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Founders: Giovanni Nigro and Lucia Ines Comi

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All correspondence should be addressed to:
Mediterranean Society of Myology - Cardiomyology and Medical Genetics - Primo Policlinico - Piazza Miraglia - 80138 Naples, Italy - Tel. +39 081 566 5300 - Fax +39 081 566 5101.
Luisa Politano, via Ferrarecce 57, 81100 Caserta, Italy; email: actamyologica2@gmail.com

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ORIGINAL ARTICLES

Ambulatory Duchenne muscular dystrophy children: cross-sectional correlation between function, quantitative muscle ultrasound and MRI

Hala Abdulhady[1], Hossam M. Sakr[2], Nermine S. Elsayed[3], Tamer A. El-Sobky[4], Nagia Fahmy[5], Amr M. Saadawy[2], Heba Elsedfy[3]

[1] Department of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [2] Department of Diagnostic and Interventional Radiology and Molecular Imaging, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [3] Department of Medical Genetics, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [4] Division of Pediatric Orthopedics, Department of Orthopedic Surgery, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [5] Neuromuscular Unit, Department of Neuropsychiatry, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Duchenne muscular dystrophy (DMD) is a progressive genetic muscle disease. Quantitative muscle ultrasound (US), muscle MRI, and functional tools are important to delineate characteristics of muscle involvement. We aimed to establish correlations between clinical/functional and above-named imaging tools respecting their diagnostic and prognostic role in DMD children. A cross-sectional retrospective study of 27 steroid-naive, ambulant male children/adolescents with genetically-confirmed DMD (mean age, 8.8 ± 3.3 years). Functional performance was assessed using motor function measure (MFM) which assess standing/transfer (D1), proximal (D2) and distal (D3) motor function, and six-minute walk test (6MWT). Imaging evaluation included quantitative muscle MRI which measured muscle fat content in a specific location of right rectus femoris by mDixon sequence. Quantitative muscle US measured right rectus femoris muscle brightness in standardized US image as an indicator of muscle fat content. We found a highly significant positive correlation between the mean MFM total score and 6MWT (R = 0.537, p = 0.007), and a highly significant negative correlation between fat content by muscle US and MFM total score (R = -0.603, p = 0.006) and its D1 subscore (R =-0.712, p = 0.001), and a significant negative correlation between fat content by US and 6MWT (R = -0.529, p = 0.02), and a significant positive correlation between muscle fat content by mDixon MRI and patient's age (R = 0.617, p = 0.01). Quantitative muscle US correlates significantly with clinical/functional assessment tools as MFM and 6MWT, and augments their role in disease-tracking of DMD. Quantitative muscle US has the potential to act as a substitute to functional assessment tools.

Key words: dystrophinopathies, quantitative magnetic resonance imaging, muscle ultrasonography, motor function measure, 6-minute walk test

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Correspondence
Hossam M. Sakr
Department of Diagnostic and Interventional Radiology and Molecular Imaging Faculty of Medicine, Ain Shams University, Abbassia square, 11381 Cairo, Egypt.
E-mail: drhossam_sakr@med.asu.edu.eg

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Introduction

Duchenne muscular dystrophy (DMD) is a severe, progressive X-linked inherited disease that affects 1 in 3600-6000 live male births [1]. DMD oc-

curs as a result of mutations (mainly exon deletions) in the dystrophin gene (DMD; locus Xp21.2). The most common presenting feature is secondary deterioration of the motor milestones typically recognized at mid childhood. With time, untreated progressive muscle weakness, joint contractures, and cardio-pulmonary compromise affect quality of life. The disease eventually runs a fatal course by approximately 20 years of age [1,2]. This is especially true for untreated or steroid- naïve patients, whereas steroid-administered patients may show some amelioration of their clinical course. The management of DMD requires a multidisciplinary approach to improve the quality of life [2-4].

Therapeutic interventions have recently demonstrated intense upswings in modifying the natural history of DMD. These interventions include; therapies that slow the decline in muscle strength and function as glucocorticoids and therapies that target the pathology of DMD or improve muscle growth and regeneration [1,5]. Furthermore, gene therapies as gene-addition, exon-skipping, stop codon read through and genome-editing therapies aim at improving the expression and functionality of dystrophin protein. Other therapies work on a cellular level and aim at replacement of damaged muscle tissue. Both of the above therapies yielded encouraging preliminary results [6,7]. Nevertheless, these therapeutic gains have accentuated the need for consistent, valid and reliable assessment tools/ outcome measures to monitor responses to treatment and to act as prognostic indicators. Widely used clinical scales that measure the motor function and activities of ambulatory and non- ambulatory patients with DMD exist [8-10]. Recently significant interest in the use of magnetic resonance imaging (MRI) of muscles as whole-body MRI, quantitative MRI and magnetic resonance spectroscopy (MRS) to monitor disease progression has evolved [11-13]. MRI is a valuable non-invasive tool to reveal distinct pathologic patterns of various hereditary muscular diseases such as facioscapulohumeral muscular dystrophy [14], sarcoglycanopathies [15], congenital myopathies [14], muscular dystrophies [16], including DMD [14,17]. The use of quantitative muscle MRI and whole-body MRI have also been shown valuable to draw clinical-imaging-genetic correlations and identify ideal sites for muscle biopsy [11,14,16,17]. The combined use of quantitative muscle US and MRI showed promising results in regard to delineating disease stage and its relevance to functional status in facioscapulohumeral muscular dystrophy patients [18,19]. In DMD quantitative muscle MRI [20-22], and quantitative muscle ultrasound (US) [23,24], were found to be a satisfactory substitute to clinical/functional assessment tools as timed function tests and so forth in regard to monitoring disease progression.

Nevertheless, we are not aware of studies that investigated the clinical utility of quantitative muscle US and quantitative muscle MRI when used simultaneously with clinical functional tests in patients with DMD. The objectives of this study are: a) to assess the role of quantitative muscle US and muscle MRI as a potential diagnostic and prognostic tool in a series of ambulatory children with genetically-confirmed DMD; b) to draw relevant correlations between these two imaging modalities and clinical outcomes namely 6-minute walk test and motor function measure.

Materials and methods

This was a cross-sectional retrospective study. Twenty-seven steroid-naive, ambulant male children/adolescents with genetically-confirmed DMD were enrolled. The mean age of patients was 8.8 ± 3.3 years (range, 3.1 to 18). Exception to the reading frame rule occurred in one patient (18 years) with frameshift deletion of exon 3-7, who presented with Becker phenotype. This was likely due to either the presence of an alternative translation initiation site in exon 8 that was activated by the mutation, or due to genetic modifiers [25-27]. The functional performance of patients was assessed using the motor function measure (MFM) [28], and six-minute walk test (6MWT) [8]. MFM consists of 32 items (20 items for children < 7 years). It assesses all three dimensions of motor performance including standing and transfer (D1) subscore, axial and proximal motor function (D2), and distal motor function (D3). 6MWT is a commonly used timed functional test that also sufficiently monitors changes in muscle function. The imaging evaluation included quantitative muscle MRI, which measured muscle fat content in the right rectus femoris at the junction of the proximal 1/3 - distal 2/3 of thigh by mDixon sequence, and quantitative muscle US which measured muscle brightness in standardized US image as an indicator of muscle fat content. Twenty-five patients completed *both* functional tests; 21 patients completed the muscle US examination and 16 completed the muscle MRI examination. The study was approved by the Medical Ethics Research Committee of Faculty of Medicine, Ain Shams University, Egypt, number FMASU R 110 / 2021. Informed consent from participants was waived by the regulatory authority.

Assessment tools

The imaging and functional evaluation took place over two consecutive days where imaging evaluations were done prior to the functional tests. The patients who had sufficient cognitive ability to comply with verbal commands pertaining to the functional tests, were evaluated by the same examiner and approximately at the same time-point of the day. The functional tests were performed uniformly in the following order: MFM followed by 6MWT.

Motor function measure (MFM)

Items of the MFM-32 and MFM-20 are classified in three domains: D1: standing and transfers (13 items for the MFM-32 and 8 items for the MFM-20); D2: axial and proximal motor function (12 items for the MFM-32 and 8 items for the MFM-20); D3: distal motor function (7 items for the MFM-32 and 4 items for the MFM-20). Scores ranged from 0 to 3 as follows: (0) cannot perform the task, or cannot maintain the starting position, (1) initiated the task, (2) performs the movement incompletely, or completely but imperfectly (compensatory movements, position maintained for an insufficient duration of time, slowness, uncontrolled movement) and (3) performs the task fully and "normally" namely the movement is controlled, mastered, directed and performed at constant speed. The calculation of scores was expressed as a percentage in relation to the maximum score (for furthers details see https://mfm-nmd.org/).

Six-minute walk test

6MWT was performed according to the ATS guidelines, modified by having two examiners, one recording time and distances, and one staying close to the patient for safety issues [29].

Quantitative muscle MRI

mDixon sequence was taken using Achieva 1.5-T MR machine (Philips Medical Systems, The Netherlands) with the following parameters; (Slice thickness 10 mm, Spacing 5 mm, Number of phase encoding steps: 268, Acquisition matrix 272/0/0/268, Flip angle 15), the images were transferred to workstation (ViewForum R 6.3). mDixon sequence was done and the workstation was used to generate the four sequences (fat, water, in phase and out of phase). The mean fat and water signal of a region of interest (ROI) within the right rectus femoris muscle were measured at the junction between the proximal 1/3 and distal 2/3, between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella).

Quantitative muscle US

US in axial plane of the right rectus femoris muscle was taken at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella), using GE Logiq p7 machine (GE Healthcare, Waukesha, Wisconsin, USA) with high resolution linear probe 7-12 MHz. All the imaging parameters (including the probe frequency, depth, and gain) were constant, the images were transferred to a personal PC and mean grayscale (i.e. muscle echogenicity) was calculated using image histogram analysis software (ImageJ), and was used as an indication of muscle fat content. The quadriceps is a large proximal muscle which is frequently and severely involved by fatty infiltration in DMD and at a relatively early stage. Technique-wise its examination is practical [17].

Statistical methods

Data was revised for its completeness and consistency. Data entry was done on Microsoft Excel workbook. Quantitative data was summarized by mean, standard deviation while qualitative data was summarized by frequencies and percentages. The program used for data analysis was IBM SPSS statistics for windows version 23 (IBM Corp., Armonk, NY, USA). Chi-square test, student t test, and Pearson correlation coefficient were used in analysis of this study. Kappa test was done to measure level of agreement. A "P value" of less than 0.05 was considered statistically significant.

Results

Six patients (22%) were < 7 years of age. 19 patients (70%) had exon deletions, 2 (7%) had exon duplications, and 6 (22%) had small variants, two of which were nonsense and four were small deletions. Comparatively, patients with exon deletions had a lower mean MFM score without statistical significance. Tables I and II show the descriptive statistics of all clinical and imaging assessment tools used. We found a

Table I. Descriptive statistics of functional assessment tools.

	N	Mean	SD	IQR	Median	Min	Max
Age	27	8.7	3.4	6.6-10.2	8.6	3.1	18
MFM							
Total %	25	74.3	17.6	63.9-88.0	78.3	26.0	96.8
D1 %	25	57.7	26.8	37.1-76.9	61.5	0	97.4
D2 %	25	86.2	17.3	83.3-97.2	94.4	30.5	100
D3 %	25	84.8	13.5	75.5-95.2	85.7	42.8	100
6MWT (meters)	25	291.4	112.4	241.5-370	304.0	40	525

N: Number of patients; MFM: motor function measure; 6MWT: 6-minute walk test; SD: Standard Deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum

highly significant positive correlation between 6MWT and the mean total MFM score (R = 0.537, p = 0.007) and its D1 subscore (R = 0.751, p = 0.000) (Figs. 1,2). We found a highly significant negative correlation between fat content by muscle US and total MFM score (R = -0.603, p = 0.006) and its D1 subscore (R = -0.712, p = 0.001) (Figs. 3-5). Additionally, we found a significant negative correlation between fat content by muscle US and 6MWT score (R = -0.529, p = 0.02) (Tab. S3). This denotes that the higher the fat replacement in muscles of the thigh, the lower the scores of MFM and its D1 subscore and the lower meters achieved by patients in 6MWT. We found a significant positive correlation between patient's age and muscle fat content by mDixon MRI (R = 0.617, p = 0.01). However, we did not find a statistically significant correlation between both muscle fat content and muscle water content by mDixon MRI and neither total MFM score nor 6MWT (Tab. S4) (Figs. 6,7) (Fig. S9). Statistical correlations between age and both mean total MFM scores and subscores, and 6MWT scores were non-significant. For additional information, see (File S1). A graphical abstract of results is shown in Figure 8.

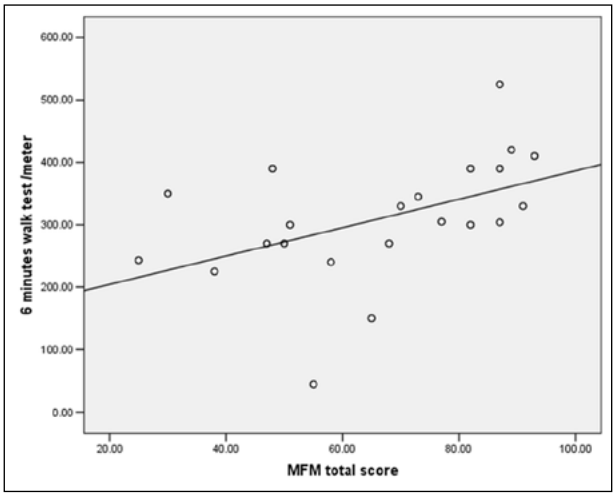

Figure 1. Correlation coefficient between Motor Function Measure (MFM) total score and 6-minute walk test showed a -p < 0.01- a highly significant **positive** correlation.

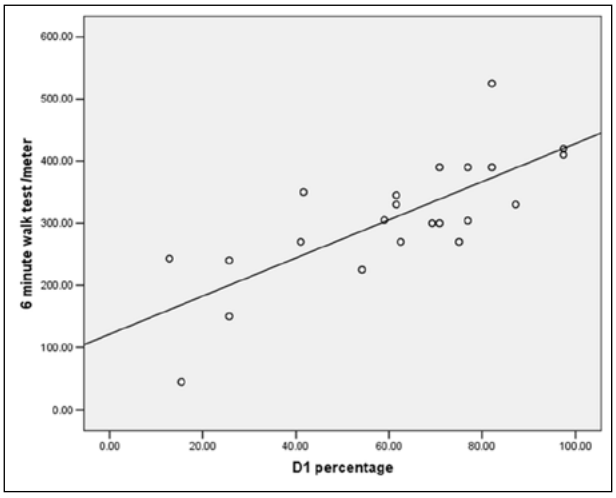

Figure 2. Correlation coefficient between motor function measure (MFM) D1 subscore percentage and 6-minute walk test showed -p < 0.01- a highly significant **positive** correlation.

