilitation:
Topic 1: Outcome measures;

Topic 2: The rehabilitation project/program: objectives and management, based on the International Classification of Functioning, Disability and Health (ICF);

Topic 3: Body function – focusing on “Functions of the joints and bones” (ICF codes b710-b729): contracture management;

Topic 4: Body function – focusing on “Muscle functions” and “Movement functions” (b730-b789): physical exercise;

Topic 5: Activities and participation – focusing on “Mobility” (d4): posture and mobility management;

Topic 6: Activities and participation – focusing on “Self-care” (d5) and “Major life areas” (d8): activities of daily living (ADL);

Topic 7: Definition of the professional figures involved in the rehabilitation project/program;

Topic 8: The rehabilitation setting: outpatient vs home therapy;

Topic 9: Duration/frequency.

In step 2, the Board reviewed the specific literature, consulting several databases (i.e., EMBASE, CINAHL, PubMed, PsychINFO and Scopus). According to their area of expertise, the Board members worked in 3 groups:

• Group 1: two child neurologists and 1 physiotherapist. This group focused on pediatric-onset forms of MD: DMD/Becker muscular dystrophy, congenital muscular dystrophy, and early-onset limb-girdle muscular dystrophy (LGMD);

• Group 2: three adult neurologists and 1 physiotherapist. This group focused on adult forms of MD: Becker muscular dystrophy, LGMD, facioscapulohumeral muscular dystrophy (FSHD), myotonic dystrophy type 1;

• Group 3: two psychiatrists and 1 physiotherapist. This group focused on the concept and content of rehabilitation projects versus programs.

The literature review was performed using the following keywords: type of MD (e.g., “Duchenne muscular dystrophy”), “exercise” and “rehabilitation”.

Reviews and studies in English, Italian, French, or Spanish, of any design, and published in peer-reviewed journals in the period January 1984 - December 2018, were included on the basis of their relevance to the topic. Literature published only in abstract form was excluded.

The third step was the formation of the expert panel (the Jury) composed of 23 experts in MD/stakeholders (representatives of the MD community). This panel comprised clinicians, researchers, and members of patients’ associations. The results of the literature review were presented to the Jury and discussed among its members at the “1stUILDM Consensus Conference on neuromuscular rehabilitation in pediatric and adult MD”, held in Rome on January 25-26, 2019. The evidence collected during the literature review and the recommendations proposed by the Board, were addressed through constructive debate involving all the participants, to ensure that all the experts/stakeholders had an active role in the consensus-reaching process.

A specific survey questionnaire was then administered to all 23 Jury members, and, under the supervision of the Board, their level of consensus on each of the proposed questions was determined, as follows:

• unanimous consensus: positive opinions expressed by 100% of the Jury members;

• majority consensus: positive opinions expressed by > 60%;

• consensus to be redefined: positive opinions expressed by between 41 and 59%;

• consensus not reached: positive opinions expressed by < 40%.

This led to the drafting of a document that was shared among all the participants for final approval. The approved draft document constitutes the basis of this paper: it extensively describes the discussion and the level of consensus reached by the panel on the above 9 questions, which apply to all forms of MD. Table I sets out specific indications for the different forms.

Consensus document

TOPIC 1: outcome measures

Discussion

Many difficulties surround the definition of, and the terminology used in, standardized outcome measures in the field of MD. There are several reasons for this, the most important being the still incomplete knowledge of the natural history of the different forms which, in turn, is due to their significant clinical heterogeneity.

Because of the low prevalence of these diseases, there are still few randomized clinical trials dealing with rehabilitation in patients with MD, and those that do exist present several methodological limitations. The studies are heterogeneous, in terms of both the populations selected and the rehabilitation programs followed. They often lack control groups or have a non-blinded study design; and precise endpoints, biomarkers, and clearly defined outcome measures are often lacking, too. Thus far, DMD is the only form in which these issues have been extensively addressed through validated international guidelines and standards of care, focusing on outcome measures, general management, secondary complications, and rehabilitation treatment. The most standardized outcome measures used to monitor motor function in DMD include the North
### Table I. Specific recommendations for different types of muscular dystrophy.

#### Duchenne muscular dystrophy (DMD)

**Standard of care** 14,15:

<table>
<thead>
<tr>
<th>Contracture management</th>
<th>Stretching/ortheses based on the natural history and stage of the disease (see standard of care for details)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Recommended frequency of stretching: at least 4 to 6 times a week, on the basis of personalized evaluation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical exercise</th>
<th>Feasibility and safety of low-intensity endurance training with assisted cycle training, during ambulatory or late-ambulatory and wheelchair-dependent phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Personalized protocols with regular, gentle aerobic exercise (like aquatics or cycling), especially in early stages of the disease</td>
</tr>
<tr>
<td></td>
<td>• Spontaneous non-structured daily activity (e.g., play)</td>
</tr>
<tr>
<td></td>
<td>• Need for specific cardiological evaluation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other issues</th>
<th>Cognitive, nutritional and psychosocial evaluation, speech therapy, and cardiac and respiratory management are fundamental for these patients (see standard of care)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Always keep in mind pain management and promotion of ADL participation, use of assistive technology, and customized powered wheelchairs</td>
</tr>
</tbody>
</table>

#### Congenital MUSCULAR Dystrophies

**Guidelines** 33,54,77:

<table>
<thead>
<tr>
<th>Contracture management</th>
<th>Joint contractures: typical in both the lower and the upper limbs, often accompanied by foot and spinal deformities, hip dislocation, and joint hypermobility. Early intervention with stretching, orthoses, standing, and assistive equipment is fundamental.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• LMNA, LMNA2, and COL6: early and adequate posture of feet and neck is of supreme importance for prevention of foot deformities and hyperextension of the neck</td>
</tr>
<tr>
<td></td>
<td>• Emery Dreifuss muscular dystrophy (EDMD): pay specific attention to early severe elbow contractures, also in ambulant patients</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical exercise</th>
<th>There are no specific conclusive data on the possible beneficial or detrimental effects of muscle exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Hydrokinesitherapy to preserve range of motion and prevent edema and swelling of extremities is recommended</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other issues</th>
<th>Pain management and promotion of ADL participation, use of assistive technology and customized powered wheelchairs</th>
</tr>
</thead>
</table>

#### Limb-Girdle muscular dystrophy (LGMD)

**Guidelines** 30,31:

<table>
<thead>
<tr>
<th>Contracture management</th>
<th>Periodic assessment to define personalized contracture program and mobility support</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Physical exercise</th>
<th>Strength training and aerobic exercise training are both safe and potentially beneficial: recommendation for combined supervised programs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Low-impact aerobic exercise (swimming, stationary cycling) improves cardiovascular performance and muscle efficiency and reduces fatigue</td>
</tr>
<tr>
<td></td>
<td>• Need to monitor the risk of damage due to supramaximal high-intensity exercise. This is very important in LGMD (in childhood, eccentric sport activities for LGMD 2B can exacerbate muscle damage progression) 78</td>
</tr>
<tr>
<td></td>
<td>• Need for specific cardiological evaluation (bear in mind the potential positive effect of aerobic training for cardiovascular function and metabolic issues)</td>
</tr>
</tbody>
</table>

| Becker muscular dystrophy (BMD) | There are no specific data concerning the management of joint contractures (see general recommendations) |

<table>
<thead>
<tr>
<th>Physical exercise</th>
<th>Endurance training is safe (also in the presence of significant cardiomyopathy) and can increase performance and daily function 79.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Aerobic/resistance training (studies including LGMD/BMD patients): both low- and high-intensity resistance training showed positive effects on muscle strength and endurance and were well tolerated 80-82.</td>
</tr>
<tr>
<td></td>
<td>• Need to monitor the risk of damage due to supramaximal high-intensity exercise. This is very important in BMD, particularly in more severely affected patients</td>
</tr>
<tr>
<td></td>
<td>• Need for specific cardiological evaluation (bear in mind the potential positive effect of aerobic training for cardiovascular function and metabolic issues)</td>
</tr>
</tbody>
</table>
Star Ambulatory Assessment, the timed function tests, the 6-Minute Walking Test (6MWT), and the Performance of the Upper Limb tool. For other neuromuscular diseases, expert networks have been created in order to seek to develop reliable and valid outcome measures. In clinical practice, the 6MWT and the Performance of the Upper Limb tool can be used in MD, as can other specific outcome measures, such as the Egen Klassifikation Scale Version 2, the Motor Function Measurement scale, and the GSGC (Gait, Stairs, Gower, Chair), as confirmed by recent validation studies.

### Panel consensus

The Jury recognizes and accepts the published standardized outcome measures for DMD, which should be performed periodically in order to monitor clinical pro-

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**Table 1. Specific recommendations for different types of muscular dystrophy.**

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Contracture management</th>
<th>Physical exercise</th>
<th>Other issues</th>
</tr>
</thead>
</table>
| Myotonic dystrophy | There are no specific data concerning the management of joint contractures (see general recommendations) | **Physical exercise**  
- Moderate physical exercise should be strongly encouraged since it does not worsen the disease progression and can minimize the disease weakness  
- Always consider the patient's basal activity level: sedentary patients may benefit from a physical exercise program, while further activity may be fatiguing for individuals with an active lifestyle  
- Equipment such as elastic bands, free weights, and machines can, very carefully, be included in the program, as can certain types of exercise, like yoga and pilates  
- To be performed at least 3 times a week  
- Low-moderate aerobic training is highly recommended after appropriate cardiological assessment. Definition: moderate exercises are defined as activities that you can perform while still continuing a conversation – without having to stop to catch your breath  
- Frequency: 2 hours and 30 minutes per week of moderate-intensity exercise, in sessions of at least 10 minutes spread throughout the week  
- Examples include: walking briskly, cycling on level ground or on a stationary bicycle, ballroom and line dancing, general gardening, household activities, canoeing, using a manual wheelchair, and water aerobics | **Other issues**  
- Balance training/reduction of falls rate/foot drop management: very important to consider, due to the specific weakness distribution and balance impairment in these patients (concomitant neuropathy, proprioceptive deficits, etc.). Also consider use of AFOs when appropriate  
- Cognitive behavior management, nutritional therapy, speech therapy, and occupational therapy: it is fundamental to include these in the neuro-rehabilitation program (OPTIMISTIC trial)  |
| Facioscapulohumeral muscular dystrophy (FSHD) | There are no specific data concerning the management of joint contractures (see general recommendations) | **Physical exercise**  
- Low-intensity aerobic exercise: safe and potentially beneficial, always target exercise on the basis of weakness distribution (to avoid falls or over-use damage)  
- Strength training: its role is controversial. Propose safe and personalized programs using appropriate low/medium weights/resistance and taking into consideration the patient’s physical limitations | **Other issues**  
- Balance training/reduction of falls rate/foot drop management: very important to consider, due to the specific weakness distribution and balance impairment in these patients (concomitant neuropathy, proprioceptive deficits, etc.). Also consider use of AFOs when appropriate  
- Surgical scapular fixation for periscapular muscle weakness: this should be considered for selected patients after careful evaluation of: potential gain in range of motion, patient’s rate of disease progression, possible adverse consequences of surgery, and prolonged postsurgical bracing |
progression of the disease and the progress of the rehabilitation program. All the members confirm the need for better definition of outcome measures for other forms of MD, in order to achieve validation of tools already proposed, or the creation of new quantitative ones. **Unanimous consensus.**

**TOPIC 2: The Rehabilitation Project/Program:**
**Objectives and management based on the International Classification of Functioning, Disability and Health (ICF)**

**Discussion**

It is widely recognized that rehabilitation should focus on patient functional status and on improvement of well-being, and not simply on the specific disease in question. The ICF is an internationally approved classification system that aims to “provide a unified and standard language and framework for the description of health and health-related states”⁹. It describes all aspects of disability (i.e., ‘impairments, activity limitations or participation restrictions’), together with possible contextual factors (environmental and personal)⁴¹. A recent study recommended using the ICF in rehabilitation studies⁴². To our knowledge, there are only 7 published studies in which the ICF was used to explore neuromuscular diseases⁴²-⁴⁹. While none of these considered use of the ICF in rehabilitation planning, a single study, applying a qualitative method, examined the content validity of the IFC Core Set as a basis for enhancing overall care in patients with neuromuscular diseases⁴⁹.