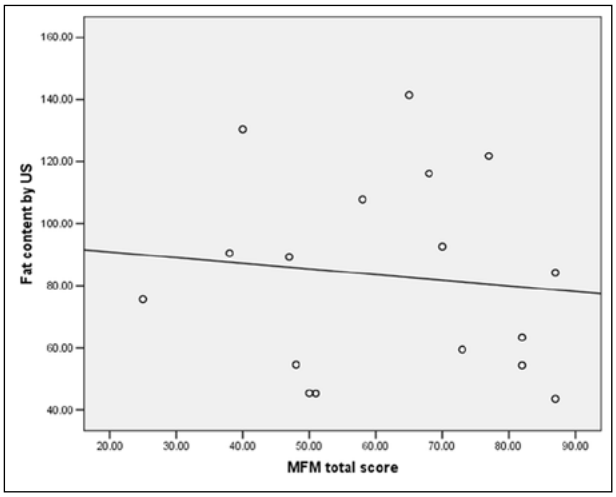

Figure 3. Correlation coefficient between motor function measure (MFM) total score and fat content by muscle ultrasound examination Showed a -p < 0.05 - a significant **negative** correlation.

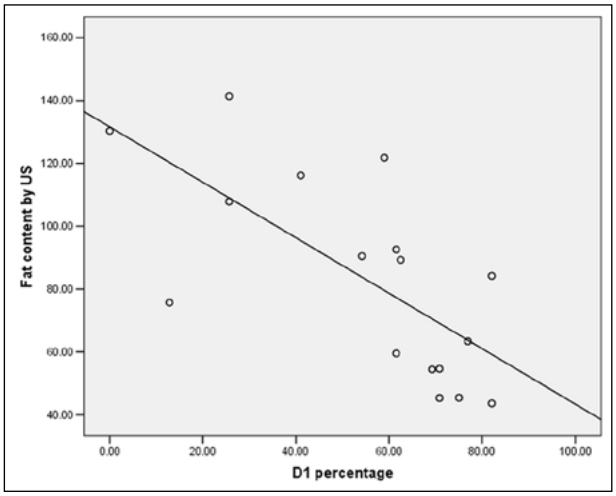

Figure 4. Correlation coefficient between motor function measure (MFM) D1 subscore percentage and fat content by muscle ultrasound examination -p < 0.01- a highly significant **negative** correlation.

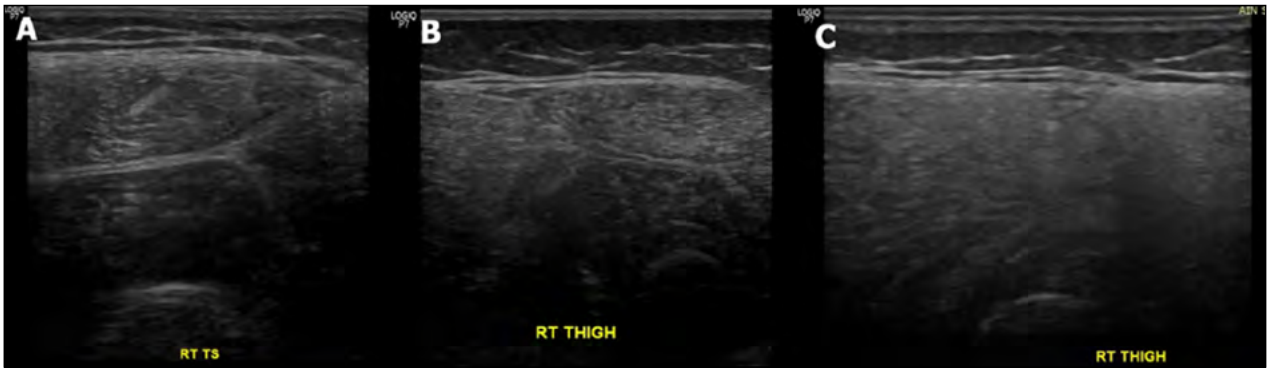

Figure 5A-C. Transverse US image of the right thigh of three different patients (A, B & C) showing different degrees of fatty infiltration of the quadriceps muscle as per increased echogenicity and blurred cortical surface of the femur. The mean histogram is 53, 76 and 80 respectively.

Table II. Descriptive statistics of imaging assessment tools.

	N	Mean	SD	IQR	Median	Min	Max
US	21	83.5	29.1	57.0-108.6	84.2	43.6	141.4
mDixon fat	16	77.9	60.7	44.1-86.5	61.6	30.2	272
mDixon water	16	375.1	145.7	321.2-468.7	431.3	52.1	535.7

N: Number of patients; SD: Standard Deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum

Discussion

Corticosteroids remain the mainstay of treatment in DMD children whereas gene therapeutic modalities are emerging [30,31], among others [32]. Some of these gene therapeutics have received regulatory approval in the USA, Europe and Japan [33]. Both treatment modalities aim at improving the child's functional status. Recent advances in therapeutics for the treatment of DMD children have sparked significant interest in finding reliable clinical and imaging assessment tools to monitor responsiveness to these treatment modalities. The current study has incorporated various clinical and imaging assessment tools to explore their diagnostic and prognostic role in genetically-confirmed ambulatory and steroid-naïve DMD children and adolescents. Whereas quantitative US depends on measuring muscle echogenicity using the mean gray scale of a ROI within a selected muscle [23], quantitative MRI depends on measuring muscle fat replacement [22,34]. Both quantitative imaging tools have proved to aid clinical assessment fundamentally.

Our study implications are twofold. Firstly, it confirmed the widely acknowledged role of MFM and 6MWT as reliable and clinically meaningful assessment tools in DMD children [8,21,28]. Secondly, our study introduced statistically significant positive correlations between scores of these clinical assessments tools, namely MFM and 6MWT on the one hand and quantitative muscle US in DMD children. This highlights the potential clinical utility of quantitative muscle US for DMD monitoring, and underscores the role of quantitative muscle US as an important complement to clinical functional assessment tools. This role has been elucidated in both cross-sectional [35] and longitudinal [23] study designs capable of monitoring disease progression in DMD children. Contrastingly, quantitative muscle fat content measured by US did not show a statistically significant correlation with patient's age.

Quantitative muscle MRI -as per T2 mapping and mDixon- has been found helpful for delineating upper [12,36] and lower extremity [22,37,38] muscle involvement, and to be a satisfactory predictor of both strength of the investigated muscles or of the overall function of the investigated extremity in DMD children. However, our results did not establish a similarly significant correlation between the scores of these clinical assessments tools, namely MFM and 6MWT and quantitative muscle MRI namely muscle fat content and muscle water content by mDixon MRI. This may be attributed to the limitations of our study. Including the genotype profile in the analysis would have been insightful for interpreting the clinical-imaging results. However, this was beyond the scope and objectives of this study, and is reserved for a separate study. Missing data of some tests were sporadic and attributed to patient-related transport barriers among others.

North Star Ambulatory Assessment and Performance of the Upper Limb Module are reliable outcome tools which are designed to assess functional mobility [39] and upper limb performance [40,41] among DMD children. Both

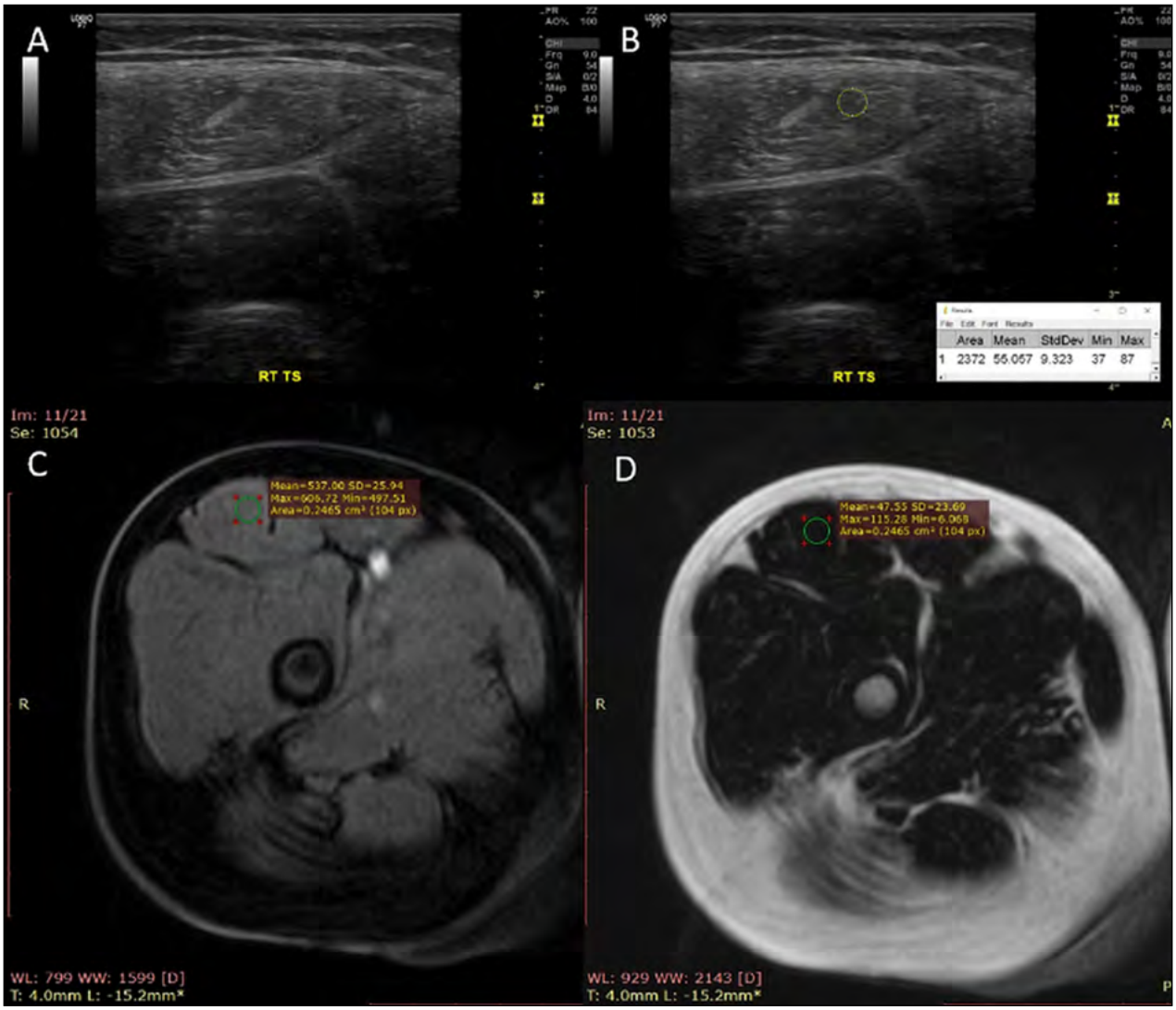

Figure 6A-D. A patient with Duchenne muscular dystrophy. A) Transverse US image of the right thigh taken at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella) showing fatty infiltration of the quadriceps muscle as shown by increased echogenicity and blurred cortical surface of the femur; B) the same image in (A) analyzed by ImageJ software with the ROI is within the rectus femoris muscle, mean histogram, 55; C,D) axial MRI image (mDixon sequence) with the region of interest (ROI) within the right rectus femoris muscle at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella), mean fat & water signals were 66 and 546 respectively.

outcome tools have been used to better characterize natural history, responsiveness to treatment and genotype-phenotype correlations in ambulatory [39,40] and non-ambulatory DMD children [41]. Although these outcome tools were not implementedin our cross-sectional study, we believe they represent valid substitutes for such.

Study limitations

Our study contained multiple clinical and imaging assessment tools (dependent variables). Additionally, its retrospective and cross-sectional nature does not allow for complete bias control in terms of standardization of patient characteristics among others. Moreover, longitudinal study designs have a greater potential to consolidate evidence for the use of both quantitative muscle US and muscle MRI as a substitute or a supplement to functional assessment tools in disease tracking and monitoring response to treatment of DMD patients. Further, the diversity of clinical assessment tools in general and functional tools in specific used across

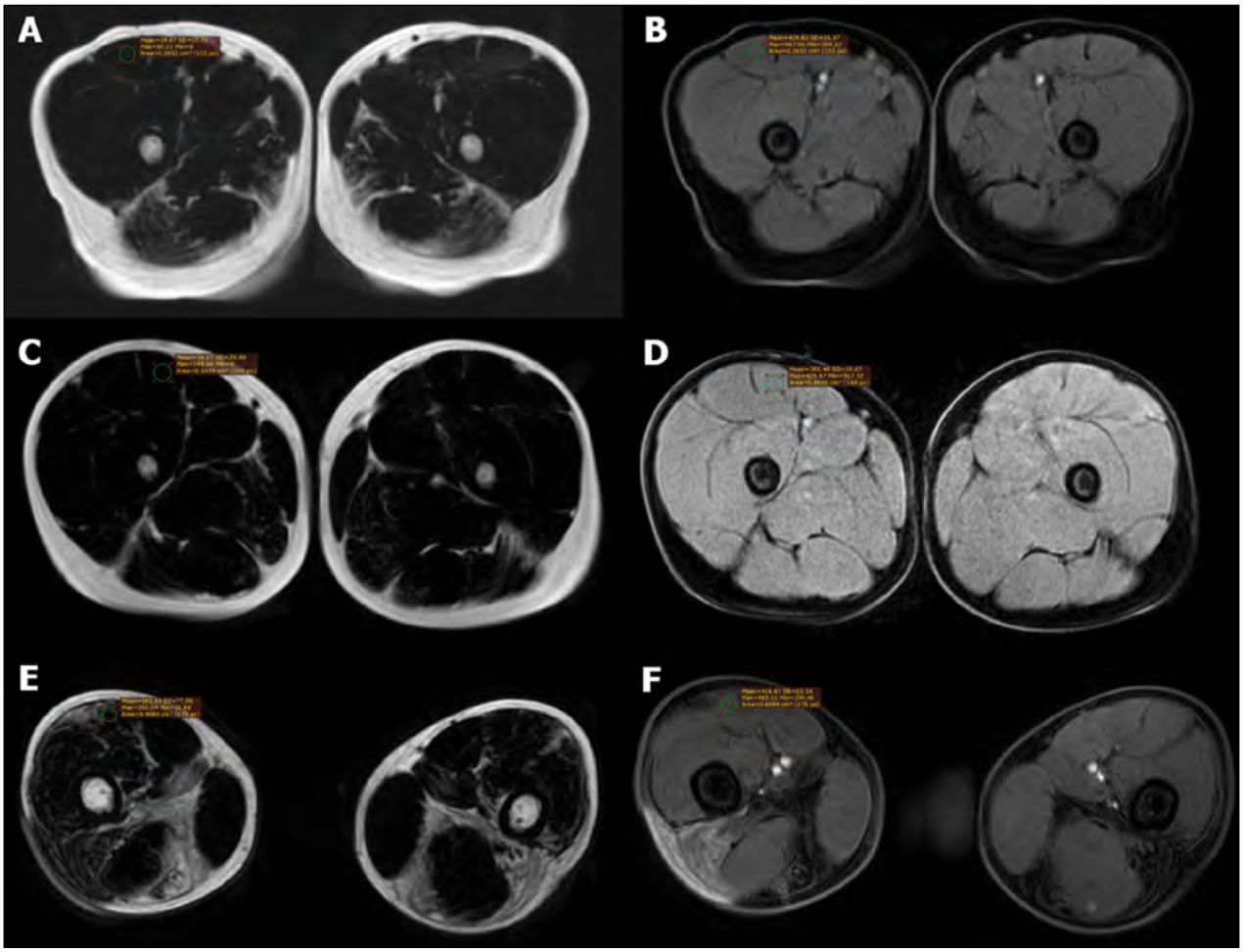

Figure 7A-F. Axial MRI image (mDixon sequence) in three different patients with DMD showing fat (A, C & E) and water (B, D & F) signal. The mean fat signals were 29, 35 and 143 respectively while those of water were 430, 351 and 416 respectively.

studies, need to be considered when interpreting the imaging-clinical correlation results [42]. Interestingly, quantitative muscle fat content measured by mDixon MRI showed a statistically positive correlation with patient's age. This is a clinically meaningful correlation, and it supports the assumption that the above-noted non-significant correlations between the clinical MFM and 6MWT scores, and quantitative fat content by mDixon MRI is mainly due study limitations. Additionally, of note is that statistical significance does not necessary equate to clinical significance. Consequently, all findings should be interpreted cautiously, and within clinical context. Results from the assessment of a single muscle by quantitative MRI and US, may not be fully representative of the overall muscle pathology.

Conclusions

Quantitative muscle US correlates significantly with clinical/functional assessment tools as MFM and 6MWT, and augments their promising role in disease tracking in DMD children. In that regard, quantitative muscle US has the potential to act as a complement to functional assessment tools. The presence of multiple clinical and imaging assessment tools (dependent variables) and study design-related limitations may have underpowered our statistical correlations of quantitative muscle MRI.

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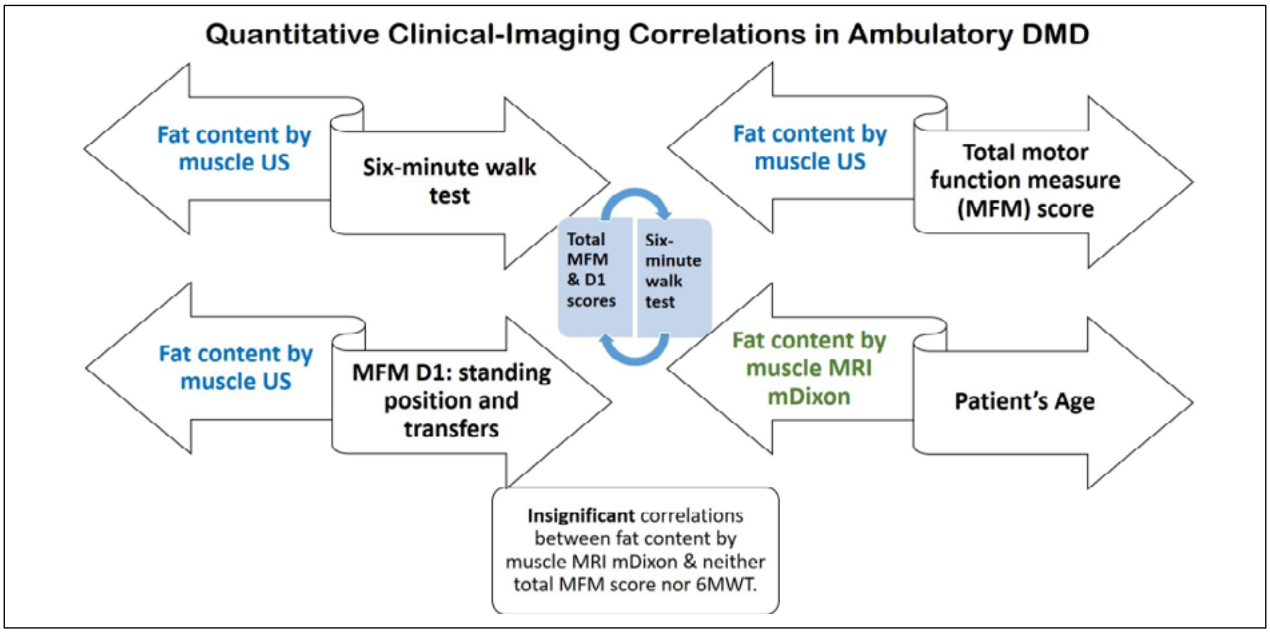

Figure 8. Graphical abstract: divergent arrows represent negative correlations while convergent the arrow represents a positive correlation.

The current manuscript has been posted on a preprint server (https://doi.org/10.1101/2021.08.17.21262119).

Conflict of interest statement

None of the Authors has any conflict of interest to disclose. No benefits in any form were received or will be received from a commercial party related directly or indirectly to the subject of this article.

Author's contributions

Conceptualization and design of study: HA, TAE, HMS, NSE; Acquisition and analysis of data: HA, NSE, HMS; Interpretation of data: NSE, HMS, NF, AMS, HE; Drafting the work: TAE assisted by NSE, HMS; revising it critically for important intellectual content; HA, HMS, NSE, NF, AMS, HE; All Authors approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical consideration

This study was approved by the Institutional Ethics Committee of the Faculty of medicine, Ain Shams University (FMASU R110/2021).

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Informed consent from participants was waived by the regulatory authority

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Supplemental material

Table S3. Correlation coefficient between age, functional assessment tools namely MFM and 6MWT and muscle fat content by ultrasound.

	US fat content
MFM total score	**R = -0.603** **P = 0.006****
D1 subscore	**R =-0.712** **P = 0.001****
D2 subscore	R = -0.127 P = 0.6
D3 subscore	R = 0.101 P = 0.6
Age	R = 0.237 P = 0.3
6MWT	**R = -0.529** **P = 0.02***

MFM: motor function measure; 6MWT: 6-minute walk test

Table S4. Correlation coefficient between age, functional assessment tools namely MFM and 6MWT and muscle fat and water content by mDixon MRI.

N = 16	**mDixon Fat**	**mDixon water**
Age	**R = 0.617** **P = 0.01***	R = -0.208 P = 0.4
MFM total score	R = -0.140 P = 0.6	R = -0.005 P = 0.9
D1 sub score	R = -0.235 P = 0.3	R = 0.056 P = 0.8
D2 sub score	R = 0.164 P = 0.5	R = -0.239 P = 0.4
D3 sub score	R = 0.152 P = 0.5	R = -0.228 P = 0.4
6MWT	R = -0.208 P = 0.4	R = 0.224 P = 0.4

*****Statistically significant; MFM: motor function measure; 6MWT: 6-minute walk test

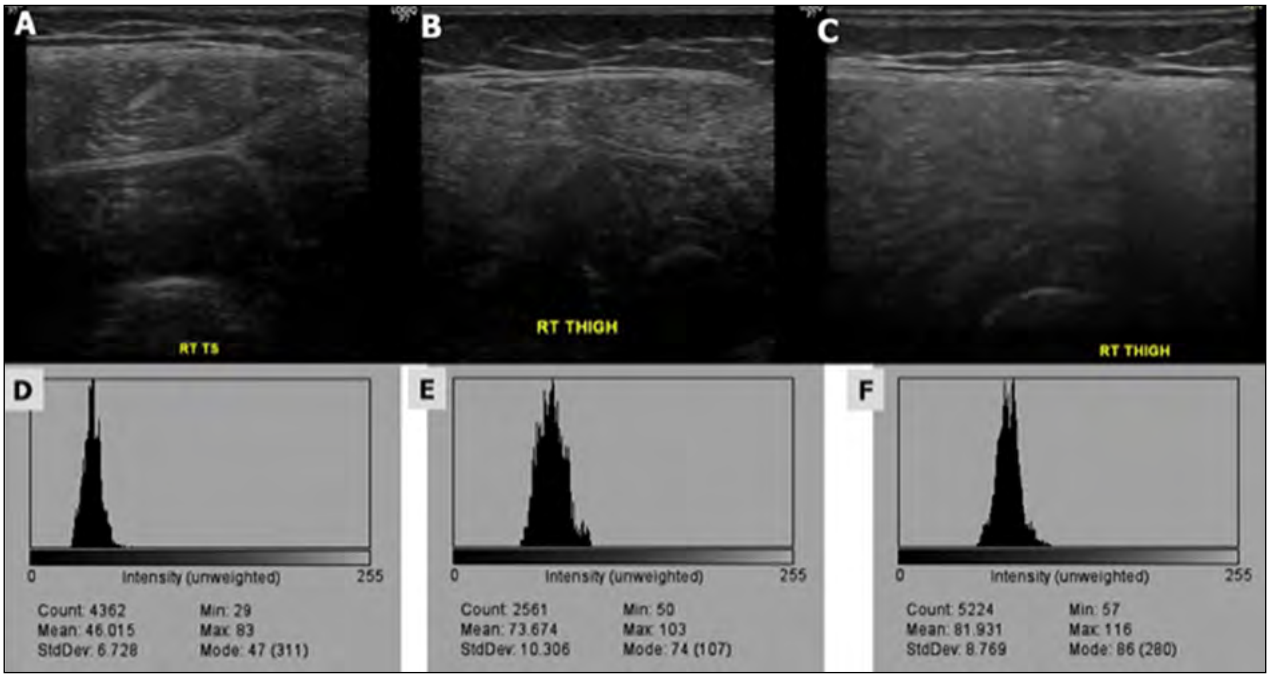

Figure S9. Transverse US image of the right thigh of three different children with DMD.

File S1. Tables S5 to S225

Table S5. Age distribution of studied patients.

N = 27	No.	%	
Age			
Below 7 years	7	25.9	
7 years or more	20	**74.1**	
	Mean	**SD**	**Range**
Age (years)	8.7	3.4	3.1-18
Median age	**8.6**		

This Table shows that 74.1% of the patients were 7 years or more of age. The mean age of patients was 8.7 years while the median age is 8.6 years.

Table S6. Distribution of type of mutation by molecular findings.

N = 27	No.	%
Deletion	19	**70.4**
Duplication	2	7.4
Small mutation	6	22.2

This Table shows that 70.4% of the cases had gene deletion and 22.2% had small mutation while 7.4% of the patients had duplication.

Table S7. Comparison between gene abnormality and the mean age of patients.

Age		Mean	SD	F	P
Deletion	N = 19	**9.3**	3.9		
Duplication	N = 2	7.5	0.07	0.7	0.4
Small mutation	**N = 6**	7.6	1.4		

P > 0.05 not significant. This Table shows a higher mean age among patients with deletion compared to other groups with no significant difference statistically. This table shows a lower mean age among cases with duplication or small mutation compared to deletion group with no statistically significant difference.

Table S8. Comparison between age of patients and type of gene mutation.

Age	Deletion No. %	Duplication No. %	Small mutation No. %	X2	P
Below 7 N = 7	5 71.4	0	1 28.6	0.8	0.6
7 or more N = 20	14 70.0	2 **10.0**	4 20.0		

P > 0.05 not significant. This table shows a higher percentage of small mutation among cases in group of 7 or above compared to cases below 7 years of age but the difference is not significant statistically.

Table S9. Comparison between the age groups in regard to the mean MFM D1 subscore.

D1 percentage	Mean	SD	t	P
Below 7 N = 7	62.5	12.5	0.5	0.8
7 or more N = 20	55.8	30.7		

P > 0.05 not significant. MFM: motor function measure. This table shows no statistically significant difference between the two age groups as regards D1 subscore of the motor function measure. A higher mean D1 subscore in younger age group compared to older age group with no statistically significant difference.

Table S10. Comparison between the age groups in regard to the mean MFM D2 subscore.

D2 percentage	Mean	SD	t	P
Below 7 N = 7	81.5	17.1	0.8	0.4
7 or more N = 20	88.1	17.5		

P > 0.05 not significant. MFM: motor function measure. This table shows a higher mean D2 subscore among older age group with no statistically significant difference.

Table S11. Comparison between the age groups in regard to the mean MFM D3 subscore.

D3 percentage	Mean	SD	t	P
Below 7 N = 7	79.7	9.4	1.1	0.2
7 or more N = 20	86.7	14.5		

P > 0.05 not significant. MFM: motor function measure. This table shows a higher mean D3 sub score among older age group patients with no statistically significant difference.

Table S12. comparison between the age groups in regard to the mean MFM total score.

MFM total score (%)	Mean	SD	t	P
Below 7 N = 7	73.5	13.2	0.1	0.8
7 or more N = 20	74.7	19.3		

P > 0.05 not significant. MFM: motor function measure. This table shows no significant difference statistically between the two age groups as regards the mean total motor function measure percentage.

Table S13. Comparison between the age groups as regards the 6-minute walk test (6MWT).

6MWT	Mean	SD	t	P
Below 7 N = 7	302.0	54.8	0.2	0.7
7 or more N = 18	287.3	129.3		

P > 0.05 not significant. This table shows no significant difference statistically between the two age groups as regards the mean meters of the 6-minute walk test. The table shows a lower mean value for 6-minute walk test among cases above seven years of age compared to younger age group with no statistically significant difference.

Table S14. Correlation coefficient between the age and MFM scores i.e. total score and subscores.

	Age
MFM total score	R = 0.044 P = 0.8
D1 subscore	R = -0.007 P = 0.9
D2 subscore	R = 0.063 P = 0.7
D3 subscore	R = 0.163 P = 0.4

P > 0.05 not significant. This table shows no significant correlation between the total MFM score or the subscores and the age of DMD patients.

Table S15. Distribution of rapid decline of ambulation according to the 6-minute walk test. Cut-off value 350 meters.

N = 25	No. %	95% CI
No Rapid decline	**7** **28**	**12.0-49.0**
Rapid decline	**18** **72**	**50.6-87.9**

This Table shows that 72% of the patients have rapid decline in ambulation in the next 48 months as predicted by the results of the 6-minute walk test below 350 m while 28% have no rapid decline in ambulation.

Table S16. Distribution of rapid decline of ambulation according to the 6-minute walk test. Cut-off value 300 meters.

N = 25	No. %	95% CI
No Rapid decline	**15** **60**	**38-78**
Rapid decline	**10** **40**	**21-61**

This Table shows that 40% of the patients have rapid decline in ambulation in the next 48 months as predicted by the results of the 6-minute walk test below 300 m while 60% have no rapid decline in ambulation.

Table S17. Correlation coefficient between the MFM results, age, 6-minute walk test and mDixon fat and water findings of MRI.

N = 16	mDixon Fat	mDixon water
Age	**R = 0.617** **P = 0.01***	R = -0.208 P = 0.4
MFM total score	R = -0.140 P = 0.6	R = -0.005 P = 0.9
D1 sub score	R = -0.235 P = 0.3	R = 0.056 P=0.8
D2 sub score	R = 0.164 P = 0.5	R = -0.239 P = 0.4
D3 sub score	R = 0.152 P=0.5	R = -0.228 P = 0.4
6MWT	R = -0.208 P = 0.4	R = 0.224 P = 0.4

*P < 0.05 significant. This table shows a significant positive correlation between mDixon fat values and age of patients. This Table shows no correlation between mDixon fat values and MFM, 6-minute walk test values. This table shows no correlation between mDixon water content and MFM, 6-minute walk test.