In the rehabilitation process, it is necessary to distinguish between the project, which aims to achieve the expected level of long-term functioning in a given patient, and the program, which identifies and sets out the short-term goals, the methodology to be used to reach them, the timing, and the milestones along the way⁵⁰.

**Panel consensus**

The Jury unanimously supports the need to define rehabilitation projects/programs on the basis of the ICF.

The main objectives of the motor rehabilitation plan should refer, in particular, to the following ICF categories:

1. **Body Functions (b):**
   - Neuromusculoskeletal and movement-related functions (b7): Mobility of joint functions (b710), Muscle power functions (b730), Muscle endurance functions (b740) and Gait pattern functions (b770);
   - Functions of the cardiovascular, hematological, immunological and respiratory systems (b4): Exercise tolerance functions (b455);
   - Sensory functions and pain (b2): Pain (b280).

2. **Activities and Participation (d):**
   - Mobility (d4);
   - Self-care (d5);
   - Major life areas (d8).

3. **Environmental Factors (e):**
   - Products and technology (e1) for personal use in daily living (e115) and for personal indoor and outdoor mobility and transportation (e120). **Unanimous consensus.**

**TOPIC 3: Body function – focusing on “functions of the joints and bones” (b710-b729): contracture management**

**Discussion**

The term “contractures” denotes lack of full passive range of motion due to joint, muscle, or soft tissue limitations. Although joint contractures may in some cases have a compensatory function, their progression over time has a significant negative impact on motor function and autonomy, leading to fixed deformities and pain. The pathogenesis involves various factors, both intrinsic (muscle structural changes and fibrosis) and extrinsic (reduced active joint mobilization due to muscle weakness associated with a static position, compensatory postures, and agonist-antagonist muscle imbalance)¹³. It is important to consider the main clinical characteristics of the different forms of MD in order to identify joint groups and muscles at greater risk of tightness. Knowledge of specific natural histories is fundamental to identifying the progression phases and providing specific need-based preventive and personalized interventions (Tab. 1). The degree of muscle pathology progression is related to the frequency and severity of contractures. Lower limb contractures appear earlier and are more frequent, while upper limb contractures usually develop later, when ambulation is lost.

Although contractures are unavoidable in some cases, a preventive rehabilitation intervention, even for mild contractures, is important to minimize their negative effects on global function. For the lower limbs, careful stretching of muscles and joints (each position should be held for at least 15 seconds, and this should be repeated 10 to 15 times during a session) and daily standing or walking (a minimum of 2 to 3 hours) are recommended; so too, if necessary, are splinting and the use of orthoses to promote body segment alignment and proper posture¹³. For upper limb contractures, careful stretching is mandatory to maintain distal functions such as wheelchair driving.

In DMD, recent updated standards of care guidelines define the rehabilitation management of contractures on the basis of the natural history and stage of the disorder¹⁴,¹⁵. Muscle and joint groups at risk of tightness are
well documented. Lower limb contractures should be managed early starting from the ambulation stages, and continued into adulthood. Upper limb contractures should be monitored mainly from the stages of loss of ambulation. All interventions must be coordinated throughout all the stages of the disease. Stretching is recommended at least 4 to 6 times a week. Night-time use of resting and stretching ankle-foot orthoses (AFOs) is recommended from the early stages of ambulation, also to improve their tolerability. Daytime use of AFOs is indicated in the stages of loss of ambulation, to ensure adequate foot position in a wheelchair, or even in the ambulation stages (during “non-loading” time) in cases where they are not tolerated at night. Knee-ankle-foot orthoses (KAFOs) have a rehabilitation and non-functional purpose. They are indicated when contractures are mild or absent, and when the trunk still has good residual strength, in the late ambulation and early non-ambulation stages, in order to maintain standing and correct lower limb alignment. It has been reported that using KAFOs may extend walking ability in DMD by between 2 and 4 years. Standing can also be promoted through the use of standing devices, which are safer than KAFOs, reducing the risk of falls. Finally, serial casting is indicated in DMD when ankle-foot contractures are not manageable by means of stretching and orthoses, but surgery is not yet indicated. As regards other childhood and adult forms of MD, there is a lack of outcome measures, well defined natural history and recommendations on the management of contractures. Table I highlights the principal issues in this regard.

Another frequent orthopedic complication in MD is scoliosis, which frequently develops in patients with childhood-onset forms (such as congenital ones) in whom the skeletal apparatus is still growing and therefore much more susceptible to deforming forces. The development of scoliosis is a frequent complication of the late or non-ambulatory stages of DMD; bracing should be considered in order to maintain midline support and encourage symmetrical spinal alignment, so as to prevent or minimize the development/progression of scoliosis. From the neuromotor development perspective, it is important to define which function or activity to promote, always bearing in mind the presence of the brace (e.g., manipulation activities are easier in the sitting position).

In severe scoliosis, surgical intervention may be recommended; candidates for surgical intervention are non-ambulatory individuals with DMD who have a spinal curve greater than 20-30° in the sitting position, have not yet reached puberty, and have not been treated with corticosteroids because the curve is expected to progress.

In other forms of MD, other spinal abnormalities can be present, such as bent spine syndrome, rigid spine, or hyperlordosis, as seen in LGMD; plaster casts or bracing could be useful to support an antigravity posture but their use should be considered in relation to the specific patient’s activities and motor performances. Moreover, it is very important to use adequate customized postural supports that ensure body alignment and counteract abnormal positions, especially when the patient spends a lot of time in a wheelchair.

**Panel consensus**

Although joint contracture management is not extensively described for all forms of MD, the Jury agrees that it is crucial to maintain the patient’s motor function. A coordinated and integrated intervention, consisting of passive or active assisted stretching, and the use of orthoses, standing devices, and customized seating solutions is strongly recommended for all forms of MD. The intervention must be preventive, preferably starting before the development of contractures, and it should target the muscles and joints at greatest risk of tightness, on the basis of the natural history and stage of the single disorder (Tab. I).

As previously mentioned, the best characterized form of MD is DMD; in other forms, in the absence of natural history data, the Jury suggests that joint function should be managed with reference to the DMD classification, on the basis of the single patient’s functional stage.

In consideration of the above, the Jury reached the following consensus on statements:

- **Terminology:**
  - Stretching can be active (involving specific muscle contraction with elongation of a joint, performed by the patient as indicated by the therapist) or passive/“manual” (performed manually by therapist or the caregiver, without muscle contraction by the patient). **Unanimous consensus.**
  - Stretching can be “self-managed” (performed, after adequate training, by the patient or by the caregiver) or “rehabilitative” (performed by the therapist). **Unanimous consensus.**

- **Frequency and duration:**
  - Both in ambulant and in non-ambulant patients, stretching (self-managed and rehabilitative) of muscles and structures at risk of tightness in the different forms of MD should be performed not less than 4 to 6 times a week. If only self-managed stretching is performed, supervision by the therapist once a month is required. **Unanimous consensus.**
- In non-ambulant patients, stretching (self-managed and rehabilitative) should be performed only for mild contractures (e.g., joint tightness with preserved range of motion) or medium contractures (e.g., joint tightness with impaired range of motion). It should not be performed in the case of fixed contractures (such as in severe deformities). *Unanimous consensus*.
- The use of orthoses can be integrated with, but cannot substitute, stretching. *Unanimous consensus*.

**TOPIC 4: BODY function – targeting “muscle functions” and “movement functions”**

**Discussion**

The most controversial issue when considering exercise training in MD is the potential for exacerbation of the muscle damage as a consequence of the exercise itself. This phenomenon has various possible underlying causes. For example, it may be a direct effect of the exercise (especially eccentric high-resistance exercise) on muscle fibers, or due to various metabolic mechanisms (hypoxic/ischemic, adenosine triphosphate (ATP) deficit, oxidative stress, nitric oxide (NO) pathway impairment).

On the other hand, muscle weakness can also be a consequence of disuse, muscular atrophy, and deconditioning due to a sedentary lifestyle. In the healthy population, physical activity exerts several benefits, such as protection from obesity, metabolic syndrome, coronary heart disease, hypertension, and (at least in part) osteoporosis, and improvement of psychological and general well-being.

The panel discussed training and physical activity in MD, considering the World Health Organization’s standard definition of different types of exercise, according to which moderate-intensity aerobic activity is a physical activity that is performed at between 3 and < 6 times the intensity of rest, and is therefore relative to an individual’s personal capacity. In a consensus on care recommendations for physical therapy in DM1, “moderate exercises” are defined as activities that the individual can perform while still continuing a conversation and without having to stop to catch his/her breath.

With regard to muscle-strengthening activity (defined as exercise that increases skeletal muscle strength, power, endurance, and mass; e.g., strength training, resistance training, and muscle strength and endurance exercises), an updated Cochrane review examined clinical trials focusing on the effects of strength and aerobic exercise training in muscle diseases. Among the studies considered, only five were randomized and met all the criteria for inclusion in the review. Two of these dealt with DM1 and one with FSHD. The authors concluded that moderate-intensity strength training and aerobic exercise training appear to do no harm since no signs of overuse were reported, and that normal participation in sports and daily activities appeared to be safe.

For other forms of MD (LGMG, Becker muscular dystrophy, etc.), the available studies are few in number, and moreover report different protocols and heterogeneous results (see Table 1). However, knowledge of specific natural histories is always fundamental before suggesting physical exercise, given the need to avoid possible harmful effects (in terms of disease progression) of strength training.

Besides classical muscle exercise, neuromuscular electrical stimulation (NMES) is widely used in rehabilitation, offering the advantage of producing activation of fast fibers. However, data regarding the possible application of NMES in MD are still controversial due to the potential harmful effects of excessive muscle stimulation.

**Panel consensus**

The Jury reached the following consensus statements:

- The main objectives of a rehabilitation project/program (with regard to b730: Muscle power functions, b740: Muscle endurance functions, b770: Gait pattern functions; b455: Exercise tolerance functions) are:
  - to prevent no-use atrophy;
  - to maintain and optimize residual muscle strength;
  - to minimize progression of weakness when possible;
  - to support and optimize cardiorespiratory function;
  - to optimize exercise tolerance, energy efficiency, and energy conservation;
  - to contain stasis edema. *Unanimous consensus*.