Table S18. Correlation coefficient between the MFM results, age, 6-minute walk test and MRS Press and MRS steam findings of MRI**.**

N = 16	MRS Press	MRS steam
Age	R = 0.058 P = 0.8	R = 0.047 P = 0.8
MFM total score	**R = 0.651** **P = 0.009****	**R = 0.660** **P = 0.01***
D1 sub score	**R = 0.594** **P = 0.03***	R = 0.469 P = 0.09
D2 sub score	**R = 0.538** **P = 0.03***	R = 0.393 P = 0.1
D3 sub score	**R = 0.534** **P = 0.04***	**R = 0.791** **P = 0.001***
6MWT	R = 0.373 P = 0.1	R = 0.310 P = 0.2

*P < 0.05 Significant, **P < 0.01 highly significant.
This Table shows a highly significant positive correlation between MRS press values and total MFM score.
This Table shows a significant positive correlation between MRS press values and D1, D2, D3 subscores of MFM. This Table shows a significant positive correlation between MRS steam and total MFM score. This table shows a highly **significant positive correlation** between D3 subscore and the MRS steam findings of MRI.

Table S19. Correlation coefficient between the MFM results, age, 6-minute walk test and DTI finding of MRI results**.**

N = 14	DTI. FA	DTI. ADC
MFM total score	R = -0.438 P = 0.1	R = 0.417 P = 0.1
D1 sub score	**R = -0.640** **P = 0.01***	**R = 0.617** **P = 0.01***
D2 sub score	R = -0.283 P = 0.3	R = 0.246 P = 0.3
D3 sub score	R = 0.004 P = 0.9	R = 0.032 P = 0.9
age	R = 0.402 P = 0.1	R = -0.404 P = 0.1
6MWT	**R = -0.701** **P = 0.004****	**R = 0.782** **P = 0.001****

*P < 0.05 Significant, **P < 0.01 highly significant. This Table shows a significant negative correlation of D1 subscore and the DTI FA findings. Which denotes the higher D1 scores associated with lower DTI FA scores. This Table shows a significant positive correlation of D1 subscore and the DTI ADC findings. Which denotes the higher D1 scores associated with high DTI ADC scores.

Table S20. Comparison between type of gene mutation and the screening of patients with 6-minute walk test. Cut-off value 300.

300	No rapid decline No. %	Rapid decline No. %	X2	P
Deletion N = 18	9 50.0	9 **50.0**	2.9	0.08
Others N = 7	6 85.7	1 14.3		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with deletion 50% compared to 14.3% among patients with other gene mutation with border line significance.

Table S21. Comparison between type of gene mutation and the screening of patients with 6-minute walk test. Cut-off value 350.

350	No rapid decline No. %	Rapid decline No. %	X2	P
Deletion N = 18	4 22.2	14 **77.8**	1.0	0.3
Others N = 7	3 42.9	4 57.1		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with small mutation or duplication 85.7% compared to 50% among patients with deletion with border line significance.

Table S22. Correlation coefficient between the ultrasound findings of fat content and the MRI findings of patients with DMD.

	US fat content
mDixon Fat	**R = 0.743** **P = 0.002****
MRS steam	R = -0.139 P = 0.6
MRS press	R = 0.294 P = 0.3
DTI FA	R = 0.396 P = 0.1
DTI ADC	R = -0.342 P = 0.2

*P < 0.05 Significant, **P < 0.01 highly significant. This table shows a highly significant positive correlation between ultrasound fat content and the mDixon fat content among patients with DMD. This table shows no correlation between ultrasound fat content and MRS steam, MRS press, DTI FA and DTI ADC.

Table S23. Comparison between type of gene mutation and the ultra sound grouping of patients (fat content of muscles). Cut-off value 84 (median value of US).

	Low fat content **No.** **%**	**High fat content** **No. %**	**X2**	**P**
Deletion N = 13	6 46.2	7 53.8	0.02	0.8
Others N = 8	4 50.0	4 50.0		

P > 0.05 not significant

Table S24. Comparison between US fat content and the screening of patients with 6-minute walk test. Cut-off value 300 for screening test.

US	**No rapid decline** **No.** **%**	**Rapid decline** **No.** **%**	**X2**	**P**
Low fat N = 10	7 70.0	3 30.0	2.5	0.1
High fat N = 9	3 33.3	6 **66.7**		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with high fat content in US 66.7% compared to 30% among patients with low fat content and the difference is border line significant.

Table S25. Comparison between US fat content and the screening of patients with 6-minute walk test. Cut-off value 350 for screening test.

350	**No rapid decline** **No.** **%**	**Rapid decline** **No.** **%**	**X2**	**P**
Low fat N = 10	3 30.0	7 70.0	1.0	0.3
High fat N = 9	1 11.1	8 88.9		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with high fat content 88.9% compared to 70% among patients with low fat content with no significant difference statistically.

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Cardiac disorders worsen the final outcome in myasthenic crisis undergoing non-invasive mechanical ventilation: a retrospective 20-year study from a single center

Erika Iori[1], Alessandra Ariatti[1], Marco Mazzoli[1], Elisabetta Bastia[2], Manuela Gozzi[3], Virginia Agnoletto[2], Alessandro Marchioni[4], Giuliana Galassi[1]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena, Italy; [2] Division of Cardiology, Baggiovara Hospital, Azienda Ospedaliera Universitaria, Modena, Italy; [3] Radiology, Azienda Ospedaliera Universitaria, Modena, Italy; [4] Respiratory disease Unit, Azienda Ospedaliera Universitaria, Modena, Italy

The study was performed to evaluate the impact of cardiological disorders on the outcome of myasthenic crisis (MC) requiring ventilation. The study includes 90 cases admitted to the Neurology Unit of Modena, Italy (January 2000 - September 2020). All patients were eligible for a non-invasive ventilation (NIV) trial. We analyzed the effect of cardiac comorbidities on the outcomes, which were the need of invasive ventilation, the risk tracheostomy for weaning failure and the duration of intensive care unit (ICU) stay Females were 58.9% and males 41.1%. Median age at diagnosis was 59 and at MC was 65. Patients were classified as early (EOMG) or late (LOMG), 34.4 and 65.6% respectively, according to age above or below 50; 85% of patients were anti- AChR antibody positive. Hypertension and cardiac diseases occurred at the diagnosis in 61 and 44.4%, respectively. Invasive mechanical ventilation (MV) was needed in 34% of cases. Nine subjects (10%) underwent tracheostomy because of weaning failure. Independent predictors of NIV failure were atrial fibrillation (AF), either parossistic or persistent (OR 3.05, $p < 0.01$), hypertensive cardiopathy (HHD) (OR 2.52, $p < 0.01$) and ischaemic heart disease (IHD) (OR 3.08, $p < 0.01$). Hypertension (HT) had no statistical effect on the outcomes. HHD was a predictor of weaning failure (OR 4.01, $p = 0.017$). Our study shows that HHD, AF and IHD increase the risk of NIV failure in MC receiving ventilation.

Key words: myasthenic crisis, atrial fibrillation, hypertensive heart disease, ischaemic cardiopathy, mechanical ventilation

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Correspondence
Giuliana Galassi
Department of Biomedical, Metabolic and Neural Sciences, University of Modena, via P. Giardini 454, 41124 Modena, Italy. Tel: + 39 059 3497325801. Fax. + 39 059 367961.
E-mail: giulianagalassi@alice.it

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Introduction

Myasthenia gravis (MG) in an autoimmune disorder characterized clinically by fluctuating weakness of skeletal muscles [1-5]. Myasthenic crisis (MC) is a complication of MG characterized by worsening of weakness, resulting in respiratory failure that may require intubation and

mechanical ventilation1 [1-5]. We previously reviewed the predictors of outcome in a large population of MC, treated with non-invasive ventilatory support (NIV) outside the intensive care unit (ICU) setting [1]. Our study showed that the clinical parameters significantly associated with NIV failure and need of tracheal intubation were male gender, infections of upper respiratory tract, older age at onset and concurrent morbidities [1]. Hypertension and cardiac diseases were widely described in our cohort of myasthenic patients [1,4]. While we assessed the overall risk factors affecting prognosis, we faced the cardiac comorbidity burden. The aim of this study was to evaluate over a 20 year follow-up whether cardiological disorders could affect the prognosis of MC.

Material and methods

Study design and patient selection

We examined demographics data from 90 MG patients with MC published elsewhere [1]. All the subjects falling into the definition of Class V of MGFA [3], requiring ventilator support in our Neurology ward between January 2000 and September 2020 were enrolled. We excluded patients aged below 15 years and cases who were ventilated prior to the study entry or within 4 weeks after thymectomy. The ventilatory support was categorized as NIV and invasive. NIV was used to deliver bilevel positive airway pressure preferably with orofacial mask. In patients who failed NIV and underwent endotracheal intubation and mechanical ventilation (MV), the time duration of MV in days was determined and dichotomized for statistical purposes: less or more than 7 days to distinguish patients who have been early and successfully extubated from those who required prolonged MV. The follow up lasted from the first to the last visit or to death. Among our 90 cases, 29 (32.2%) exhibited more than 1 episode of MC for total number of 131 crisis. The study design was approved by the local Ethical Committee (N914/2020).

Examination at baseline and cardiological assessment

For age at onset, gender, antibody status and treatments we refer to previously reported data [1]. The cardiological assessments included clinical evaluation, basal and serial blood pressure, 12 -lead ECG and transthoracic echocardiography, when indicated. Cardiological disorders were categorized in ischemic heart (IHD), hypertensive heart (HHD), valvular diseases, rhythm and conduction abnormalities, requiring implantation of a pacemaker or of implantable cardioverter defibrillator. To prevent statistical bias, the type of incidental cardiac disease was defined for each patient enrolled at the time of diagnosis of MG and not during follow-up. Hypertension (HT) was defined following published guidelines [6,7]; we consider hypertensive all patients with resting and sustained blood pressure level above 140/90 mm Hg and/or those who were using antihypertensive medications [6,7]. In respect of HHD, the most widely accepted model of includes chronic pressure overload, development of left ventricular hypertrophy (LVH), due to progressive fibrotic changes, ultimately causing diastolic dysfunction, elevated LV filling pressures and diastolic heart failure [6,7]. LVH by ECG in our study was categorized using Sokolow-Lyon or Cornell criteria [8]. By echocardiography, an abnormal left ventricular mass (LVM) index was defined as greater than 110 gr/m^2 in women and 125 gr/m^2 in men [9]. The assessment of IHD was obtained from the patient's medical history and ECG, having in mind that the spectrum of myocardial ischemia ranges from no symptoms to myocardial infarction [10]. Cardiac conduction abnormalities included the atrial-ventricular (A-V) blocks II and III and cases, who needed implantable electric devices. We searched for history of atrial fibrillation (AF), either parossistic or permanent [11]. Valvulopathies aortic, mitral-tricuspidal, either congenital or acquired were classified according to current guidelines [12].

Outcome definition

Primary outcome of the study was NIV failure, defined as the need of tracheal intubation and invasive MV [1,3,5]. The decision to intubate the patient and start MV was made by the attending physicians on the basis of clinical and physiological criteria. In our Institution, the criteria used for elective intubation and MV in patients suffering from neuromuscular diseases are standardized as follows: vital capacity (VC) < 20 ml/kg or negative inspiratory force (NIF) < - 20 cmH2O, persistence of unsustainable work of breathing, refractory hypoxemia, persistent hypercapnia or acidemia despite NIV trial [1]. Other factors considered for elective tracheal intubation are bulbar dysfunction with inadequate airway protection, ineffective cough, retention of bronchial secretions and altered consciousness [1]. Secondary outcomes were the length of ICU stay, defined as the need for MV for a period of time longer or shorter than 7 days and the need of tracheostomy due to weaning failure. In patients who underwent MV, a weaning trial was considered when physiological parameters showed a clear evidence of improved respiratory muscles strength: maximum inspiratory pressure (MIP) > -20 cm H_2O, maximum expiratory pressure (MEP) > 40 cmH20, forced vital capacity (FVC) > 10 ml/kg [1]. Extubation was attempted when patients showed no clinical signs of respiratory fatigue during a spontaneous breathing trial with pressure support ventilation (PSV), in conjunction with physiologic parameter improvement (FVC of at least 15 ml/kg, MIP > - 20 cmH_2O, arterial

blood gases showing normal gas exchange). In patients who failed several weaning trials and/or showed no improvement of physiological respiratory muscle parameter after 15 days of MV, tracheostomy was performed with percutaneous dilatational technique.

Statistical analysis

Statistical analysis was performed using Stata 14.2 (Stata Corporation, College Station, TX, USA).Patient characteristics were analysed using descriptive statistics. Data were presented as median with minimum-maximum range or as mean with standard deviation (SD). For comparison between groups, Mann-Whitney U test was used for continuous variables and Mantel-Haenszel Chi Square Exact test for categorical variables. The impact of clinical variables was evaluated using logistic regression model. Crude and adjusted odd ratios (OR) were obtained after adjustment with risk factors for NIV. Interactions between variables were tested as well. Violin plot was used in explanatory data analysis visually showing the numerical distribution of data and skewness through displaying the percentiles and averages. Margins statistic computed marginal predictions based on a previously fitted regression model. We calculated the area under the receiver operating characteristic curve (AUC) which ranges from 0.5 to 1 (perfect discrimination). Receiver Operator Characteristic (ROC) curves were obtained from plotting true positive rate versus false positive rate. Multiple imputation chained equation method (MICE) was used to impute and estimate missing data regarding cardiological predictors using [13].

Results

Baseline clinical characteristics of the patients

Table I summarizes the demographic data at baseline. Median age of onset was 59 (Range 15-88, IQR 33). Median age of MC was 65 (IQR 31). Duration of follow up ranged from 13 (2 cases) to 577 months (IQR 150). HT vas detected in 61.1% and selected cardiological disorders in 44.4%, whereas missing data in 11% of cases were imputed and estimated statistically with the MICE method [13]. Table II summarizes the cardiological diagnoses with the prevalence of each phenotype at the time of diagnosis of MG. The cardiological disorders prevailed significantly in LOMG (p = 0.001), whose mean age was 67 years. The patients with AF were 17% of overall MC; among those, 86.6% had LOMG and only 2 (13.3%) were females. Patients with LOMG represented 84.6% of IHD and only 3 (27%) were females. HHD represented 62 % of overall proven cardiac disorders at diagnosis and 88% had LOMG.

By the end of follow-up, HHD and AF were coincidentally observed in 37.5% of patients; indeed HHD and AF share common pathogenetic risk factors [10,11]. In respect of the antibody status, 93% of patients with AF had antibodies to AChR and subjects with HHC and IHD were respectively 84% and 77% antibody positive. Among the 25 patients with thymomas, 4 cases had AF (16%) whereas 12 and 28% had IHD or HHC, respectively. Valvulopathies involving mitral-tricuspidal and aortic valves were never severe and always incidentally found by echocardiography.

Table I. Demographic characteristics at baseline (n = 90).

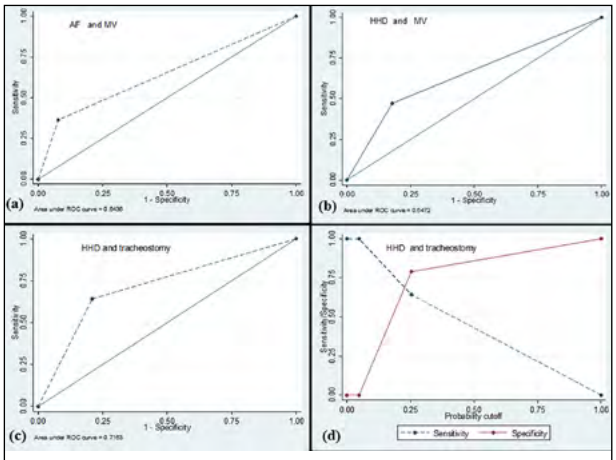
Variables		**n %**
Gender	Male	37 (41.1%)
	Female	53 (58.9%)
Age at onset (years) [median (range)]		59 (16-88, IQR 33)
	Early onset (EOMG)	31 (34.4%)
	Late onset (LOMG)	59 (65.6%)
Antibody profile [n (%)]	Anti-AChR Abs	77 (85.6%)
	Anti-MuSK Abs	3 (3.3%)
	Double seronegative	10 (11.1%)
Cardiovascular comorbidities at diagnosis	Hypertension	55 (61.1%)
	Cardiological diseases	40 (44.4%)
Other comorbidities at diagnosis	Autoimmune diseases Metabolic, endocrine diseases	34 (37.8%) 21(23.3%)
Multiple comorbidities ≥ 3 at diagnosis		39 (43.3%)
Thymectomy Thymoma Thymic hyperplasia		45 (50%) 25 (27.6%) 15 (16.7%)

Abbreviations. Abs: Antibody; AChR: AcetylCholine Receptor; EOMG: Early onset onset myasthenia gravis; LOMG: Late onset myasthenia gravis; MuSK: Muscle Specific tyrosine Kinase; MGFA: Myasthenia Gravis Foundation of America; MC: Myasthenic crisis; IS: Immunesuppressant; IQR: Interquartile range

Table II. Cardiac morbidities detected at MG diagnosis in our 90 patients.

Type of cardiopathy	Incidence [n (/%)]
Conduction defects	14 (15%)
Arrhythmias (AF)	15 (17%)
Ischemic heart disease (IHD)	13 (14%)
Hypertensive heart disease (HHD)	25 (28%)
Valvular diseases (mitral – tricuspid or aortic)	14 (15%)
Not defined cardiological phenotypes	10 (11%)
Hypertension (HT) without HHD	55 (61.1%)
Overall cardiac disorders	40 (44.4%)

Abbreviations. AF: Atrial Fibrillation; IHD: Ischaemic Heart Disease; HHD: Hypertensive Heart Disease; HT: Hypertension: MG: myasthenia gravis. The number in brackets illustrate the prevalence of each types of cardiac disorders at the time of MG diagnosis.



Abbreviation. AF: Atrial Fibrillation; AUC: Area under the curve; HHD: Hypertensive Heart Disease; MV: Mechanical ventilation; ROC: Receiver Operator Characteristic

Figure 1. A-B) ROC curves indicating a good discrimination for MV of AF and HHD (AUC = 0.64); C) ROC curve shows a good discrimination of HHD for the risk of tracheostomy (AUC = 0.71); D) ROC curves indicates sensitivity and specificity for the best cut -off of HHD as predictor for tracheostomy.

Primary and secondary outcomes

NIV failure followed by tracheal intubation were documented in 34 patients (37.7%). Table III depicts the crude and adjusted odd ratios (OR) for the risk of MV in each cardiological phenotype. Independent predictors of ventilation were AF (OR = 3.05; p < 0.01), HHD (OR = 2.52 p = < 0.01) and IHD (OR = 3.08; p = 0.01), whereas the presence of HT had no statistical effect on this outcome (p = 0.52). Interestingly, we found that the presence of conduction abnormalities almost doubled the risk of MV (OR = 2.14, p = 0.025), but this variable was not an independent predictor after adjustment with sex and age. Our female patients exhibited a lower risk of ventilation than males already at the base line (p = 0.003). By testing the interactions between variables (i.e AF, HHD and IHD), sex or age at onset, no results were statistically significant. Figures 1A and B depict the ROC curves, which showed good discrimination of AF and HHD as predictors of MV (AUC = 0.64). Figure 1C shows good sensitivity of HHD for risk of tracheostomy (AUC = 0.71) and figure D) depicts the sensitivity and specificity for the best cut-off of HHD as predictor for tracheostomy. Among the 34 patients who showed NIV failure and need of invasive MV, 64.7% had ICU stay longer than 7 days. Table IV shows the crude and adjusted ORs for the risk of ICU duration more than 7 days; neither AF nor IHD nor HHD had a statistical significant effect. Interestingly, patients who experienced more than 2 episodes of MC needed longer ICU stay (OR = 12, p < 0.01). Figures 2A and B illustrate the impact of recurrent MC on the risk of longer duration of ICU stay (AUC = 0.72). Tracheostomy due to failure of extubation was needed in 10% of the whole cohort and in 26.4% of the patients, who had undergone previously to invasive MV. HHD was an independent predictor of extubation failure (OR = 4.01, p = 0.017), as shown in Table V. Figure 2C illustrates the interaction between probability of tracheostomy, male gender and HHD. The probability increases in males with HHD. The violin plots

Table III. Crude and adjusted odd ratios for the risk of mechanical ventilation (MICE * see methods for details).

Variables	Crude OR	95% CI	*p*	Adjusted OR	95% CI	*p*
Atrial fibrillation (AF)	5.08	3.01-8.56	**< 0.01**	3.05	1.6-5.53	**< 0.01**
Hypertensive heart disease (HHD)	4.1	2.30-7.4	**< 0.01**	2.52	1.32-4.81	**< 0.01**
Ischaemic heart disease (IHD)	5.7	2.69-12.3	**< 0.01**	3.08	2.69-12.3	**< 0.01**
Hypertension (HT)	1.5	0.64-3.8	0.32	1.45	0.46-4.6	0.52
Altered conduction	2.1	1.1-4.1	**0.025**	1.13	0.52-2.48	0.74
Valvulopathies	1.20	0.50-2.86	**0.68**	1.22	0.47-3.16	0.67

Abbreviations. AF: Atrial Fibrillation; IHD: Ischaemic Heart Disease; HHD: Hypertensive Heart Disease; MICE*: multiple imputation chained equation. OR: odd ratio. Significant results in bold.

Table IV. Crude and adjusted odd ratios for the risk of more than 7 days in ICU (MICE* see methods for details).

Variables	Crude OR	95% CI	*p*	Adjusted OR	95% CI	*p*
Atrial fibrillation (AF)	1.5	0.87-2.64	0.13	0.65	0.15-2.79	0.56
Hypertensive heart disease (HHD)	2.83	0.86.-9.30	0.086	3.4	0.89-13.2	0.072
Ischaemic heart disease (IHD)	1.16	0.29-4.6	0.82	1.12	0.29-4.32	0.86
Hypertension (HT)	1.5	0.32-7.1	0.59	1.72	0.29-10.2	0.54

Abbreviations. AF: Atrial Fibrillation; IHD: Ischaemic Heart Disease; ICU: Intensive Care Unit; HHD: Hypertensive Heart Disease; MICE*: multiple imputation chained equation. OR: odd ratio

Table V. Crude and adjusted odd ratios for the risk of tracheostomy. (MICE * see methods for details).

Variables	Crude OR	95% CI	*p*	Adjusted OR	95% CI	*p*
Atrial fibrillation (AF)	1.15	0.30-4.2	0.83	0.29	0.07-1.81	0.084
Hypertensive heart disease (HHD)	6.7	2.37-19.2	< 0.01	4.01	1.27-12.5	0.017
Ischaemic heart disease (IHD)	1.27	0.34-4.7	0.72	1.4	0.51-3.8	0.51
Hypertension (HT)	2.4	0.47-123.3	0.29	2.5	0.35-18.4	0.34

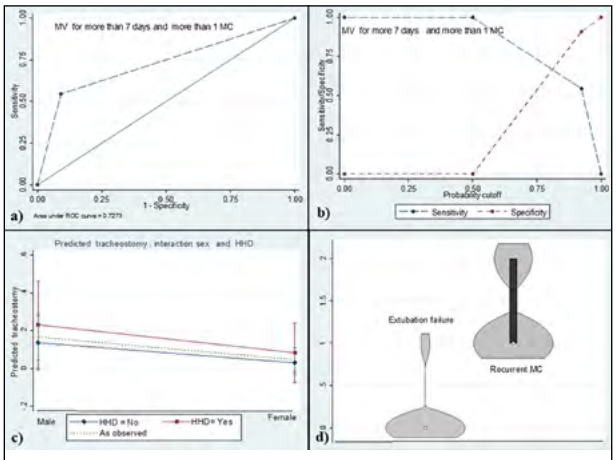
Abbreviations. AF: Atrial Fibrillation; IHD: Ischaemic Heart Disease; HHD: Hypertensive Heart Disease; MICE*: multiple imputation chained equation. OR: odd ratio

in Figure 2D (supplementary material) illustrate the overall occurrence of extubation failure and MC recurrence. The white circles represent the median.

Discussion

We previously demonstrated that in patients with MC, NIV failure may occur only in a third of cases [1]: NIV failure was significantly associated with male gender, respiratory tract infections, older age at onset, concurrent morbidities [1]. Here, we retrospectively analyzed whether cardiac comorbidities in MCs demanding ventilation could affect the need of MV, the length of ICU care and the risk of extubation failure. Notably, mostly of our patients had old age; indeed this finding is unavoidable, fully reflecting the prevalence of LOMG in recent years, in the world [4,14-17]. Age and morbidity rate are considered risk factors for longer intubation and poor outcome by several authors [14,17,21-24]. Misra et al. [17] found a significant incidence of coronary artery diseases and hypertension among their LOMG patients, whereas Sivadasan et al. [14] described cardiac disorders only in 12 patients (19%) out of 62 followed in Neuro-ICU; possibly, such low incidence is due to the fact that these authors [14] did not analyse in details the types of cardiopathies.

Extubation failure in MC reached in some studies the incidence of 35%, increasing with older age and pneumonia [20,22]. Murthy in his editorial [21] observed that cardiac complications are predictors of mortality in MC; moreover in the work of Thomas et al. [22], the precipitating factors causing death were sepsis and respiratory failure, due to several conditions, including congestive heart failure. Older age and respiratory failure were predictors of death in a large US cohort of 5,502 patients, whereas the cardiac complications, although highly observed, did not independently predict mortality [23]. Liu et al. [24] found that the non-MG related factors, including preceding strokes,



Abbreviations. AUC: Area under the curve; MV: Mechanical ventilation; MC: Myasthenic crisis; HHD: Hypertensive Heart Disease; ICU: HHD. Intensive care unit

Figure 2. A) Receiver Operator Characteristic (ROC) curves indicating the sensitivity of more than 1 episode of MC for predicting more than 7 day duration of ICU stay (AUC = 0.72); B) The ROC curve for best cut-off that maximizes sensitivity and specificity of recurrent MC for this outcome; C) Predicted risk of trachestomy: interaction between gender and presence or absence of HHD. The graph shows that the probability of tracheostomy increases with male gender and presence of HHD. Dashed line. as observed in the model. Line with diamond symbol = without HHD. Line with square = with HHD; D) The violin plots illustrate the overall occurrence of extubation failure and MC recurrency. The circles are the median.

AF, hyperlipidemia, myocardial infarction were closely linked with death. In our study AF, HHD and IHD independently predicted NIV failure, whereas only HHD had impact on extubation failure.

The effect of cardiological morbidities on MV need in MC requires attention [21,22]. The heart and lung are anatomically coupled as they occupy the same thoracic cavity, connected via blood vessels. Pressure changes within the thoracic cavity during the respiratory cycle affect the pressure systems to the heart and from the heart to the extra-thoracic spaces [25,26]. Johannessen et al. [27] showed that LV diastolic filling differed significantly in patients with MG compared to age and heart-rate matched controls; however in their patients, the systolic function was normal at the baseline. These authors [27] concluded that age, filling pressure and myocardial ischemia influenced the LV compliance. Later on, Owe et al. [28] reported that the frequency of heart disease or increased mortality due to heart disease between 1951 and 2011 in MG patients was equal in Norway to the prevalence among the general population. According to Gilhus et al. [29], MG patients do not seem to have more frequent manifest heart disease or increased heart disease mortality; however, attention should be given to patients with thymoma associated MG and to LOMG, exhibiting antibodies to titin, striated muscles, anti-Kv 1.4, ryanodine receptors, where the heart muscle can be target of an autoimmune inflammation [29,30].

Why, in our study, some cardiologic morbidities showed significant impact on weaning failure is still under discussion. Patients suffering from MC show a reduction in strength of respiratory muscles and might be more prone than normal subjects to develop diaphragm fatigue and respiratory pump failure [31,32]. In addition, current evidences demonstrate that inspiratory dysfunction may occurs with aging and is accentuated by overlapping chronic heart failure [31,32]. In particular, physiological studies showed that age is inversely correlated with maximal inspiratory pressure (MIP), with faster decline for subjects older than 65 years. The pathophysiological process behind this age-related inspiratory dysfunction probably reflects muscular abnormalities due to sarcopenia [33].

Moreover, different studies suggest that inspiratory muscle dysfunction due to age may be exacerbated by an overlapping, even subclinical chronic heart failure [32-34]. Indeed, respiratory muscle test obtained from volitional as well as non-volitional procedure (through phrenic nerve stimulation), showed clearly a decreased of maximal inspiratory pressure generated by diaphragm in patients affected by chronic heart failure, which is age dependent [34]. Furthermore, patients affected by chronic heart failure showed reduced ability to perform inspiratory efforts against a sub-maximal pressure threshold load [35]. Despite the molecular mechanisms underlying diaphragm abnormalities in humans with chronic heart failure remain poorly understood, a recent study analysing diaphragm biopsies revealed a severe diaphragm myopathy that occurs independently from disuse, aging or obesity [36]. Furthermore, the same study showed that diaphragm weakness was associated with intracellular abnormalities, as fiber atrophy, oxidative stress, mytochondrial dysfunction, impaired Ca2+ homeostasis and elevated proteasome-dependent proteolysis [36]. Indeed, the molecular and physiological changes of diaphragm, associated with aging and cardiovascular disorders, could promote the diaphragm task failure during the MC.

Our study analyses a predominantly elderly population; the prevalence of AF and cardiovascular disorders in the Western world general population during the past 20 years showed increased rate for both men and women [10,11]. According to ESC guideline [6,10,11], the heart failure is defined as a clinical syndrome due to a structural and/or functional abnormality of the heart, resulting in elevated intracardiac pressure and/or inadequate cardiac output at rest and/or during exercise. Historically, studies investigating HHD have primarily focused on LV hypertrophy, but it is increasingly apparent that HHD encompasses a range of target-organ damage beyond LV, including other cardiovascular structural and functional adaptations that may occur separately or concomitantly [7,37]. Chronic heart failure is the result of cardiological disorders of different aetiology; in our study the definition HHD could hide patients with diastolic dysfunction, falling within the phenotype of chronic heart failure with preserved ejection fraction, as previously state in the original work by Johannssen et al. [27].