- Terminology:
  - Physical activity: this includes “spontaneous non-structured activity” (i.e., normal activity during daily life), sports and “structured activity” (i.e., therapeutic exercise). *Unanimous consensus*.
  - Therapeutic exercise, prescribed by a specialist, should be defined by the following components: frequency, intensity, time, and type (F.I.T.T.). *Unanimous consensus*.
  - Both non-structured and structured activities and sports can include the two main exercise types: aerobic/cardiovascular fitness training (designed to improve cardiorespiratory endurance) and strength/resistance training (performed to im-
prove muscle strength and endurance). The latter can consist of concentric (shortening), isometric or eccentric (lengthening) contractions. Unanimous consensus.

- The term “muscle activation” should be used in rehabilitation programs rather than “muscle strengthening” or “strength training” or “resistance training”, to underline the importance of avoiding excessive loading (overload work) of dystrophic muscle. Unanimous consensus.

• General recommendations:
  - Spontaneous non-structured physical activity (ADL, free play) should always be encouraged. Unanimous consensus.
  - Sports activities:
    - Avoid contact sports, and competitive and non-competitive sports involving mainly eccentric exercise/activities. Unanimous consensus.
    - Competitive sports without specific eccentric exercise can be considered, exceptionally, in selected situations after critical clinician evaluation. Majority consensus.
  - Sports activities should always be readily accepted by the patients; swimming/water sports and low-resistance cycling are particularly recommended, while regular football training and tennis should be avoided. Wheelchair hockey and use of new technologies (e.g., Wii) are also encouraged. Unanimous consensus.

• Therapeutic exercise:
  - Eccentric exercise must be avoided, whereas concentric sub-maximal resistance exercises (“muscle activation”) and moderate aerobic training are recommended. Balance training should be included when deemed indicated and as prescribed by the clinician and rehabilitation team (patients can be evaluated by means of specific functional balance scales, such as the Berg Balance Test, gait analysis, and by recording the number of falls, which can indicate a balance impairment). Unanimous consensus.
  - Mean frequency: at least 3 times a week, for at least 30 minutes per session. Unanimous consensus.
  - Always consider patient-specific conditions, including compliance and any relational issues, and avoid unnecessary clinical interventions, which could have a negative impact on ADL. Unanimous consensus.
  - The 6MWT can be used as an outcome measure for endurance. Conversely, no reliable and feasible outcome measure for aerobic training is available at present, and more focused investigation is needed to fill this gap. Unanimous consensus.

- Postural hygiene and lymphatic drainage techniques including massage and compression garments should be promoted whenever these are deemed indicated by clinical experts. Unanimous consensus.

• Personalization and monitoring:
  - The patient’s status (including disease genotype, concomitant diseases, severity of weakness, pre-training level of activity – sedentary versus active) must always be carefully evaluated by the multidisciplinary team before a rehabilitation project/program or sports activities are prescribed. Outcome measures of muscle function (e.g., strength, endurance) and aerobic capacity (e.g., work capacity), and functional assessments are necessary. This evaluation must also include cardiological and respiratory assessment. Unanimous consensus.
  - Clinicians, patients and caregivers should bear in mind the possible risk of overwork weakness, and should be extensively warned about and trained to promptly recognize the following red flags:
    - significant muscle pain/soreness/cramps during or after exercise, or myoglobinuria in the 24 hours following a specific activity;
    - significant and prolonged weakness/fatigue after exercise (compared with basal condition);
    - significant (as per clinical judgment) elevation of CK compared with the patient’s basal CK level. N.B. The panel agreed that no specific or absolute cut-off values of CK can be established as a basis for clinical management decisions, given that this parameter is highly variable (being influenced by the specific form of MD, its phase, the severity of the disease, etc.). With regard to this parameter, the judgment of the physician in charge of the patient remains mandatory. Unanimous consensus

TOPIC 5: Activities and participation – focusing on mobility (d4): posture and mobility management

Discussion

Few studies have specifically explored the management of gait, balance, and manual abilities in MD. Øygard and co-authors demonstrated some improvements in gait spatiotemporal parameters after Bobath sessions in pa-
tients with LGMD and FSHD. Targeted exercises (focusing for example on ankle dorsiflexion, hand/finger movements, the diaphragm), balance training (to prevent falls), and aquatic therapy could be particularly appropriate especially in certain forms of MD.

Supported ambulation involving the use of assistive devices of different types (such as body-weight-supported treadmill, robotic-assisted training with exoskeleton) is anecdotally reported in MD patients, but these systems need further investigation.

The most updated Cochrane review on foot drop management evaluated possible therapeutic approaches that included ‘wait and see’ (i.e., no intervention), physiotherapy, surgery, and drug treatment. It was concluded that targeted strength training shows no positive effects in the treatment of foot drop in myotonic dystrophy and FSHD patients, and that early lower limb surgery in DMD children lacks consensus and remains controversial.

Loss of ambulation is a frequent complication in MD and, due to the significant variability of the different forms, can occur at different ages and be associated with different degrees of general motor disability. In these cases, products and technologies codified in the Environmental Factors chapter of the ICF (e.g., “Products and technology for personal use in daily living” – “Products and technology for personal indoor and outdoor mobility and transportation”) are very important to support mobility. The choice of a personalized manual or electric powered, indoor/outdoor wheelchair is fundamental and related not only to mobility factors, but also to the single patient’s expectations in terms of community participation at different stages of his/her life.

The guidelines for LGMD recommend the “prescription of assistive devices that are adapted specifically for the patient’s deficiencies”; in the same way, standards of care for DMD underline the importance of assistive technology and manual/powered wheelchairs as part of the rehabilitation management of these patients.

The Consensus Statement on Standard of Care for Congenital Muscular Dystrophies highlighted the importance of appropriate wheelchair prescription and customization, according to the child’s needs and level of disability. Standing and ambulation should be encouraged if deemed achievable on the basis of the individual child’s assessment.

The benefits of powered mobility are universally recognized, and consist of greater independence, increased QoL, and potential savings in social costs. Indeed, powered wheelchairs are no longer seen as simple mobility aids but as facilitators of participation and occupation. Additionally, they have direct therapeutic effects: powered wheelchairs are fundamental in optimizing medical management of patients with chronic disabilities, and they are equally important for minimizing discomfort and postural abnormalities. In the late stages of DMD, patients lose postural control, and need to use personalized adaptive seats, even in addition to wearing a brace. When the use of a brace is no longer feasible or tolerable, the half-reclining position, facilitated by the use of a chair with an anatomically adjustable back or with a padded seating shell, remains the only possible solution.

In these stages, the major clinical issues are orthopedic complications, including fractures and (kypho)scoliosis, cardiopulmonary involvement, and pain. At this point, powered wheelchairs offer major therapeutic benefits, particularly in the management of pain and for pressure relief.

Tilt-in-space systems are necessary to reduce pain and prevent bedsores, always bearing in mind the degree of spinal deformities, and should be considered even before the loss of independent pressure relief.

In patients with respiratory involvement, the wheelchair often requires specific adjustments to accommodate ventilatory equipment. For self-feeding, anterior trunk support and an elevated support for leverage to enable propping and leaning forward can also be necessary. To facilitate toileting routines, the use of a semi-reclining wheelchair can be helpful.

Panel consensus

In consideration of the above, the Jury reached the following consensus statements:

- The main objective of the rehabilitation project/program (with regard to d4: Mobility) is:
  - to maintain and optimize movement skills, manual skills, and postural changes and transfers. **Unanimous consensus.**

- General recommendations:
  - Functional orthoses should be considered to improve mobility and autonomy. **Unanimous consensus.**
  - Training for walking safety, including balance exercises, is suggested for as long as is possible; training for safe postural changes and transfers is essential. **Unanimous consensus.**
  - Appropriate manual or powered electric wheelchair prescription and customization is essential. **Unanimous consensus.**

**TOPIC 6: Activities and participation – focusing on “self-care” (d5) and “major life areas” (d8): activities of daily living (adl)**

**Discussion**

Improvement of QoL is one of the main targets in MD due to the progressive nature of these diseases.
ADL and function should be regularly assessed, so as to be able to increase the patient’s independence and safety through the use of transfer aids and adaptive equipment. Assistive devices, including ones incorporating robotic technologies, can play a significant role in increasing the daily-life autonomy of individuals with disability, but there are no specific studies on their use in MD patients.

Assessing cognitive and psychosocial aspects in relation to patient autonomy is also important as some forms of MD are also characterized by cognitive impairment, which further impacts on ADL management. Dany and colleagues, after investigating QoL in people with slowly-progressive neuromuscular diseases, emphasized that issues concerning the environment, social relationships, and the individual’s psychological state can be much more important than physical symptoms, which, from the patients’ perspective, do not always reflect their overall wellbeing. The psycho-emotional dimension of disability can include feelings like anger, disability non-acceptance, and in some cases feelings of rejection or humiliation. These elements are often difficult to address, but rather than allowing them to be overlooked, the rehabilitation project/program should take into account the psychosocial dimension.

Panel consensus

The Jury reached the following consensus statements:

- The main objectives of the rehabilitation project/program (with regard to d5: Self-care; d8: major life areas) are:
  - to support functional independence in ADL;
  - to support and optimize participation at school, work, and in the social environment;
  - to optimize and improve QoL. Unanimous consensus.

- General recommendations:
  - Promote sport to improve participation;
  - Include transfer aids and adaptive equipment to ensure the highest possible degrees of independence and safety;
  - Assistive technologies (e.g., ergonomic support, robotic manipulators, home automation, environmental control) should be considered in order to improve autonomy. Unanimous consensus.

TOPIC 8: The rehabilitation setting: outpatient vs home therapy

Discussion

When available, outpatient settings offer several advantages for the realization of the rehabilitation program, such as appropriate equipment, appropriate environments and devices, opportunities for socialization, and easier collaboration between the members of the multidisciplinary team. However, in some cases, for clinical and/or logistic reasons, a home therapy program can be required (e.g., when patients depend on vital equipment or lack adequate transportation, or when the journey would take too long).

Panel consensus

The Jury reached the following consensus statement:

- Home therapy should be considered for patients with severe motor impairment (i.e., bedridden patients, or those with severe cardiorespiratory impairment), for those needing very frequent treatments, and in situations where significant problems getting to the rehabilitation center (transport and travel problems, including lengthy or complex journeys) could un-
Management of motor rehabilitation in individuals with muscular dystrophies

dermine the objectives of the treatment. Unanimous consensus.

TOPIC 9: Duration/frequency

Discussion

Given the chronic and progressive nature of MD, the management of patients with these diseases needs to be understood as a life-long process. However, in defining the timing of rehabilitation projects/programs, it is very important to consider several clinical and logistic variables. The Jury agrees that rehabilitation interventions in children should generally be ongoing, as reported for all stages of DMD. According to expert opinion in this growing area of research, MD can interfere with several levels of neurodevelopment. However, it is important to avoid unnecessary and excessive interventions, so as to safeguard socialization and ADL participation, which are equally important.

With regard to adult patients, there is a heated debate, given the greater clinical variability in this population, even within single types of MD. It was generally agreed that, when drafting a rehabilitation project/program, it is very important to consider the specific rehabilitation objective of the treatment, which is based on the patient’s clinical condition, motor function, and compliance. Since all forms of MD are progressive, it is inappropriate to speak of a “stabilization” or “maintenance” phase.

Panel consensus

After extensive discussion, the Jury reached the following consensus statements:

- In children, the rehabilitation project/program should generally be ongoing, while avoiding excessive interventions that can interfere with socialization/ADL participation. Unanimous consensus.
- In adults, the definition of the rehabilitation objectives, and of the duration and frequency of interventions, must be the result of a careful multidisciplinary evaluation of the characteristics of the MD and of the patient’s clinical and functional conditions. Majority consensus.

Conclusions

The pressing need for appropriate and precise clinical recommendations for use in drawing up rehabilitation projects/programs is felt daily in the management of patients with MD. The purpose of this document, based on practical recommendations shared by a multidisciplinary panel of MD experts, is to provide clinicians, patients and caregivers with detailed, updated indications on the rehabilitation of MD patients, both children and adults. It is based on the main literature evidence and on expert opinions; it outlines the specific roles and responsibilities of the professional figures involved in the rehabilitation project/program, and provides technical indications in line with the F.I.T.T. model of physical therapy. Furthermore, it details practical measures for managing contractures, mobility and ADL. The document is valuable both for clinicians, being a tool that can be rapidly consulted in order to counsel patients, and for patients themselves, who need to be sure they are getting the right care at the right time in their disease history.

This study presents some methodological limitations, in part due to the heterogeneity of the scientific literature and outcome measures, and the lack of a precise definition of natural history data in most forms of MD. Moreover, the analysis does not cover important “modern tools” such as robotic assistive technology, digital platforms, and telerehabilitation systems, which are increasingly being developed, and whose importance has been especially appreciated in the course of the COVID-19 pandemic.

Despite these limitations, we anticipate that this Italian consensus document, commissioned by UILDM, may provide a basis for official standardized guidelines and open up a new scenario with regard to the patient-clinician alliance.

Ethical consideration

None.

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Conflict of interest

The Authors declare there are no conflict of interest.

Author contributions

ME Lombardo participated in the consensus conference and wrote the text.
All the Authors and The Italian Consensus Conference Groupe participated in the consensus conference and reviewed and approved the text.

References


44. Bendixen RM, Senesac C, Lott DJ, et al. Participation and quality of life in children with Duchenne muscular dystrophy using the International Classification of Functioning, Disability,


Splicing mutation in TAZ gene leading to exon skipping and Barth syndrome

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Barth syndrome is a monogenic X-linked disorder characterized by cardiomyopathy, skeletal myopathy and neutropenia. It is caused by deficiency of cardiolipin and associated with mutations in the tafazzin gene (TAZ). A 3 years old boy with dilated cardiomyopathy, neutropenia and growth retardation was investigated. Genetic screening found a new variant in the junction of intron 2 and exon 3 of the TAZ gene - c.239-1_239delinsTT. Functional analysis of the variant revealed the aberrant splicing of exon 3 leading to its complete excision from mature mRNA and frameshift at the beginning of tafazzin. Variant c.239-1_239delinsTT can be classified as pathogenic based on splicing alteration and typical clinical phenotype observed in TAZ mutation carriers.

Key words: Barth syndrome, TAZ, aberrant splicing, dilated cardiomyopathy, exon skipping

Introduction

Barth syndrome (BTHS) was originally described in 1983 as an X-linked syndrome of dilated cardiomyopathy, skeletal myopathy and neutropenia causing death in male infancy or early childhood. This syndrome is associated with mutations in the tafazzin (TAZ) gene at Xq28 that lead to cardiolipin deficiency and abnormal mitochondria. Tafazzin participates in the synthesis of mature cardiolipin (CL) – the necessary component of the mitochondrial membrane, critical for high-energy demand tissues. Lowered tafazzin activity destroys the formation of OXPHOS supercomplexes, mainly in the myocardium tissue and to the cardio-specific loss of the SDH, all these defects lead to the cardiomyopathy.

Several phenotypes have been associated with BTHS: dilated cardiomyopathy (most common), left ventricular noncompaction, endocardial fibroelastosis, hypertrophic cardiomyopathy. All these types are associated with the lack or massive suppression of CL synthesis and, as a result, with mitochondrial dysfunction.

Neutropenia, another main sign of BTHS, is typically observed in ~ 70% of patients and can be chronic or cyclic and doesn’t depend on patient’s age. It can be more (< 500 cells/mcl) or less (1,000-1,500 cells/mcl) severe; as a result various infections have been reported: recurrent mouth ulcers (4 and more episodes per year), pneumonia, blood infections and others.
Splicing mutation in TAZ gene

Muscle weakness – the third sign of the syndrome, is predominantly proximal and non-progressive during childhood. Most boys have more or less delayed gross motor milestones, then in adolescence they are able to walk but often find it hard to kick the ball or to run; up to 18 they usually reach normal height and body mass index 4,9.

We present a clinical report of dilated cardiomyopathy (DCM), neutropenia and skeletal myopathy associated with a TAZ splice site mutation in a 3 years old male patient.

Case presentation

The male patient was born with a weight of 2800 g and a body length of 50 cm. He is the second child in young unrelated parents. Since birth growth retardation (Tab. I) and the motor delay were observed. The boy suffered from increased fatigue, physical intolerance. He showed mild microcytic, hypochromic anemia, cyctic neutropenia with neutrophil 6-18%. There was also a history of infectious illnesses. In the first year of his life, he was treated from acute bronchitis, pneumonia, obstructive bronchitis; in the second year – acute bronchitis, carbuncle of the upper lip. At the age of 3 years 6 months, the boy was admitted to the intensive care unit with lethargy, pallor, puffy face, groaning breathing. Transthoracic echocardiography showed left ventricular dilatation: left ventricle (LV) diameters in end-diastole 38 mm (LVEDD indexed to BSA – 69,1 mm/m²), LV end-diastolic volume index 126ml/m², LV ejection fraction 39%. Moderate hypertrophy of the LV myocardium, predominantly of the posterior wall was observed. Moderate dilatation of the left atrium (LA) and right atrium (RA) was revealed (LA anterior-posterior and lateral-lateral diameters in the four-chamber 25*28 mm, RA anterior-posterior and lateral-lateral diameters right atrium 22*27 mm), right ventricle (RV) dimension in the four-chamber view was 20*36 mm. Right ventricular function wasn’t impaired (Tricuspid Annular Plane Systolic Excursion (TAPSE) – 12 mm). Mitral and tricuspid regurgitation corresponded with II and I degrees. The aortic valve is tricuspid, a function is not impaired. The pulmonary artery pressure is not increased. A small amount of pericardial effusion was detected with separation of pericardial sheets along the anterior wall of the RA up to 3 mm. Acute myocarditis of unspecified aetiology was diagnosed.

At the age of 4 years, the proband had hypostatura, blonde hair, rounded face, high, wide forehead, deep-set eyes, full cheeks, pointed chin, dimple under the lower lip, slightly protruding large ears. Speech development appropriated for his age. Parents noted the child prefer salty food with spices.

Cytogenetic study of his peripheral lymphocytes showed normal karyotype 46, XY. Tandem mass spectrometry of dry blood spot didn’t reveal any significant disturbances.

Clinical examination of family members showed that proband’s parents and sister didn’t have any cardiac symptoms or abnormal cardiac studies.

Genetics study

Genomic DNA was obtained from buccal epithelium by phenol/chloroform extraction. We performed the targeted next-generation sequencing (NGS) using the TruSight Cardiomyopathy sequencing kit on the MiSeq System (Illumina Inc., USA). List of 174 genes included in the NGS panel is presented in Table II.

The quality of raw NGS data was estimated with FastQC. The alignment was carried out with BWA against the reference genome NCBIbuild37 (UCSC hg19), the VCF files were generated with a GATK4 HaplotypeCaller. Variants were annotated by ANNOVAR using dbSNP IDs, NHLBI Exome Sequencing Project, The 1000 Genomes Project, the Genome Aggregation Database, ClinVar (2020), InterVar and REVEL. All the variations were classified according to the recommended method of the American College of Medical Genetics and Genomics.

A variant c.239-1_239delinsTT was detected in junction of intron 2 and exon 3 of the TAZ gene (NM_000116). It is located in the canonical splice site and predicted to alter splicing according to the Human Splicing Finder. Sanger sequencing confirmed this variant. Segregation analysis revealed that the mutation appeared de novo. The patient’s mother and sister showed a normal sequence of the TAZ gene (Fig. 1).

To confirm the alternative splicing of exon 3, total RNA of the patient and his mother were purified from

Table I. Patient’s physical development data.

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (g)</th>
<th>Percentiles</th>
<th>Body length (sm)</th>
<th>Percentiles</th>
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<td>4 years 4 months</td>
<td>11,900</td>
<td>&lt; 3</td>
<td>92</td>
<td>&lt; 3</td>
</tr>
</tbody>
</table>
whole blood using TRI reagent (Sigma-Aldrich, USA) according to the manufacturer’s instructions. Reverse transcription of the total RNA was performed with oligo-dT primers. RT-PCR was carried out with primers specific to exon-exon boundaries in the mature mRNA (transcript variant NM_000116; primer sequences available upon request). The expected size of the amplicons was 249 bp including the exons 1-4. The PCR-fragments were analyzed by electrophoresis in 2% agarose gel, extracted from gel and directly sequenced.

### Results and discussion

We identified new splice variant c.239-1_239delinsTT in TAZ gene of the boy with dilated growth retardation. He suffered from cardiomyopathy, cyclic neutropenia and various infections, both local and systemic. We confirmed the alternative splicing of exon 3 due to this variant. RT-PCR analysis showed two different amplicons: 249 bp in healthy mother (corresponds to the wild type) and 204 bp in patient (corresponds to the PCR-product without exon 3). Sequence analysis confirmed the variant c.239-1_239delinsTT led to the skipping of the exon 3 in the mature mRNA (Fig. 2). Thus the genetic reason for DCM in our patient was the complete excision of exon 3 causing a frameshift at the beginning of tafazzin.

To date, 12 splicing mutations in the TAZ gene have been reported in ClinVar as pathogenic or likely pathogenic. Several of them were found in the junction of intron 2 and exon 3 of the TAZ gene. Probably all of them result in the aberrant splicing of exon 3, but the functional analysis has been conducted for few. Interestingly, other

### Figure 1.

A) Pedigree of the studied family. The solid symbol indicates clinically affected subjects. The arrow denotes the proband. Symbols (+) and (-) indicate TAZ mutation carriers and non-carriers in X-chromosome, respectively. The absence of a symbol denotes that genetic analysis was not performed; B) NGS reads detecting mutation in intron-exon junction; C) Electrophoregram showing the DNA sequence for the junction of intron 2 and exon 3 in TAZ gene.