Recent studies highlighted the fact that cardiac dysfunction is cause of weaning failure. Indeed weaning shares some similarities with a cardiac stress test [32,35]. Moreover, the shift from MV to spontaneous breathing might induce several events. Firstly, a negative intrathoracic pressure increases the systemic venous return pressure gradient, the RV preload, the central blood volume and the LV preload; secondly it increases the surrounding pressure of the LV with increase in LV afterload [32,35] and finally, an increase in the work of breathing is accompanied by an enhanced adrenergic tone, as documented by serum catecholamine levels [37-40]. Overall these mechanisms could provoke an increase in pulmonary arterial occlusion pressure and an increase in LV filling pressure. In addition in case of MV need, inspiration-associated over-inflation of lung volume will enhance the pulmonary vascular resistance with increased RV afterload, ultimately leading to pulmonary edema [39,40]. Given that, we can conclude that cardiological disorders in patients with MCs may increase the risk of NIV failure via several dynamic mechanisms.

Intravenous immunoglobulins (IVIg) was the first-line therapy administered in 71% of our MC during the last decade, whereas the cases with faster neurological deterioration and/or required longer ICU stay were more frequently treated with plasma exchanges (PE) [1]. Treatment with IVIg in our hands was preferred to PE in older patients, especially if affected by sepsis [2,29]. During MC, we avoided steroids because patients with poor control of MG and eventually associated pneumonia are at risk of worsening [1,17,18,21,22].

As stated by Murthy [21], there is no specific therapy for cardiac involvement in MG and in MC. Over the years, the cardiologists in our Institution tended to maintain the baseline therapies given for AF, IHD and HHD. Indeed, the natural history of MG could be modified by drugs given because of associated pathologies, but this issue in patients remains uncertain, because the data are limited with a lack of systematic studies [14]; for instance, we are not sure whether the usage of aspirin and statins in MG patients with associated cardio-cerebrovascular diseases could change their long-term outcome. At this point, we have to remember that MG patients often undergo lifelong therapy with anticholinesterase agents (AChEI) and we should have clear the potential adverse action of AChEI in worsening cardiological symptoms, including coronary vasospam and A-V blocks [42,43]. Prado et al. [44] stated that cardiac dysrhythmia attributed to hypercholinergic crisis is one of the fatal complications observed in MC. In addition, the concerns about sudden cardiac death secondary to cardiac autonomic neuropathy in the elderly, warrant a close follow-up with attention to drugs as cardioselective beta-blockers and amiodarone [14].

Conclusions

Our single center study was retrospective, based on serial assessments by the same physicians over 20 years. There are limitations in the study: first, the results might not be extended to MG in general. Second, it was impossible to acquire accurate weaning details, because decisions came certainly from judgment of clinicians having in charge the patients. Third, the evolution of cardiac parameters after MC was not obtained as the patients were followed with an ambulatory care. The impact of cardiac comorbidities on MC should be known by clinicians, because the interactions between respiratory muscles and heart are challenging for patients and doctors.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Authors' contributions

EI,MM, AA provided data and revised drafts. EB,VA,MG evaluated the patients details. GG wrote and revised the draft and made statistical analysis. AM revised the final drafts.

Ethical consideration

This study was approved by the Institutional Ethics Committee AVEN (914/2020).

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Written informed consent was obtained from each participant/patient for study participation and data publication.

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Frailties and critical issues in neuromuscular diseases highlighted by SARS-CoV-2 pandemic: how many patients are still "invisible"?

Giulia Ricci[1], Francesca Torri[1], Francesca Bianchi[1], Lorenzo Fontanelli[1], Erika Schirinzi[1], Elisa Gualdani[2], Paolo Francesconi[2], Delia Gagliardi[3], Gigliola Serra[4], Tiziana Mongini[5], Gabriele Siciliano[1]

[1] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [2] Agenzia Regionale Toscana, ARS; [3] Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy; [4] Clinica di Neuropsichiatria Infantile, Dipartimento di Scienze Mediche, Chirurgiche e Sperimentali, Università degli Studi di Sassari, Sassari, Italy; [5] Department of Neurosciences Rita Levi Montalcini, Università degli Studi di Torino, Torino, Italy

Almost 90% of neuromuscular diseases (NMDs) are classified as rare diseases, defined as conditions affecting less than 5 individuals in 10.000 (0.05%). Their rarity and diversity pose specific challenges for healthcare and research. Epidemiological data on NMDs are often lacking and incomplete. The COVID-19 pandemic has further highlighted the management difficulties of NMDs patients and the necessity to continue the program of implementation of standard of care. This article summarizes the Italian experience during pandemic.

Key words: COVID-19 pandemic, neuromuscular diseases

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Correspondence
Giulia Ricci
Neurology Unit, S. Chiara Hospital, via Roma 67, 56126 Pisa, Italy. E-mail: giulia.ricci@unipi.it

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Note of the Editor

This paper summarizes the speeches on *Fragility and critical issues related to the management of patients with neuromuscular disorders*, presented during the webinar organized by the Italian Association of Myology (AIM) in September 2021. They had as focus the management of the patients with neuromuscular disease in the period of the pandemic, at the National level.

Introduction

Rare diseases (RDs) are defined as conditions affecting less than 5 individuals in 10.000 (0.05%) [1]. Despite their low prevalence, RDs are collectively common conditions involving from 6 to 10% of the European population, raising critical issues for the whole community in terms of best clinical practices, diagnosis achievability, health services planning and public health programs.

RDs are often complex conditions requiring integrated, long-term care delivery settings and highly specially designed management. Although their chronic and often progressive course, long-term complications of RDs can be lessened or delayed by early diagnosis, allowing an optimal management and prompt supportive and/or targeted therapies if available. In addition,

appropriate and timely diagnosis ameliorates patient health status reducing psychological and social burden of the diseases and allows proper genetic counselling.

Almost 90% of neuromuscular diseases (NMDs) are classified as rare diseases. NMDs are a broad group of neurological disorders that represent a major cause of mortality and lifelong disability in children and adults. NMDs are caused by acquired or genetic defects of motoneurons, peripheral nerves, neuromuscular junctions or skeletal muscle. They may be difficult to recognize, result in progressive muscle weakness and wasting, often leading to difficulties in swallowing and breathing or in cardiac failure. Patients can experience long delays in diagnosis and, in some cases, may be forced to move from their own towns in order to access to the best medical care. Their rarity and diversity pose specific challenges for healthcare and research [2].

NMDs and standard of care in Europe

In order to strengthening the fight against complex and rare diseases, in 2017 the European Commission created the European References Networks (ERNs). These are virtual networks, each focused on a specific group of rare or low-prevalence diseases, that involve national Health Care Providers (HCPs) across Europe. ERNs seek in tackling rare diseases by easing diagnostic processes and equalizing therapeutic approaches, making national and European health systems more efficient, accessible and resilient. There are 24 ERNs involving 25 European countries, more than 300 hospitals with over 900 HCP units covering all major disease groups so far. Though each ERN focuses on a specific set of diseases, HCPs belonging to different ERNs can easily interface from each other. In these networks, medical specialists across different disciplines are connected in a sort of 'virtual' advisory board trough a dedicated IT platform and telemedicine tools. EURO-NMD is a European Reference Network for the thematic grouping of rare neuromuscular diseases (NMDs) [3].

The expertise for diagnosis and management available in each HCP could be enriched by data sharing, thus resulting in an increasing request for cross-border e-health technologies providing means for teleconsulting and exchanging knowledge across medical experts. The use of telemedicine is at the basis of the ERN network to diagnose and treat rare and complex diseases at a continental level, providing the critical advantage of expanding the set of knowledge available to any patient. Periodic meetings and boards are instrumental for networks maintenance, but one of the most outstanding telemedicine tools in ERN's network strengthening is the Clinical Patient Management System (CPMS) (https://ern-euro-nmd.eu). The CPMS is a digital platform where clinicians belonging to different HCPs can discuss about clinical cases in a secure way for patients' data protection. Using the CPMS, it is possible for health professionals to discuss about patients by sharing images, clinical reports and organizing video meeting where clinicians can discuss vis à vis. The CPMS is the foundation of one of the most important ERN core tasks: reducing health care inequalities within the European Union by providing access to expert specialized care to all patients with rare and complex diseases. In short, cases discussions happen through the opening of so-called "panels", where the clinician requiring a medical consultation provides a detailed description of clinical case. Data sharing (e.g. radiological images, exams reports, laboratory findings) via panels is possible, so that advices on virtual consultation can be easily provided by the specialists invited both using the chat tool and via video meetings. Moreover, patient's data are available for databases and registries. Overall, the CPMS could represent a new digital tool to improve data collection of patients with rare diseases across different ERNs and countries, also implementing national registries.

Efforts and levels of criticality in collecting epidemiological data on NMDs

It is estimated that in Italy at least 80,000-100,000 patients are affected by NMDs[4]. There are likely to be at least over 200 different forms of NMDs. However, epidemiological data on NMDs are often lacking and incomplete. The classifications of NMDs, also in the light of diagnostic advances and the best molecular characterization by the discovery of new causative genes, is constantly evolving. On the other hand, the lack of unambiguous indicators (for example, incomplete hospital discharge forms *Schede di Dimissione Ospedaliere* SDO, ICD-9 classifications, exemption codes for rare diseases that are not always diseases specific, out-patient cases which escape correct disease classification) makes data collection complicated, also from an administrative point of view. In last decade, this has led to an increasing awareness, also by the institutional entities, of the need of tracing rare patients, for example with development and implementation of disease registries.

In Italy the National Register of Rare Diseases (RNMR) has been established by the Istituto Superiore di Sanita' (ISS) (art. 3, Ministerial Decree 279/01) with aims to carry out the surveillance of RDs, to obtain epidemiological data, and finally support research and promoting the comparison between healthcare professionals. All Italian Regions, with different times and methods, have formally identified accredited clinical referring centers of

the National Rare Diseases Network and have established the regional/interregional registers. Since 2001 the RNMR has collected data from 20 regional or interregional registers. However, these data can be still incomplete and not to fully describe the clinical reality in the different regions. We can recognize several criticalities. It is conceivable that a submerged number of undiagnosed or not taken on care of cases continues to exist, especially in the peripheral areas far from NMDs referring centers. Moreover, there is the possibility that patients remain untracked as rare patients since they can benefit also from a non-specific disease exemption code. Therefore, the lack of unique indicators to track rare NMDs patients makes difficult to collect and merge data coming from different administrative sources.

In collaboration with the Agenzia Regionale di Sanità (ARS), a technical organism of the Tuscany Region with consultancy and research purposes (authors EG and PF), the clinicians of Neurology Unit- Department of Clinical and Experimental Medicine (GR, FT and GS) performed an epidemiological survey on NMDs patients residing in Tuscany. To identify the prevalence of patients affected by NMDs and overcome the possible limitations in using only data coming from the Tuscany Region Register of rare diseases (as discussed above), established *current health flows* have been used as data sources, originally thought for administrative-financial purposes but with secondary potential applications also for epidemiological purposes: a) hospitalizations in facilities affiliated with the regional health system converging in the SDO flow (hospital discharge forms, ICD9 CM encoding); b) exemptions codes from rare disease register; c) data of civil registry office. Analysis results opportunely filtered and thus extracted are summarized in Table I. This analysis showed that the prevalence of patients with NMDs x 1000 Tuscan residents as of January 1, 2019 is equal to 1.8 (about 0.2%), which corresponds to expectations based on literature [5-10]. The analysis also made it possible to stratify the patients according to the different forms of diseases, data also consistent with the expectations. This would therefore confirm that the algorithm selected on the basis of administrative data is sensitive in tracking patients with NMD diseases. Nonetheless, for some diseases, such as spinal muscular atrophy (SMA), we obtained higher rate of prevalence than expected, also based on cases known in our Center. In ruling out any founder effect on this result, we interpreted that as due to a possible bias linked to the lack of unique disease codes for SMA that also select patients with other diagnoses. In fact, if we further stratify the selected cases in the different subgroups of clinical codes/forms, as shown in Figure 1, we observe that the number of registered cases diagnosed with Werdnig-Hoffmann disease and Kugelberg-Welander disease is lower and closer to the real estimates of the clinical practice [8]. Nevertheless, it is likely, however, that there are still "invisible patients", that is cases not known to the referring centers. Notably, concerning SMA, it is considerable that in this last years the availability of new therapies and communication also through non-institutional channels such as patient associations should have led to more opportunities to contact by patients themselves with the referring centers.

COVID-19 pandemic and vaccination programs: the Italian experience and roles of patients association

NMDs patients felt the impact of SARS-CoV-2 pandemic in many ways, including concern for worsening of disease course and respiratory status with infection [11,12]. Since the vaccines against COVID-19 have being available, great hope for protection against the virus but also numerous questions and uncertainties about safety and possible side effects in NMDs emerged among patients' community.

From December 2020 - January 2021 European countries had started to develop plans to roll out their vaccine programs, and have necessarily had to adopt strategies with prioritization.

The scientific community (on behalf of World Muscle Society: www.worldmusclesociety.com, TREAT NMD, and in Italy the Italian Association of Myology and Peripheral Nervous System Association) strongly advocated that patients with NMDs should be considered as a high risk, priority group and supported statements made from organizations around the world calling for prioritization for vaccination for individuals affected with NMDS and their care givers.

In Italy the first Ministerial Decree on February 2021 regarding national vaccination program, that identified patients' priority groups, did not include the majority of NMDs forms, which then led to a subsequent rapid correction and inclusion of these diseases in the following weeks, thanks also to the prompt intervention of technical scientific committee of health ministry, scientific associations and patients' community. Moreover, in the early phases of the vaccination program, the different regions along the country had not yet equipped themselves with IT platforms which then allowed and facilitated the direct access for patients with their exception codes to booking the vaccine in the following months.

Therefore, the first recruitment of patients had been difficult with a non-standardized methodology on the national territory. For example, in Tuscany the clinical referring centers have had the task of contacting by themselves patients directly, and this had led to the identification of some cases that were not known to the centers because they were fol-

Table I. Prevalence of neuromuscular disorders in Tuscany.