### Table II.

| ABCC9, ABCG5, ABCG8, ACTA1, ACTA2, ACTC1, ACTN2, AKAP9, ALMS1, ANK2, ANKRD1, APOA4, APOA5, APOB, APOC2, APOE, BAG3, BRF, CACNA1C, CACNA2D1, CACNB2, CALM1, CALR3, CASQ2, CAV3, CBL, CBS, CETP, COL3A1, COL5A1, COL5A2, COX15, CREB3L3, CRELD1, CRYAB, CSRP3, CTT1, DES, DMD, DYNAC19, DOLK, DPPE, DSC2, DSG2, DSP, DTNA, EFEMP2, ELN, EMD, EYA4, FBN1, FBN2, FHL1, FHL2, FKRIP, FKTN, FXN, GAA, GATAD1, GCKR, GJAS, GLA, GPD1L, GPIHPBP1, HAHA, HCN4, HFE, HRAS, HSPB8, ILK, JAG1, JPH2, JUP, KCN5, KCND3, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCQ1, KLF10, KRAS, LAMA2, LAMA4, LAMC1, LDB3, LDLR, LDLRAP1, LMF1, LMNA, LPL, LTBP2, MAP2K1, MAP2K2, MI1B, MURC, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLK, MYLK2, MYO6, MYOZ2, MYPN, NEXN, NXX2-5, NODAL, NOTCH1, NPFA, NRAS, FCSK9, PDLIM3, PKP2, PLN, PRDM16, PRKAG2, PRKAR1A, PTP11, RAF1, RANGRF, RBM20, RYR1, RYR2, SALL4, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SC02, SEPN1, SGCB, SGCD, SGCG, SHOC2, SLC25A4, SLC2A10, SMAD3, SMAD4, SNAT1, SOS1, SREBF2, TAZ, TBX20, TBX3, TBX5, TCFP, TGFBI2, TGFBI3, TGFBR1, TGFBR2, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TRDN, TRIM63, TRPM4, TTN, TTR, TXNRD2, VCL, ZBTB17, ZHX3, ZIC3 |
Splicing mutation in TAZ gene

Effects on splicing were shown for the base changes identified in the same locus - c.239-1G>C and c.239-1G>A. The first one abolishes splicing of intron 2, the second one reconstitutes the splice site with a 1 base shift. In any case, the mutations interfere with the translation of tafazzin and result in BTHS.

Mutations causing BTHS are of several types including splice site mutations, frameshifts, insertions, deletions, nonsense, and missense mutations. They lead to decreased or missing tafazzin enzymatic activity, with correspondingly more or less global changes in cardiolipin content and composition associated with the disease severity. Unfortunately, not for all TAZ gene mutations the correlation between genotype and phenotype is apparent.

Whited et al. (2013) developed and applied a BTHS-mutant panel in the yeast Saccharomyces cerevisiae. The authors introduced disease-causing variants into the Taz1p yeast ortholog to investigate loss-of-function mechanisms of tafazzin. As a result, seven functional classes of BTHS mutations were defined: (1) non-functional proteins resulted from frameshifts and splice-site variants, (2) submitochondrial mislocalization and erroneous aggregation of products, (3) altered assembly of tafazzin, (4) catalytically inactive proteins, (5) low expression of tafazzin, (6) products unable to engage in stable productive assemblies (7) temperature-sensitive proteins. The processes of cardiolipin biosynthesis and remodeling are conserved from yeast to humans and therefore identified mechanisms bring us closer to the understanding of the genetic basis and clinical variability of BTHS. Later some of these functional classes were documented in human tafazzin within human cells.

In conclusion, this is the first description of c.239_1_239delinsTT variant causing the Barth syndrome. This splice-site variant can be considered as pathogenic for the following reasons: abnormal splicing
of the TAZ leading to the skipping of the exon 3 (PVS1), de novo variant in the family without a history of DCM or heart failure (PS2), typical clinical phenotype observed in TAZ mutation carriers (PP4), absence in population databases Exome Sequencing Project, 1000 Genomes Project and Exome Aggregation Consortium (PM2).

Ethical consideration

Clinical surveillance and genetic investigations were performed by the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was duly obtained from all participants.

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Conflict of interest

The Authors declare no conflict of interest.

Author contributions

LS, ND and NZ conceived and planned the research. IM and LS carried out the clinical and genetic analysis. NZ contributed to the interpretation of the results. LS wrote the manuscript with support from ND and IM.

References


A novel DMD intronic alteration: a potentially disease-causing variant of an intermediate muscular dystrophy phenotype

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Pathogenic germline variants in DMD gene, which encodes the well-known cytoskeletal protein named dystrophin, are associated with a wide range of dystrophinopathies disorders, such as Duchenne muscular dystrophy (DMD, severe form), Becker muscular dystrophy (BMD, mild form) and intermediate muscular dystrophy (IMD). Muscle biopsy, immunohistochemistry, molecular (multiplex ligation-dependent probe amplification (MLPA)/next-generation sequencing (NGS) and Sanger methods) and in silico analyses were performed in order to identify alterations in DMD gene and protein in a patient with a clinical manifestation and with high creatine kinase levels. Herein, we described a previously unreported intronic variant in DMD and reduced dystrophin staining in the muscle biopsy. This novel DMD variant allele, c.9649+4A>T that was located in a splice donor site within intron 66. Sanger sequencing analysis from maternal DNA showed the presence of both variant c.9649+4A>T and wild-type (WT) DMD alleles. Different computational tools suggested that this nucleotide change might affect splicing through a WT donor site disruption, occurring in an evolutionarily conserved region. Indeed, we observed that this novel variant, could explain the reduced dystrophin protein levels and discontinuous sarcolemmal staining in muscle biopsy, which suggests that c.9649+4A>T allele may be re-classified as pathogenic in the future. Our data show that the c.9649+4A>T intronic sequence variant in the DMD gene may be associated with an IMD phenotype and our findings reinforce the importance of a more precise diagnosis combining muscle biopsy, molecular techniques and comprehensive in silico approaches in the clinical cases with negative results for conventional genetic analysis.

Key words: DMD gene, muscular dystrophy, dystrophinopathies, intronic sequence variant
**Introduction**

Dystrophinopathies are X-linked recessive disorders associated with pathogenic variants in the *DMD* gene (OMIM # 300377) which result in abnormal synthesis of dystrophin protein, a cytoskeletal protein with a major structural role in muscle. These disorders lead to muscle weakness and progressive degeneration of muscle function. Although most of the pathogenic variants in *DMD* gene are large rearrangements, it is estimated that 25-35% of affected patients have small-scale sequence variants affecting the dystrophin structure and/or function. These disorders are progressive neuromuscular commonly diagnosed between the ages of 2 and 6 years due to delay in walking, unsteady gait, frequent falls, and difficulty at climbing stairs as well as increased levels of serum creatine kinase (CK). The functional impact of genetic variants is believed to be the main driver of variability in clinical manifestations. Based on that, these disorders can be classified as Duchenne muscular dystrophy (DMD; OMIM #310200), when a patient presents the severe form or Becker muscular dystrophy (BMD; OMIM #300376) and intermediate muscular dystrophy (IMD), both characterized by early-onset, and by milder forms caused by a partially functional dystrophin.

Several clinical cases with an IMD phenotype have recently been reported in the literature mainly due to widely spread, availability and cost reduction of genetic analysis such as multiplex ligation-dependent probe amplification (MLPA) and next-generation sequencing (NGS) techniques. These molecular methods provide precise and early diagnosis combined with the microscopic study of invasive muscle biopsy. Also, advancements in NGS technologies have allowed improvements in molecular diagnosis and identification of new sequence variants in gene regions not previously evaluated, such as intronic alterations which constitute less than 0.5% of the currently reported causative variants but their value is presumably underestimated in dystrophinopathies.

In this study, we described a novel point variant in intron 66 of the *DMD* gene in a patient with intermediate manifestation of dystrophinopathy.

**Case presentation**

A 9-year-old boy patient from Rio Grande do Sul state (Southern region) of Brazil was born by cesarean delivery at 36 weeks, weighing 2495 g, head circumference 34 cm and discharged from hospital 48 hours later. His parents were not consanguineous. He sat independently at 9 months, never crawled, and walked at 22 months. He had no other comorbidities, never suffered any surgical procedure. His parents noticed that since he was three years old, he often fell on the ground, and had difficulties to go up the stairs, stand up or do physical activities.

On physical examination, the patient exhibited head circumference of 53 cm, medium and photoreactive pupils, eye movements preserved in all directions, posture with hyperlordosis and global hypotonia. Moreover, he had proximal muscle weakness in upper limbs and deep hyporeflexia, bilateral flexion-cutaneous-plantar reflex, calf pseudohypertrophy, and Gowers sign. Wechsler Intelligence Scale for Children IV (WISC-IV) intelligence tests revealed a low intelligence quotient (IQ) of 59, characterizing mild cognitive deficit. An elevated determination of creatine phosphokinase (CPK; 11.150 U/I) and aldolase (10.9 IU/L) enzymes were also observed. Other laboratorial tests showed: aspartate transaminase (AST) 282 mg/dL; thyroid stimulating hormone (TSH) 4.06 nIU/L; B12 vitamin 494 pg/mL; lactic acid 6.5 mg/dL. Skull magnetic resonance imaging (MRI) and transthoracic Doppler echocardiogram indicated no evidence of significant abnormalities. Considering these clinical features, specially impaired muscle function and high levels of CPK, this patient was referred to a molecular analysis of the *DMD* gene and muscle biopsy. There was no family history of muscle weakness or cardiac abnormalities. Indeed, the patient’s mother showed normal CPK levels (62 U/I) and normal echocardiogram.

**Methods and results**

Muscle biopsy was collected at the quadriceps muscle, frozen in isopentane cooled in liquid nitrogen and fresh-frozen cryostat sections were used for histochemistry, enzyme histochemistry and immunohistochemistry (IHC) analysis. Transverse serial frozen sections were stained with hematoxylin-eosin (HE) (Fig. 1A) and modified trichrome gomori (Fig. 1B) revealed great variation in muscle fiber sizes, round hypotrophic fibers and myonuclei internalization. Necrotic muscle fibers surrounded by myophagocytosis, increased endomysial connective tissue (masson trichrome stain) and interstitial adipose tissue was observed in several muscle areas. No intracytoplasmic vacuoles and no rods or ragged red fibers (RRF) were observed. The normal distribution of glycogen in muscle biopsy was evaluated by Periodic Acid Schiff (PAS) staining. Enzyme histochemistry did not show cytochrome c oxidase (COX) -negative/SDH- positive muscle fibers (Fig. 1C). Additionally, no conspicuous intra-vacuolar or perimysial amyloid deposits were revealed by Congo Red staining. Core- and target-defects in succinate dehydrogenase (SDH) (Fig. 1D) and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) were not observed in this sample.
A novel DMD intronic alteration: a potentially disease-causing variant of an intermediate muscular dystrophy phenotype

Muscle fibers exhibited diffuse sarcolemmal immunoreactivity for dysferlin (DYSF), neuronal nitric oxide synthase (nNOS) and laminin alpha 2. Strong diffuse immunoreactivity with alpha-sarcoglycan (data not shown) and caveolin-3 (Fig. 1E) in the membranes of muscle fibers were accompanied by diffuse emerin staining in myonuclei. Reduced and discontinuous sarcolemmal staining on hypotrophic fibers were evidenced in the immunoreactivity using a human -dystrophin monoclonal antibody that reacts with the N-terminal domain of this protein (Fig. 1F). Sparse satellite and regenerated muscle fibers were highlighted by diffuse emerin staining in myonuclei. A significant hypotrophy of type 1 fibers was verified in the slow myosin heavy chain (MHC) class I immunoreaction (data not shown). The source, clone and dilution of antibodies used for each staining are as follows: anti-dystrophin (Accurate Chemical & Scientific Corporation; Dy10/12B2;1:20), anti-nNOS (Santa Cruz Biotechnology; R-20; 1:70), anti-caveolin-3 (Abcam; ab2912; 1:100), anti-laminin-2 (Enzo; 4H8-2; 1:100), anti-SGCA (Sigma-Aldrich; polyclonal; 1:50), anti-DYSF (Abnova; Ham1/7B6; 1:20), anti-MHC class I (Abcam; W6/32; 1:130), anti-CD56 (Bio-Rad; Eric-1; 1:50), anti-CD68 (Dako; KP1; RTU) and anti-CD45 (Dako; 2B11 + PD7/26; RTU).