ICDIX disease code	Rare disease identification code	Disease description	Hospital Discharge Form Codes Number (SDO)	Disease-specific Identification Codes Number (SEA)	SDO ∩ SEA	Total	Prevalence x 1000 Tuscany residents
35781	RF0180	**Chronic inflammatory demyelinating polyneuropathy**	**1.069**	**27**	**15**	**1.111**	**0.3**
3580	034	Miasthenia gravis	387	219	219	825	
35800		Miasthenia gravis without acute exacerbation	295	0	146	441	
35801		Miasthenia gravis with acute exacerbation	145	0	67	212	
		Miasthenia gravis	**827**	**219**	**432**	**1.478**	**0.4**
33520	RF0100	Amyotrophic lateral sclerosis	303	26	107	436	
33529		Other motor neuron diseases	87	0	26	113	
3352	RF0100	Motorn neuron disease	78	0	7	85	
33524	RF0110	Primary lateral sclerosis	39	6	15	60	
33522		Progressive bulbar palsy	16	0	6	22	
33521		Progressive muscular atrophy	39	0	6	45	
		Amyotrophic lateral sclerosis/motor neuron diseases	**562**	**32**	**167**	**761**	**0.2**
3350	RFG050	Werdnig-Hoffmann disease	30	11	6	47	
33510	RFG050	Spinal muscular atrophy, not specified	23	5	8	36	
33519	RFG050	Other spinal muscular atrophies	22	8	5	35	
3351		Spinal muscular atrophy, not specified	14	0	12	26	
33511	RFG050	Kugelberg-Welander disease	7	0	4	11	
		Spinal muscular atrophy	**96**	**24**	**35**	**155**	**0.0**
3591	RFG080	Hereditary progressive muscular dystrophy	376	116	162	654	
		Muscular dystrophy	**376**	**116**	**162**	**654**	**0.2**
3592	RFG090	**Myotonic disorders**	**134**	**78**	**106**	**318**	**0.1**
3560	RFG060	Hereditary peripheral neuopathy	152	108	28	288	
3561	RFG060	Muscular-peroneal atrophy	143	16	43	202	
		Hereditary peripheral neuropathy	**295**	**124**	**71**	**490**	**0.1**
3570	RF0183	**Infectious acute polineuritis**	**114**	**0**	**0**	**114**	**0.0**
27787		**Mitochondrial metabolism disorders**	**134**	**0**	**5**	**139**	**0.0**
2710	RCG060	**Glicogenoses**	**112**	**21**	**23**	**156**	**0.0**
7103	RM0010	Dermatomyositis	209	49	93	351	
7104	RM0020	Polimyositis	344	44	87	475	
		Inflammatory myopathies	**553**	**93**	**180**	**826**	**0.2**
			4.272	**734**	**1.196**	**6.202**	**1.ß8**

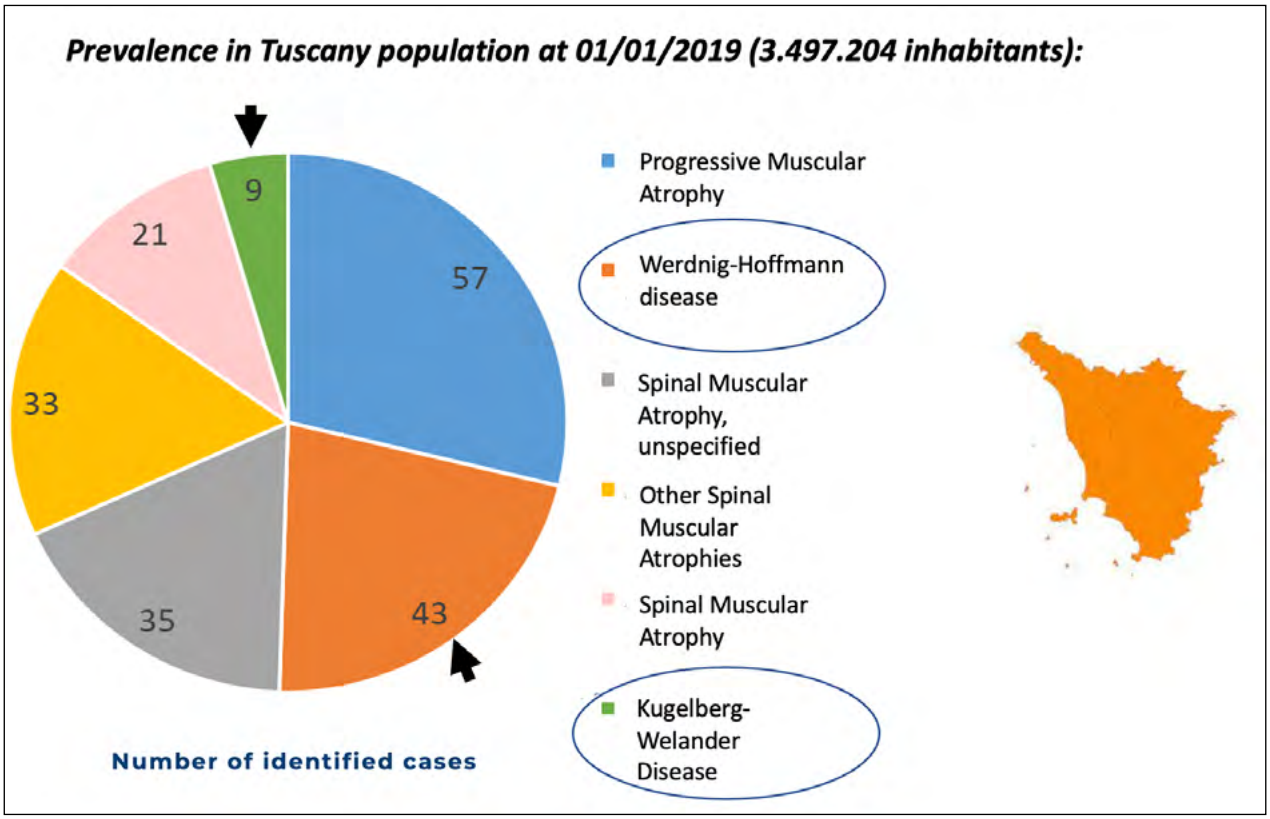

Figure 1. Prevalence of neuromuscular disorders in Tuscany population at 1st January 2019.

lowed outside the region or not taken in charge. However, although without a defined and planned methodology, the transversal and synergic actions carried out at different levels by specialists, general practioners and patients' association allowed to partially fill up these health system gaps.

How to overcome the health system frailties that the SARS-CoV-2 pandemic has brought out: future prospective and challenges towards a new standard of care NMDs

The clinical management of NMDs patients is an articulated and complex process, which makes use of numerous specialist figures alongside the central figure of the neurologist or neuro-pediatrician. The definition of complete and multidisciplinary paths for diagnosis and treatment becomes a fundamental requirement to ensure care and standards of care.

The clinical pathways named diagnostic, therapeutic and integrated care programs (Percorsi Diagnostico-Terapeutici Assistenziali, PDTA) can be multidisciplinary tools aimed at sharing decision-making processes and organizing care for a specific group of patients during a well-defined period of time, thus improving the quality of care [13]. PDTA should:

- include a clear explanation of the objectives and key elements of evidence-based care;
- facilitate communication between team members, caregivers and patients;
- coordinate the assistance process through the coordination of roles and the implementation of the activities of the multidisciplinary assistance teams. PDTA should also include documentation, monitoring and evaluation of outcomes and identify the resources necessary for the implementation of the path itself. Overall, the purpose of the PDTAs is therefore, in this view, to increase the quality of assistance perceived and effectively delivered, improving outcomes and promoting patient safety through the use of the right resources needed.

In these last years, for instance, the Tuscany Region has started the promotion and the definition of several PDTAs at regional level for various forms of NMDs, with the collaboration of a working group that involves specialists from different areas and regional territorial companies, as well as patient associations.

The COVID-19 pandemic has further highlighted the management difficulties of NMDs patients and the necessity to continue the program of implementation of standard of care yet started in Europe with the definition

of the ERNs and with different applications in the various European countries. It will be increasingly necessary to favor and further develop *smart* management care through the implementation of IT platforms, telemedicine services and other eHealth technologies [14,15].

The Europe has responded to the pandemic crisis with an unprecedented investment program having highly ambitious objectives, where Italy will be one of the first recipients.

Among the missions of the Italy's recovery plan (*Piano Nazionale di Ripresa e Resilienza*, PNRR), there will be the digitalization of health system. In the near future we are moving towards a health system that supports scientific research, strengthens prevention, and brings "medicine home". The view is that of a digital health service which integrates health and social services. The PNRR aims to renovate healthcare in Italy by upgrading its technological infrastructure, strengthening the training of operators and creating contact points with patients also through telemedicine. Overall, this will offer a proactive top-quality opportunity to network with organizations and people that value quality health information as well as effective integrated system and innovative solutions in order to improve diagnosis, treatment and care of patients with NMDs.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Authors' contributions

GR, FT, FB, GS: writing the manuscript, acquisition of data, analysis and interpretation of data; EG, PF: acquisition of data, analysis data; LF, ES, DG, TM: revising the manuscript.

Ethical consideration

Not applicable.

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Telemedicine applied to neuromuscular disorders: focus on the COVID-19 pandemic era

Melania Giannotta[1], Cristina Petrelli[2], Antonella Pini[1]

[1] Neuromuscular Pediatric Unit, UOC di Neuropsichiatria dell'età pediatrica, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy;
[2] Neurological Unit ASUR Marche AV3, Macerata, Italy

Neuromuscular diseases are rare and usually chronic progressive disorders that require a multidisciplinary clinical evaluation and functional monitoring. The patient-physician relationship and therapies are also key elements to be provided. The COVID-19 pandemic dramatically changed the way patients' health was managed and national health care services underwent a radical reorganization. Telemedicine, with the use of Information and Communication Technology (ICT) by health professionals, became the main strategy to ensure the continuation of care. However, the experience regarding the use of Telemedicine in neuromuscular disorders is very limited and the scientific literature is extremely scarce. From the first experiences in the '50s, the development of Telemedicine has been supplemented and supported by the implementation of ICT to guarantee the secure and effective transmission of medical data. Italian national guidelines (2010-2020) describe the technical and professional guarantees necessary to provide Telemedicine services. Nevertheless, at the time the pandemic appeared, no guidelines for clinical evaluation or for the administration of functional scales remotely were available for neuromuscular diseases. This has been a critical point when clinical evaluations were mandatory also for the renewal of drug prescriptions. However, the common opinion that telemedicine basic services were important to overcome the change in medical practice due to COVID-19 in neuromuscular diseases, even in pediatric age, emerged. Moreover, alternative digital modalities to evaluate patients at home in a kind of virtual clinic were considered as a field of future development.

Key words: telemedicine, COVID-19 pandemic, neuromuscular disease, remote neurologic assessment, pediatric

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Correspondence
Melania Giannotta
Neuromuscular Pediatric Unit, UOC di Neuropsichiatria dell'età pediatrica, IRCCS – Istituto delle Scienze Neurologiche di Bologna, via Altura 3, 40139 Bologna, Italy. Tel. +39 051 6225111.
E-mail: melania.giannotta@isnb.it

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Note of the Editor

This paper summarizes the speeches on *Telemedicine in the COVID-19 era* presented during the webinars organized by the Italian Association of Myology (AIM) in September 2021. They had as focus the management of patients with neuromuscular diseases in the period of the pandemic: experiences emerged in Italian central-southern macro-areas.

Introduction

The Advance Informatics in Medicine defined in 1990 the Telemedicine as "the integration, monitoring and management of patients, and the education of patients and health care professionals, using systems that provide ready access to expert advice and patient information, regardless of where the individual, or the information, resides".

Later, in 1997, the World Health Organization described Telemedicine as "the provision of health services where distance is a critical factor, requiring the use of Information and Communication Technology (ICT) by health professionals to exchange information for the diagnosis, treatment and prevention of disease, to ensure the provision of ongoing information to health care providers, and to support research and evaluation of care" [1,2].

If face-to-face consultation between physician and patient remains the gold standard of clinical care, Telemedicine may represent in some circumstances the only modality to evaluate the patient. It should be emphasized that Telemedicine services may represent a possible integration to the medical evaluation and it must be assimilated to any diagnostic/therapeutic health service, but do not replace the traditional healthcare service with an in-person relationship.

Telemedicine can take place between a physician and a patient or between two or more physicians, including other healthcare professionals, in situations where they are not in the same location.

The provision of healthcare services, using ICT, involves the secure transmission of medical data and information, through text, sound, images, or other forms needed for the prevention, diagnosis, treatment, and follow-up of patients. However, the use of ICT tools for the processing of health information or for data treatment, does not constitute Telemedicine in itself. Health information portals, social networks, forums, newsgroups, e-mails do not constitute Telemedicine (National Telemedicine Guidelines 2010) [3].

History of telemedicine

Telemedicine started about in the '60s, to monitor patients far from the treatment centres. The first reports date back to W. Darrah, who described a mobile health service for migrant families [4]. Lately, telecommunications (telephone and radio at the beginning) have played an important role in the development of Telemedicine.

"Telognosis" the acronym for "teleo-roentgen-diagnosis", was the diagnosis by telephone-transmitted roentgenograms which started in 1950 in Philadelphia.

"Telepsychiatry" began in Nebraska in 1964 when a two-way closed-circuit microwave television system was established between the Nebraska Psychiatric Institute and Norfolk State Hospital in Nebraska providing consultations, education, training, and research. In 1967 the Massachusetts General Hospital provided medical consultation over TV cameras to the Boston Logan Airport Medical station a close hospital in a very traffic area.

The Italian tradition of experimental Telemedicine dates back to early 1970s when the Catholic University Hospital of Rome sets up a teleconsulting service for poisoning. In 1976 the University of Bologna introduced a prototype system for the acquisition and transmission of electrocardiograms (ECGs via telephone). In the same year, a teleconsulting service was established by the Research Centre of the Telecommunications Company between the Emergency Department of the Hospital S. Giovanni of Turin and the Civil Hospital of Susa.

In 1995 it was decided to fund a research and training project in the field of Telemedicine with the aim to improve the quality of health care and reduce costs [5].

Due to technological progress, the dissemination and development of Telemedicine experiences have increased since 1995, in particular as a consequence of the growing of Internet network and the development of new digital technologies such as:

- transmission according to communication protocols (TCP/IP) up to WEB 2.0 http21st century;
- social networks;
- interoperability between electronic systems;
- collaboration and communication technologies that help to communicate and work together in real-time (synchronous) or at different times (asynchronous);
- Internet Voice over protocol for the provision of voice communications and multimedia sessions over Internet by using Internet Protocol networks;
- mobile phones that increased potential for real-time communication between professionals and patients.

The progressive increase of scientific publications with a peak in COVID-19 pandemic documented this technological development.

However, despite the positive experiences in the clinical practice, the technological advances and the good results in terms of cost-benefits balance, the diffusion of Telemedicine service was still not adequate for the need of health care in Italy as documented by an Italian Survey in 1995 [5].

Between the late '90s and early 2000s, Telemedicine had an important development so that the European Commission has issued the decree COM 2008/689 of the 4th of November 2008 ("Telemedicine for the benefit of patients, health systems and society") to support the Member States in the implementation of Telemedicine services [6]. In 2010 the Italian Health Ministry finally edited national guidelines for Telemedicine services.

Telemedicine applied to the discipline of neurology (teleneurology) regarding neuromuscular patients has certainly seen a huge push forward, because these patients have been defined as fragile since the first moments of the pandemic, as they were considered at high risk of developing severe symptoms in case of SARS-CoV-2 infection.

On the 17th of December 2020, the State-Regions Conference implemented the Ministry of Health document

"National guidelines for the provision of Telemedicine services", thus allowing digital medicine to enter definitely among the services provided and payable by the National Health System (NHS) [7]. This new document highlights the applicability of Telemedicine in an innovative way also regarding the improving health services in favour of people whose pathology or condition is relevant for the governance of the NHS such as rare diseases (in adulthood and pediatric age), including neuromuscular diseases.