**Molecular and in silico analysis**

Afterwards, genomic DNA of the proband was isolated from peripheral blood leukocytes and the screening for exon deletions or duplications in the DMD gene was performed by MLPA technique using the SALSA® MLPA® P034 and P035 (DMD/Becker) kits (MRC Holland, Amsterdam, Netherlands), according to the manufacturer’s instructions. A reference DNA (no deletions and/or duplications in the DMD gene) was used as a normal copy number control. MLPA amplified fragments were separated by capillary gel electrophoresis in an ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and the results were analyzed using the Coffalyser.Net Software (https://coffalyser.wordpress.com/) (MRC®,

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**Figure 1.** Quadriceps muscle biopsy with features of dystrophy. A) Muscle fibres showing variation in size and atrophic fibres (white arrow) surrounded by endomysial connective tissue/adipose tissue (dark arrow) (Hematoxylin and Eosin, 10x); B) Muscle section with variation in fibre size, internal nuclei, increased endomysial connective tissue and adipose tissue (modified Gomori trichrome, 10x); C) and D) Oxidative enzymes showing variation in fibre size with slight predominance of type 1 fibres (open arrow) [cytochrome c oxidase/succinate dehydrogenase (COX/SDH) and succinate dehydrogenase (SDH), respectively; 10x]. (E) Immunolabelling of caveolin-3 using peroxidase label in the same muscle (20x). (F) Immunolabelling of dystrophin revealing some fibres with weak and uneven (open arrow; 20x) labelling compared with controls.
To confirm the origin of this DMD intronic sequence variant, genomic DNA was obtained from his mother (an obligatory carrier) and, after amplification by PCR using primers that flank the variant region previously described by Lenk and colleagues, the purified PCR product was analyzed by Sanger sequencing. The sequencing analysis showed the presence in heterozygosis of the c.9649+4A>T variant (Fig. 2).

Considering the lack of information regarding DMD c.9649+4A>T variant, an in silico approach was employed. First, the search for this intronic alteration in several population databases, including 1000 genomes Project, Exome Sequencing Project (ESP), Exome Aggregation Consortium database (ExAC), Genome Aggregation Database (gnomAD), and Online Archive of Brazilian Mutations (AbraOM), indicated that c.9649+4A>T was not previously reported in healthy individuals. Additionally, the variant was not described neither in Leiden Open Variation Database (LOVD), ClinVar nor in a specific DMD/BMD mutation database (TREAT-NMD DMD Global database). Based on this, it was considered a novel DMD sequence variant. Next, in silico analysis was performed in order to investigate the biological effect on splicing motifs (including exonic enhancers and silencers). Mutation Taster and Berkeley Drosophila Genome Project (BDGP) algorithms suggested that the c.9649+4A>T intronic variant might affect splicing through a WT donor site disruption. Supplementary Figure 1 depicts the output provided by Human Splicing Finder algorithm, showing the reduction in the splicing prediction raw scores comparing WT DMD sequence vs. variant sequence. Moreover, PhyloP score derived from alignment of 46 vertebrate species genomic sequences indicated that this nucleotide change occurs in an evolutionarily conserved region. The SpliceAI, a deep learning-based tool, predicts the identified variant to affect most probably the splicing (Delta score = 0.58). Finally, the c.9649+4A>T was classified according to the guidelines proposed by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) and by Sherloc classification system for the interpretation of sequence variants. After careful analysis of all available evidence about this novel DMD variant, it was classified as a variant of uncertain significance (VUS) by both classification approaches. Briefly, PM2 (pathogenic moderate evidence: absent from controls), PP3 and PP4 (supporting pathogenic evidence: multiple lines of computational evidence support a deleterious effect and patient’s phenotype is highly specific for a disease with a single genetic etiology, respectively) were the applied criteria using the ACMG guidelines. In contrast, EV0135, EV0184 and EV0024 (pathogenic evidence: absent from general population; variant involving a donor +3A/G, +4A or +5G; and weak functional evidence for protein function disrupted, respectively), as well as EV0211 (neutral evidence: first case report with the variant) were the selected criteria based on Sherloc framework.

Figure 2. Schematic representation of the DMD gene region encompassing the novel sequence variant c.9649+4A>T (indicated by the red arrow) in the splice donor site within intron 66 (upper panel; Created with BioRender.com), and bidirectional Sanger sequencing analysis from maternal DNA showing the presence of both variant and wild-type alleles (lower panel). Sense and antisense DMD sequences are shown by indicating the orientation of the DNA strands in the panels, as well as a range of 4-8 base pairs are underlined in both directions at the exon-intron junction in order to highlight this boundary. WT, wild-type sequence.
Discussion

More than 890 DMD pathogenic variants have been described so far (detailed information in: https://clinvarminer.genetics.utah.edu/variants-by-gene/DMD/condition/Duchenne%20muscular%20dystrophy/pathogenic), covering the different functional domains of dystrophin protein. The majority (~65%) of these causative variants are intragenic deletions/duplications that often lead to frameshift errors. Among the remaining ones, we find intronic alterations that usually create cryptic exons by activating potential splice sites. Of note, the pre-mRNA of DMD gene is composed by 99% of introns that exhibit a very complex pattern of expression and different alternative splicing, contributing to high rates of point variants, insertions and deletions.

This study presents a novel intronic sequence variant in the DMD gene, c.9649+4A>T in a patient at 9 years of age with an intermediate manifestation of muscular dystrophy. Remarkably, the variant was absent from all queried databases (1000 Genomes Project, ExAC, ESP, GnomAD, LOVD).

The novel DMD sequence variant described here is at the 5′ donor splice site of intron 66 which is essential during the post-transcriptional modifications, specifically the pre-mRNA splicing process. Disruptions on donor site, defined by the three terminal nucleotides of each exon and the first seven bases of the downstream intron, tend to generate an alternative 5′ splice site, resulting in different protein isoforms that leads to a wide variety of clinical Duchenne phenotypes.

Importantly, at the same splice donor site of intron 66, two different germline DMD variants at the nucleotide position +5 were previously reported in the LOVD database, namely c.9649+5 G>T and c.9649+5 G>A, being classified as pathogenic and likely pathogenic alterations, respectively. In another sequence variant, also in the same position, c.9649+5G>C, was classified as pathogenic in the ClinVar database. This finding represents an indirect evidence that nucleotide changes at c.9649+4 position might have functional impact associated with the splicing efficiency alteration in the DMD intron 66. In accordance with it, previous studies showed that the point intronic variants c.9649+1G>A, c.9649+2T>C and c.9649+2insT also at the 5′ donor splice site of intron 66, led to severe phenotype (Duchenne dystrophy). Furthermore, other point variants within or in the vicinity of the intron 66 region also leads to a range of variety clinical Duchene phenotype as variants c.9649+15T>C, c.9807+5G>A and c.9857+15C>T but its pathogenic effects are unknown.

Indeed, the novel DMD intronic variant is located within the cysteine-rich domain of the protein, consisting in a region required for β-dystroglycan interaction with dystrophin complex. Therefore, we can speculate that this genetic alteration might destabilize this complex, a functional consequence which could explain the discontinuous sarcolemmal staining observed in our muscle biopsy analysis, leading to the clinical manifestation of muscle weakness observed in the proband. As note, it is well known that germline DMD pathogenic variants in the cysteine-rich domain are among the possible underlying genetic defects associated with DMD phenotype.

As recently well reviewed, the global cognition functions are often affected in DMD patients, even in severe or mild phenotypes and, likewise, our patient also shows a mild cognitive deficit. At the same time, our patient has a healthy heart function, suggesting that the intronic variant reported here produces enough amounts of dystrophin protein in the cardiac muscle as observed in normal echocardiogram. Based on the muscle biopsy analysis and in silico results obtained in the current study, we may suggest that, even considering its uncertain clinical significance using both ACMG-AMP and Sherloc criteria, the c.9649+4A>T variant leads to a decrease in the dystrophin protein production levels and hypotrophic fibers as showed in Figure 1. Moreover, the reduced levels of dystrophin on patient’s muscle might explain his high levels of serum CK which can be released from dystrophic fibers. Further functional and molecular approaches such as patient-derived induced pluripotent stem (iPS) cells and analyses based on dystrophin reporter minigene are needed in order to characterize the tissue-specific effects of this novel variant in the dystrophin protein in detail. Indeed, segregation of clinical phenotype within the family may clarify the impact of this variant on its classification.

As limitations of this study, the RNA isolation from the biopsied muscle tissue of proband could not be performed due to the small amount of material collected. It would be important in order to evaluate the DMD transcript expression levels and abundance of specific isoforms. Furthermore, the amount of dystrophin protein in the brain or cardiac muscle in our patient was not evaluated. Finally, although this is the first report of the intronic DMD variant c.9649+4A>T and the clinical suspicion of molecular alterations in this gene was strong, our molecular approach was based on the single-gene analysis of one patient, not involving additional whole exome or genome sequencing tests to screen for potential causative variants in other genes.

Conclusions

Our data show that the c.9649+4A>T intronic sequence variant in the DMD gene may be associated with an IDM phenotype and further studies are needed.
to clarify the complete functional effects of this genetic alteration, specially its functional impact in the mRNA processing. Overall, our findings reinforce that the variant described here initially classified as VUS, may be re-classified in the future as likely pathogenic or pathogenic. Indeed, our study reinforces the importance of a more precise diagnosis combining muscle biopsy, new generation molecular techniques and comprehensive in silico approaches in the clinical cases with negative results for conventional genetic analysis.

**Ethical consideration**

Written informed consent was obtained from the parents of the index patient/proband for publication of this case report and accompanying laboratory tests results and images.

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We appreciate the cooperation of the patient and his parents for this study. Schematic representations were created with BioRender.com.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

**Author contributions**

APBS, IAV, GB and RS designed the study, coordi-
nated the project, performed sequence analysis, analyzed the data and wrote the paper; RS, FQ, provided patient samples and patient data. IVA designed and performed bioinformatics analysis. JCB, MLB performed immunohistochemical experiments and assisted in drafting and critical reading. ACBF, GB performed molecular experiments, assisted in drafting and critical reading. All authors read and approved the final manuscript.

References


33 Doorenweerd N. Combining genetics, neuropsychology and neuroimaging to improve understanding of brain involvement in Duchenne muscular dystrophy – a narrative review. Neuromuscul Disord 2020;30:437-442. https://doi.org/10.1016/j.nmd.2020.05.001


Combined high flow nasal cannula and negative pressure ventilation as a novel respiratory approach in a patient with acute respiratory failure and limb-girdle muscular dystrophy

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We describe the case of a 56-year-old-man with limb-girdle muscular dystrophy affected by acute hypercapnic failure secondary to pneumonia treated with high flow nasal cannula, intermittent abdominal ventilation, and negative pressure ventilation. The patient did not tolerate noninvasive positive pressure ventilation and refused invasive ventilation and tracheostomy. We successfully experienced a novel approach combining high flow nasal cannula with cycles of intermittent abdominal pressure ventilation and negative pressure ventilation.

Key words: high flow nasal cannula, intermittent abdominal pressure ventilation, negative pressure ventilation, limb-girdle muscular dystrophy

Abbreviations

HFNC: High flow nasal cannula
IAPV: intermittent abdominal pressure ventilation
NPV: negative pressure ventilation
NVS: noninvasive ventilatory support

Introduction

NVS (Noninvasive Ventilatory Support) is usually the therapy of choice for chronic respiratory failure in patients with muscular dystrophies, but some patients are intolerant of its use. In such patients, the high flow nasal cannula (HFNC) supportive therapy has emerged as a safe, useful therapy in acute and chronic respiratory failure, improving oxygenation and comfort. There are no data on the use of HFNC in patients with neuromuscular diseases.

Respiratory involvement is an almost constant feature of several muscular dystrophies, in particular of Duchenne muscular dystrophy (DMD) but also of some types of Limb- Girdle-Muscular-Dystrophy (LGMDs) and congenital types. Respiratory muscle weakness develops insidiously during the disease and patients need support that HFNC alone (Fisher & Paykel Healthcare AIRVO 2) cannot guarantee.
We now report a case of hypercapnic respiratory failure with intolerance to NVS where the alternate association of HFNC with cycles of intermittent abdominal pressure ventilation (IAPV with LunaBelt Dima Italia and negative pressure ventilation (NPV), PegasoVent DimaItalia with nylon poncho surrounding semi-cylindrical tent-like support) was successful and well-tolerated.

Case presentation

A 56-year-old-man affected by LGMD still in genetic staging, having acute respiratory failure, refused intermittent NVS because of intolerance, claustrophobia, and psychological reasons (fear of not being able to call family).

The patient was hospitalized with acute respiratory failure. At first, he was sedated and treated with NIV in pressure support ventilation with parameters: positive end-expiratory pressure 5 cm H\textsubscript{2}O, pressure support 10 cm H\textsubscript{2}O, respiratory rate 14/min, the fraction of inspired oxygen (FiO\textsubscript{2}) 63%; Arterial Blood Gas (ABG) showed pH: 7.45, pCO\textsubscript{2}: 43 mmHg, pO\textsubscript{2} 93 mmHg, Lac: 1,1, HCO\textsubscript{3}- 30,1 Mmol/l.

Clinical condition and ABG remained stable for two days during NIV, but after the onset of a nasal pressure sore, the patient became shaken and no longer tolerant to NIV, with worsening of ABG parameters (FiO\textsubscript{2} 60%, pH: 7.30, pCO\textsubscript{2}: 80 mmHg, pO\textsubscript{2}: 78 mmHg, HCO\textsubscript{3}-: 35.3 mmol/L).

We tried to replace the old mask with one that avoided nasal lesions, but the patient categorically refused to wear any type of mask.

The patient refused intubation and tracheostomy, too. Therefore, we started the HFNC with the following parameters: flow: 60 L/min FiO2 60%, temperature 37\textdegree C and IAPV (Pressure belt: 20 cm H\textsubscript{2}O; T inspiratory: 1,3 sec; respiratory rate: 14 bpm; rise time: 0,6 sec).

After 1 h of this ventilatory approach, ABG parameters improved: pH: 7.43, pCO\textsubscript{2}: 63 mmHg, pO\textsubscript{2}: 86 mmHg, HCO\textsubscript{3}:- 41 mmol/L. Alternate of cycles of HFNC - with a reduction of FiO\textsubscript{2} to 50% -and of IAPV lead to a stabilization of ABG parameters: FiO\textsubscript{2} 50%, pH: 7.47, pCO\textsubscript{2}: 56 mmHg, pO\textsubscript{2}: 68 mmHg, HCO\textsubscript{3}:- 37.4 mmol/L.

After 48h, the patient refused the IAPV treatment, so we alternated HFNC (Flow: 60 L/min, FiO2 50,% T 37\textdegree C) with NPV (P1: 40; PE: -01; F:14 I/E: 2.1:1) for 3/ day of 3 hours per cycle getting a significant improvement of ABG: (FiO\textsubscript{2} 50%, pH: 7.47, pCO\textsubscript{2}: 51 mmHg, pO\textsubscript{2}: 76 mmHg, HCO\textsubscript{3}: std: 31.7 mmol/L).

In the subsequent weaning, only NPV guaranteed support. Table I shows an arterial blood gas. The patient tolerated the latter treatment well and agreed to continue NPV at home. He reported more comfort and a feeling of better control over his condition.

Discussion

Limb-girdle muscular dystrophies are progressive muscular diseases in which respiratory complications may be one of the main causes of death\textsuperscript{1}. NVS should be the standard of care for respiratory support in patients with muscular dystrophies with survival benefit and upgraded quality of life. Many patients do not tolerate NVS and may have some episodes of acute respiratory failure during their disease with the risk of intubation or tracheotomy\textsuperscript{2}. NVS intolerance is one of the major elements for high intubation rates\textsuperscript{3}. Decubitus lesions, claustrophobia, or fear of not being able to call for help, can cause rejection or failure of NVS.

There are at least two reasons for not using dry oxygen via a nasal cannula: the FiO2 used is initially too high, and also the low-flow oxygen predisposes to epithelial lesions and dryness of the mucous membranes leading to scabs and crusts formation, some discomfort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NIV (FiO\textsubscript{2} 63%)</th>
<th>Ventimask (FiO2 60%)</th>
<th>1 hour: HFNC (FiO\textsubscript{2} 60%)-flow rate 60 L/min+IAPV</th>
<th>After 24 hour: (FiO\textsubscript{2} 50%)-flow rate 60 L/min+IAPV</th>
<th>1 hour: HFNC (FiO\textsubscript{2} 50%)-flow rate 60 L/min+NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.45</td>
<td>7.30</td>
<td>7.43</td>
<td>7.47</td>
<td>7.47</td>
</tr>
<tr>
<td>pO\textsubscript{2} (mmHg)</td>
<td>93</td>
<td>78</td>
<td>86</td>
<td>68</td>
<td>76</td>
</tr>
<tr>
<td>pCO\textsubscript{2} (mmHg)</td>
<td>43</td>
<td>80</td>
<td>63</td>
<td>56</td>
<td>51</td>
</tr>
<tr>
<td>SO\textsubscript{2} (%)</td>
<td>100</td>
<td>94.8</td>
<td>95.7</td>
<td>93.2</td>
<td>96</td>
</tr>
<tr>
<td>Lac (mmol/L)</td>
<td>1.1</td>
<td>1.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>HCO\textsubscript{3} (mmol/L)</td>
<td>30.1</td>
<td>35.3</td>
<td>41</td>
<td>37.4</td>
<td>31.7</td>
</tr>
</tbody>
</table>

ABG: arterial blood gas analysis; P\textsubscript{O\textsubscript{2}}: partial pressure of oxygen; P\textsubscript{CO\textsubscript{2}}: partial pressure of carbon dioxide; SO\textsubscript{2}: oxygen saturation; Lac: lactates, HCO\textsubscript{3}:- Bicarbonate
and irritation. Sometimes, it can also cause nosebleeds. HFNC oxygen treatment is effective in the management of adults with acute hypoxemic respiratory failure, and to a minor extent, in patients with acute hypercapnic respiratory failure or weaning. One of the major effects of HFNC in the nasopharynx is to wash CO\textsubscript{2}, which reduces dead space and increases the ratio of alveolar ventilation to minute ventilation, decreasing resistive work of breathing. The dead-space wash-out, nasopharyngeal resistance reduction, positive pharyngeal pressure, alveolar recruitment, oxygen dilution reduction, decreased work for breathing, and patient comfort. In muscular diseases with reduced functionality of the central drive, there is no sign of the use of HFNC. Despite the benefit of oxygenation and the small level of pressure generated, when muscle breakdown is advanced, HFNC cannot be used alone as the oxygen. Using HFNC during IAPV, and the use of a combined system, may enhance the advantages of both techniques. IAPV comprises an elastic inflatable bladder incorporated into a corset surrounding the abdomen. Through a ventilator which inflates bladder, the abdominal content and diaphragm move upward, assisting the expiration phase. With bladder deflation instead, the inspiration occurs passively. Only scattered reports on the use of IAPV are available and only one paper concerning its use in large populations of patients, in a regimen of noninvasive ventilator support. IAPV facilitates diaphragmatic motion and may be useful in patients with bilateral diaphragmatic weakness or paralysis. Negative pressure ventilation was successfully and predominantly used for long-term mechanical ventilation until the mid-1980s. Later, the interest waned, partly because the noninvasive positive pressure ventilation has proven to be more effective in patients with altered pulmonary or chest wall mechanics, and in those with obstructive sleep comorbidities apnea-hypopnea. Technology evolution has developed small and portable devices, while NPV needs a poncho, an interface more voluminous than a mask. NPV is a ventilation model in which sub-atmospheric pressure during inspiration affects chest surface, which determines the expansion of the thorax and a pressure decrease in the pleural space. This creates a pressure gradient that allows air to move from the airways to the alveolus. When the pressure around the thorax becomes less negative, the expiration takes place passively, thanks to the return of the lung and the rib cage.

IAPV was used in ALS tracheotomized patients to facilitate speech, and NPV is currently used in Duchenne muscular dystrophy patients and in post-polio syndrome. As far as we know, this is the first case of combined application of IAPV or NPV, in patients with limb-girdle muscular dystrophies.

**Ethical consideration**

None.

**Acknowledgement**

None.

**Funding**

None.

**Conflict of interest**

The Authors declare no conflict of interest.

**Author contributions**

PI and AA conceptualized the study, performed a literature review and drafted the manuscript. PI and LM performed a literature review and drafted the manuscript. AA and LM performed a literature review and collected data. GF critically revised the article. All authors read and the final manuscript.

**References**


9 Thomson A. The role of negative pressure ventilation. Arch Dis Child 1997;77:454-458. https://doi.org/10.11/adc.77.5.454


Anti-HMGCR antibodies and asymptomatic hyperCKemia. A case report

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Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) related myositis is a form of immune-mediated necrotizing myopathy (IMNM). Anti-HMGCR autoantibodies target HMGCR, a glycoprotein linked to the endoplasmic reticulum implied in the cholesterol synthesis pathway, and exert a pathogenic effect on skeletal muscle cells. More than 60% of patients affected by HMGCR-related myositis shares statin-exposure in their medical history. Patients commonly experience CK levels elevation, myalgia, muscle weakness and soreness at variable extent, which manifest acutely or sub acutely with a progressively worsening course, in some cases mimicking limb-girdle muscular dystrophies (LGMD) phenotype and treatment is based on an immunosuppressive strategy. Here we present the peculiar case of a previously statins-exposed 72 y.o. asymptomatic man with persistent moderate hyperCKemia and high levels of anti-HMGCR, in which pharmacotherapy has not been initiated yet, while a wait-and-see approach has been adopted instead.

Key words: necrotizing, myopathy, HMGCR, hyperCKemia, statin, antibodies

Introduction

Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) related myositis is a form of immune-mediated necrotizing myopathy (IMNM)¹ along with anti- signal recognition particle (SRP) myositis. Anti-HMGCR autoantibodies, first identified in 2010, target HMGCR, a glycoprotein linked to the endoplasmic reticulum, implied in the cholesterol synthesis pathway via conversion of HMG-CoA to mevalonic acid; the autoantibodies recognize the intracellular C-terminal end of the enzyme and exert a pathogenic effect on skeletal muscle cells². Strikingly, more than 60% of patients affected by HMGCR-related myositis shares statin-exposure in their medical history moreover, unlike anti-SRP myositis, anti-HMGCRs are not strictly related to malignancy³. Diagnostic criteria for anti-HMGCR IMNM include elevated serum creatine kinase (CK) levels, presence of anti-HMGCR autoantibodies and proximal muscle weakness mainly in the lower limbs. Muscle biopsy is not required for diagnosis if autoantibodies are detected, nevertheless distinctive histopathological features are the presence of scattered myofibers at various stages of necrosis, fibres regeneration and macrocytes infiltration, while lymphocytic infiltrate is usually absent or poorly represented, and staining for MHC I and Csb-C9 molecules can be positive⁴. On the clinical side, patients experience myalgia, muscle weakness and soreness at variable...
extent, which manifest acutely or sub acutely with a progressively worsening course, in some cases mimicking limb-girdle muscular dystrophies (LGMD) phenotype. Treatment is based on an immunsuppressive strategy in which corticosteroids but also methotrexate, rituximab and intravenous immunoglobulins have been safely and effectively employed in many cases. Here we present the peculiar case of a 72 year old asymptomatic man with persistent hyperCKemia and high levels of anti-HMGCR.

Case presentation

A 72 year old male patient referred to Neuromuscular Unit of Santa Chiara Hospital in Pisa in March 2018, for the detection of persistent moderate hyperCKemia (1000 U/L) on routine blood tests performed since December 2017, without muscular symptoms. The neurologic physical examination was normal. The patient’s medical history included two episodes of intracerebral haemorrage, hypertension, benign prostatic hyperplasia, type 2 diabetes, retinal thrombosis in the right eye. Notably, he also suffered from hypercholesterolemia, for which the patient had been taking Atorvastatin 40 mg per day for 2 years, discontinued soon after hyperCKemia discovery. The patient had been taking also Metformin 500 mg per day and Amlodipine 5 mg per day. No family history for neuromuscular disorders was reported. Subsequent dosages of blood CK levels confirmed an increase of values up to a maximum of 1500 U/L, while, at the six-monthly neurological assessments the patient remains asymptomatic. An electromyography study was made showing a myopathic pattern, with polyphasic, low amplitude MUPs, no spontaneous activity at rest and normal sensory and motor conduction velocities. In November 2019 a muscle biopsy on quadriceps femoris was performed, that showed rare hypo-atrophic, rounded and angled fibres, slight increase of perimysial and endomysial connective tissue and minimal peripheral rimmed positivity at oxidative enzymes staining (Fig. 1). Considering the muscle biopsy features, a genetic testing for LGMDs-associated genes (CAPN, DYSF, ANO5, CAV3, GAA) did not reveal DNA mutations. Myositis-associated autoantibodies including anti-HMGCR were dosed that detected a high anti-HMGCRs title (108.8 cU/ml, normal value < 20 cU/ml). The immunohistochemistry analysis for inflammatory markers was then conducted on the biopsy sample, showing that HLA (Mouse monoclonal anti-Human HLA-ABC Class I Antigen, clone W6/32, MHC I) and MAC staining were negative (Fig. 2). The muscle magnetic resonance imaging (MRI), performed on December 2020, showed a normal muscle trophism with mild fatty infiltration on the proximal part of the right biceps femoris and a minimal oedema on the triceps surae of both legs (Fig. 3). At the last follow-up visit, held in April 2021, the patient was persistently asymptomatic, without signs of muscle weakness but with a persistent hyperCKemia (1900 U/L). In consideration of the absence of symptoms, it was decided to follow the patient periodically to evaluate the clinical and biochemical trend, without any specific treatment.

Discussion

To our knowledge only few cases of completely and persistently asymptomatic patients diagnosed with anti-HMGCR IMNM have been reported in literature. Soares et al. recently described a patient with anti-HMGCR, increasingly high CK levels up to > 6000 U/L in the time of six years and no sign of muscle weakness. He was treated with intravenous immunoglobulins (IVIg) with consequent CK levels decreasing to 2700 U/L.
Anti-HMGCR antibodies and asymptomatic hyperCKemia. A case report

Anti-HMGCRs tend to be a very specific finding of inflammatory process, as it was not found in patients with other related diseases other than myositis. In our case, statins may be the cause of a cytotoxic myopathy frequently observed, while the other medications that the patient was taking (Meforin and Amiodipine) to our knowledge are not associated to hyperCKemia, neither alone nor in combination. The peculiarity of the case here presented is that CK values are slightly, but steadily increased through the last three years of clinical monitoring. According to the scientific literature, statin withdrawal is effective in the remission of myositis only in rare, mild cases, and many patients require immunosuppressive treatment. Notably, in our case muscle biopsy did not show any necrotic or inflammatory features but only minor and not specific myopathic changes. Even muscle MRI also did not show any specific pattern of inflammation or extensive involvement, but only minor and non-specific aspects that could be age-related and non-pathologic. Furthermore, the case we describe highlights the opportunity and importance of including the anti-HMGCR test in cases of hyperCKemia and previous exposure to statins, even when the clinical examination is unremarkable over time. The pathogenetic role of anti-HMGCRs was described by Bergua et al. in 2018, in an in vivo study that examined the effects of IMNM patients’ IgGs in mice. They observed muscle damage and complement activation, thus demonstrating the direct effect of antibodies on tissues and providing the basis for hypothesizing plasma exchange as an effective treatment in severe forms. Given the pathogenic role of autoantibodies, we believe that in these cases it could be useful to timely monitor their serum levels and compare them to CK levels. In milder or asymptomatic stable cases, like the one we describe, in our opinion, the timing and extent of the treatment should be questioned, because none of the therapeutic options currently in use does not carry potential side effects or risks, especially in current times of pandemic, when immunosuppressive therapy should be a considered choice. Consequently, in our case, we chose to proceed with clinical and laboratory follow-up instead of starting with drug administration, although we are aware that a broader longitudinal follow-up is necessary to establish more solid management indications, to identify early signs of clinical deterioration and initiate therapy promptly. In our view this case highlights the opportunity of testing anti-HMGCR antibodies in patients statin-exposed promptly. Notably, in our case muscle biopsy did not show any necrotic or inflammatory features but only minor and not specific myopathic changes.

**Ethical consideration**

The manuscript in part or in full has not been submitted or published anywhere. The manuscript has been at the EAN 2021 conference and is not under copyright.

**Acknowledgement**

Not applicable.

**Funding**

The study did not receive any funding.

**Conflict of interest**

No potential conflict of interest was reported by the authors.

**Author contributions**

FT wrote the paper.

GA processed and analyzed muscle tissue involved in the study.

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**Figure 2.** Muscle biopsy. HLA and MAC (C and D) were negative.
LC processed muscle tissue involved in the study and provided genetic analysis.
GS drafted the work and revised it.
GR approved the work for publication.

References
NEWS FROM AROUND THE WORLD

AIM

In the period between April and June 2021, several meetings held with the regional coordinators of the Association led to the organizing four webinars, one for each “Macro Areas” representative of Northern, Central and Southern Italy.

The webinars, aimed at disseminating and discussing issues related to neuromuscular diseases with local doctors and sharing information that allow an early diagnostic suspicion and the correct sending of the patient to the Reference Centers, will take place simultaneously on September 16, 2021. The topic of the webinars will be the management of patients with neuromuscular diseases in the territorial situations during the pandemic period: from the vaccination experience to telemedicine.

In the same period, six webinars took place under the patronage of AIM on topics discussed within the Advisory Board and addressed to general practitioners, neurologists, pediatricians, physiotherapists, nurses. Three of these events were in May 2021 (6/7, 20 and 27) and three in June (10, 17, 24). All the meetings, individually MCE accredited, had a great success of participation (further information is available at https://www.aim-fad2021.it/).

Prof. Carmelo Rodolico
AIM Secretary

MSM

Due to pandemics, the 14th Meeting of the Mediterranean Society of Myology (MSM) is moved to 2022. Proposals to organize and host the event are welcome.

WMS

The 26th WMS congress will take place, as a virtual meeting between 20 and 24 September. The 5-day congress week will be an opportunity to catch up on the latest developments in neuromuscular diseases from around the world. The Programme Committee has done a fantastic job to come up with an exciting scientific programme and they expect the quality of the submitted abstracts on all aspects of neuromuscular disease to be as outstanding as always. Controversial debates, oral lectures and e-poster presentations through the virtual platform and a range of stimulating industry symposia on a dedicated day are expected. The usual WMS 2021 Virtual Pre-Congress Teaching Course will be held on the neuromuscular field, so everyone who is interested is encouraged to register and participate. To learn more, submit an abstract and register for the congress, please visit the congress website: https://www.wms2021.com
FORTHCOMING MEETINGS

2021

June 12-15
The European Human Genetics Conference. Glasgow, United Kingdom. Information: website: https://eshg.org

June 19-22
7th Congress of the European Academy of Neurology (EAN), Vienna, Austria. Information: website: www.ean.org

June 25-26

July 16
262nd ENMC Preparatory Workshop: Standards of Care for the Dysferlinopathies. Information: website: https://www.enmc.org

July 20-22
12th Annual Congress of Cardiology-2021 (ICC-2021), Lisbon, Portugal. Information: website: www.bitcongress.com

September 17-19

September 20-24

September 20-24

October 1-3
The 3rd ENMC workshop on Dystroglycan and the Dystroglycanopathies. Information: website: https://www.enmc.org

October 3-7
XXV World Congress of Neurology (WCN 2021), Rome, Italy. Information: website: https://wfneurology.org/world-congress-of-neurology-2021

October 15-16
Mitochondrial Diseases Virtual Conference 2021. Information: website: www.mitocon.it

October 15-16

October 19-23

October 29-31

November 19-21

December 10-12

2022

January 28-30
254th ENMC Workshop: Formation of a European network to initiate a European data collection, along with development and sharing of treatment guidelines for adult SMA patients. Information: website: https://www.enmc.org

February 11-12

February 13-17

March 25-27

April 28-May 02
14th European Paediatric Neurology Society Congress, Glasgow, UK. Information: website: www.epns.org

May 13-15

October 10-15
INSTRUCTIONS FOR AUTHORS

Acta Myologica publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, case report, and advances in applied (translational) and basic research.

Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within eight weeks. All rebuttals must be submitted in writing to the editorial office.

Starting from 2020, a publication fee of 200 Euros is required. The Corresponding Author must fill in the appropriate form and send it with the corrected proofs. 50% off is offered for members of Associazione Italiana di Miologia (AIM) and/or Mediterranean Society of Myology (MSM) in good standing with dues. A copy of the payment receipt for the current year is mandatory to prove the membership.

On-line submission
Manuscript submission must be effected on line: www.actamyologica.it according to the following categories:

Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.
Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.
Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 5 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.

Lecture. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.
Congress Proceedings either in the form of Selected Abstracts or Proceedings will be taken into consideration.
Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.

The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1).

Title page. The AA are invited to check it represents the content of the paper and is not misleading. A short running title is also suggested.

Key words. Supply up to six key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.

Text. Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.

Illustrations. Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.

If the figure has been previously published a credit line should be included and permission in writing to reproduce should be supplied. Color photographs can be accepted for publication, the cost to be covered by the authors.

Patients in photographs are not to be recognisable

Tables: Tables should be self-explanatory, double spaced on separate sheets with the table number and title above the table and explanatory notes below. Arabic numbers should be used for tables and correspond with the order in which the table is first mentioned in the text.

References. Indicate all Authors, from 1 to 3. If their number is greater than 3, indicate only the first 3, followed by “et al.”. Arabic numbers in the text must be superscript. References in the list must be numbered as they appear in the text, with the reference number superscript. DOI number must be included with each reference (when available). If not available, indicate the PMID number.

Examples of the correct format for citation of references:

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