Types of telemedicine

We know different types of Telemedicine:

- *Tele-consultation (Tele-consult):* real-time or deferred request for a remote diagnostic consultation;
- *Tele-visit:* one of the main telemedicine healthcare services that can be provided within the NHS. This is an outpatient activity made after a careful assessment of the situation and according to specific criteria;
- *Tele-reporting* of instrumental examinations, i.e. the identification of instrumental examinations carried out in peripheral departments that require specific experiences for diagnostic interpretation;
- *Tele-monitoring* of treatments, under the supervision of specialists, even in peripheral facilities;
- *Remote emergency services* of telematic communication with remotely located assistance centres in emergency and dangerous situations.

Telemedicine offers many advantages. In chronic disabled disorders it guarantees the continuity of care for isolated or distant patients. In acute situations it allows quicker medical solutions or responses. Overcoming the barrier of time and distance according to a hub and spoke model, it does not exclude a face-to-face evaluation, if necessary. A medical Teleconsultation (personalized healthcare) could be very important in emergency situations or when a specialist evaluation is needed, and this is not available locally.

Only an outpatient service that does not require a complete examination of the patient may be provided through a tele-visit. Indeed, although a tele-visit is carried out in real-time it does not replace the first visit that must be always done in a face-to-face way and it cannot be the exclusive doctor-patient relationship modality. It can be assessed only if a complete physical examination of the patient is not required. Moreover, a tele-visit is not a simple video call. Like the face-to-face visit, a tele-visit must develop according to an articulated and multi-professional process to ensure the safety of medical activities, the protection of personal data, the regularity for reporting purposes and use immediate and easy access tools for the patient.

It must be organized to ensure a real benefit for health but also logistical and operational for the patient and the entire organization. Tele-visit must always be reported and the cost is the same as a visit. Unlike the tele-visit, teleconsultation is considered an integral part of the medical work, it is not included in the tariff nomenclator and it does not need a prescription. It is usually registered into a local database, but it is not detected in the institutional medical activity registry. Teleconsultation can be done with many specialists.

Telemedicine may be synchronous or asynchronous.

"Synchronous Telemedicine" is like a "live video-conferencing," which is a "two-way" audiovisual link between a patient and a care provider. Synchronous Telemedicine requires the presence of both parties at the same time and a communication link between them that allows a real-time interaction to take place. Usually, during real-time telehealth sessions, operators such as technicians or nurses, can handle special telehealth-enabled tools in order to remotely perform a neurophysiologic examination (e.g.: EMG) under consulting provider's direction and according to national recommendations [8].

"Asynchronous telemedicine" is like a "store-and-forward video-conferencing" and consists of the recorded health history transmission to a health practitioner, usually a specialist. It involves acquiring medical data, then transmitting this data to a doctor or medical specialist at a convenient time for assessment offline [9].

As an assimilated health care service, it must also comply with all the rights and obligations of a health service.

Professional guarantees must be provided:

- organization guarantees concerning the set of procedures to offer a quality service;
- guarantees of access to the service in a clear and verifiable modality;
- clinical guarantees that the activity is designed and implemented in accordance with evidence-based medicine;
- technological guarantees concerning the integrity of the information transmitted.
- Another very important aspect to consider is that Telecommunication infrastructure must assure:
- interoperability of networks and protocols according to guidelines;
- continuity of service during the supply period;
- safety for the citizen by ensuring data protection;
- source verification (authentication);
- information security [3,7].

Telemedicine in neurologic-neuromuscular disorders

The quality of patient-physician relationship, the modality of remote clinical assessment and monitoring, and

the administration of therapies are the key elements to be provided in neuromuscular Telemedicine. Regarding the doctor-patient relationship, Telemedicine permits the maintenance of a close patient-physician relationship and can be a simple tool to facilitate the resumption of normal follow-ups at the end of the pandemic. It is reported in literature [10-12] that thanks to telemedicine there has been a decrease in accesses to the hospital, with positive effects also on the psychological sphere of the patients. The direct clinical evaluation of the neurological patient is very important to first make a correct diagnosis and then to evaluate the course of the pathology, especially for the neuromuscular patient. Hence the development of TV models, instructing patients and caregivers to evaluate some parameters of the neurological physical examination (e.g.: muscle strength assessment). Furthermore, scales have been identified to assess the severity of polyneuropathies and the impact of functional limitations on the patient's quality of life such as VANS scale (the Veterans Affairs Neuropathy Scale), ONLS scale (Overall Nlimitations Scale) and i-RODS scale (Inflammatory Rasch-built Overall Disability Scale).

The second element of Telemedicine concerns remote monitoring. This means identifying the parameters that can be monitored over time, such as heart rate, arterial saturation, the extent of movements (the latter through dedicated instrumentation, such as accelerometers, actigraphs, etc.), the exact measurement of the strength of individual fingers or the whole hand using the vigorimeter.

The third element is digital therapies: i.e., access in hospital settings to perform medical therapies that can be performed only and exclusively in protected settings (such as antisense oligonucleotides for the treatment of SMA or Patisiran for genetic amyloidosis). Some treatments can be home-based, such as subcutaneous immunoglobulins instead of the intravenous formulation for forms of dysimmune polyneuropathy [13].

During the COVID-19 pandemic, one of the most significant problems was physical therapy, which has been reduced by 93% due to the closure of rehabilitation centers and/or facilities. Starting from this finding tele-rehabilitation was developed, according to the guidelines of the Italian Society of Physical Medicine and Rehabilitation, which was aimed more at patients with vascular problems (ischemic events), degenerative diseases (such as Parkinson's disease) and multiple sclerosis. Tele-rehabilitation for neuromuscular patients has very limited experiences due to difficulties in managing electronic devices, inhomogeneity among families and countries and technical problems related to the network and the possibility of access to various devices. As a consequence, the scientific production is extremely limited.

Another important limitation of Telemedicine for neuromuscular patients is represented by the impossibility to perform remotely a key test for the neurophysiologist, which is electromyography. There are, however, few clinical situations that require an EMG-ENG examination with urgency and they are:

- Guillain-Barré syndrome;
- Myasthenia Gravis;
- rapidly progressive inflammatory neuropathies/myopathies;
- misdiagnosed motor neuron diseases.

In many of these cases, patients require hospital management from both a diagnostic and therapeutic point of view; in all other cases, EMG-ENG examination can be postponed.

The Bologna Pediatric Neuromuscular Unit experience, in the first phase of the pandemic era

In March 2020 the Bellaria Hospital (which hosts most of the neurological activities of the IRCCS Institute of Neurological Sciences), became a COVID-19 Hub Service and all the planned activities of the Neurological Services were suspended. Urgent neurological activities were guaranteed in other hospitals.

One of the consequences of the pandemic has been the emptying of general practice clinics and, to a large extent, specialist clinics as well. The fear of contagion has drastically decreased access to diagnostic services and hospital facilities, reducing the relationship with one's general practitioner or specialist only for burocratic reasons: due to all that, Telemedicine had a faster acceleration in the last two years.

Nevertheless, since national guidelines for Telemedicine services appeared only on the 17th December 2020, in the meanwhile, to cope with all the afore mentioned, we evaluated by ourselves different modalities to ensure the early diagnosis and monitor the health and the evolution of pediatric patients affected by rare neuromuscular diseases. In particular, we needed to carry out functional and neurological evaluations to prescribe innovative therapies. Exchanging points of view between colleagues of the team and from other neuromuscular centres was essential to find new creative strategies.

The first step was patients' stratification for their gravity. Patients with respiratory weakness (with or without non-invasive ventilation or cough machine), multi-organ involvement (cardiac, pulmonary), susceptibility to worsening during fever or infections, feeding difficulties or treated with immunosuppressive or disease-modifying therapy, required greater attention and close monitoring [13,14].

Afterward, each patient received a tele-visit to check the state of health and the absence of urgent needs. A tele-consultation to monitor and manage specific issues

was set up, to obtain informations particularly about blood pressure, heart rate, Sat% O^2 and psychological status.

Rehabilitation was stopped in many patients, but those with special needs received an online tele-visit by the physiotherapist of the Childhood Rehabilitation Medicine Unit and by the Territorial Child and Adolescence Neuropsychiatric Services which in Italy treat the patients in their outpatient's rehabilitation clinic. One of our main efforts was also to guarantee the continuation of clinical monitoring with functional scales, necessary for the prescription and the administration of intrathecal Nusinersen therapy for Spinal Muscular Atrophy.

We decided to develop alternative digital modalities (such as the use of smartphones or video platforms) to evaluate patients at home, in a kind of real-time virtual clinic. This modality needed parents to be instructed by the physiotherapist to perform ad-hoc maneuvers or functional scales at home. First, we assess the Internet connection (both for the family and for the team composed by child neuropsychiatrist and physiotherapist in the referral center). Technological devices capable of supporting a videocall were essential; moreover, even the quality of the image can significantly influence the clinical evaluation of the child, but also the possibility for the family to follow the practical demonstrations carried out by the operators, in order to perform the various items in front of the camera.

In order to carry out a reliable assessment, information was provided on adequate setting, furniture arrangement and number of caregivers needed to assist the child in performing the scales' items.

Emotional responses from children and language barriers that could hinder the understanding and therefore also the execution of the test were also considered.

The family was contacted by phone to make an appointment for the tele-evaluation. During this telephone interview, instructions were given to parents for the preparation of a proper setting and of the necessary tools to carry out the assessment.

Different assessment settings were defined according to the characteristics of the child: pathology, age, motor skills, etc. Different settings and tools were required according to the rating scale to be administered (North Star Ambulatory Assessment or Hammersmith Functional Motor Scale Expanded). Moreover, the type of videoplatform to be used has been agreed in advance with parents.

Results obtained from the online evaluation were very satisfactory, the majority of the functional scales items were well understood and realized. The monitoring of the clinical outcome was obtained. Family satisfaction was also high. It is important to consider that the population tested was the one formerly belonging to our centre, and therefore consisted in people (children and parents) who already know the functional scales chosen for the tele-evaluation.

Conclusions

The COVID-19 pandemic has dramatically changed the way patients' health is managed, imposing new methods of visit and follow-up.

Telemedicine basic services were important to overcome the change in medical practice due to COVID-19. Advantages and disadvantages were noticed. Telemedicine is applicable in the monitoring of patients with rare neuromuscular diseases, even in developmental age. Unnecessary assessments can be reduced. Frequent evaluations are allowed in a family environment. Moreover, Telemedicine can be a useful tool in the management of ordinary care (monitoring of exams and parameters, comparison about patients and caregivers' doubts) and in the early identification of patients who require a face-to-face evaluation and/or to be directed to the Emergency Services.

In addition, Telemedicine can be useful also for multidisciplinary tele-consulting between the referral hub and peripheral centers specialists (child neuropsychiatrists, neurologists, physiatrists, pediatricians, palliative-care specialists, nutritionists, cardiologists, pulmonologists, endocrinologists, psychologists), both in a real-time way during the patients' visit at their residence centers (synchronous) or in a store-and-forward way (asynchronous), accessible to the professionals interested at a later time.

In this way, patients can get timely specialty care without the need to travel between the locations of their primary care providers and wait times for specialty care are lessened, especially in areas with shortages of medical specialists.

However, as regards the clinical tele-evaluation in neuromuscular disorders, some critical issues must be considered: functional scales could be incomplete or imprecisely administrated by the caregiver. It is not possible to evaluate articular range and the possible presence or worsening of joints contractures. Moreover, data collection from an online evaluation is not reliable for any ongoing experimental study.

It is mandatory to reach a consensus on the management of Telemedicine and the interoperability of networks at least at a regional level.

Further progress in Telemedicine applied to neuromuscular diseases in developmental age are possible: for example, by the creation of specific apps, remotely controlled by doctors, useful in the daily monitoring of changes in neuromuscular performances.

It is also important to train territorial physical therapists who perform the functional motor assessments at the patient's home or at the clinical centre for the tele-evaluation, in order to ensure a proper data collection.

According to the hub-spoke model, multidisciplinary tele-visit and tele-assistance (such as physical therapy) with local specialists can be extended near to the patient's place of residence, supported by technological platforms conform to regional administrative rules and authorization.

Although it is not a substitute for a face-to-face visit, Telemedicine can become an integral part of the follow-up of active patients, workers, parents, caregivers, minimizing the overall impact of the disease on their lives. It can also be an alternative for disease monitoring and ongoing chronic therapies.

Saving a journey (and consequently working hours and in general time and money) may improve patients and caregivers' compliance.

This may also lead to think about other scenarios of certain social validity, for example a "work break" to have an online medical assessment which takes just an hour off or less instead of a day off, or the possibility for a working parent or both to "virtually" accompany the minor child [15-17].

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Authors' contributions

MG, CP wrote the draft. AP reviewed the final version of the paper.

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No mention is made of sensitive data referable to patients.

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CASE REPORTS

A novel compound heterozygous mutation in *PYGM* gene associated with McArdle's disease

Salvatore Iacono[1], Antonino Lupica[1], Vincenzo Di Stefano[1], Eugenia Borgione[2], Filippo Brighina[1]

[1] Section of Neurology, Department of Biomedicine, Neuroscience, and advanced Diagnostic (BiND), University of Palermo, Palermo, Italy; [2] Unit of Neuromuscular Diseases, Oasi Research Institute-IRCCS, Troina, Italy

McArdle's disease is an autosomal recessive glycogenosis due to mutation in the myophosphorylase gene (*PYGM*) resulting in a pure myopathy. The clinical onset typically occurs in childhood with cramps, myalgia, and intolerance to physical exercise, although late onset forms are also reported. We describe a case of a 17-year-old male complaining of cramps and myalgia following brief and intense exercise. The patient reported marked improvement in muscle fatigability few minutes after starting aerobic exercise. When he was a child, he had experienced few episodes of vomiting, nausea, and black colored urine following physical activity. Laboratory testings revealed high creatine kinase serum levels. Genetic testings for metabolic myopathies demonstrated a compound heterozygous for two *PYGM* mutations (p.R570Q and p.K754Nfs*49) allowing the diagnosis of McArdle's disease. To date, 183 mutations in the PYGM gene are listed in Human Gene Mutation Database Professional 2021.2, but this novel compound heterozygosis has never been reported before.

Key words: McArdle's disease, glycogenosis, *PYGM*, hyperCKemia, second wind phenomenon

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Correspondence
Salvatore Iacono
Section of Neurology, Department of Biomedicine, Neuroscience, and advanced Diagnostic (BiND), University of Palermo, via Del Vespro 143, 90127 Palermo, Italy.
Tel. +39 091 6554780; Fax. +390916552974.
E-mail: salvo.iak@gmail.com

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Introduction

McArdle's disease, also known as glycogen storage disease (GSD) type V, is an autosomal recessive GSD due to mutations in the gene of myophosphorylase (*PYGM*) (NM_005609), located on chromosome 11q13; this condition leads to myophosphorylase deficiency and the subsequent reduction of glycogen breakdown in voluntary muscles thus blocking glycogenolysis [1]. As a consequence, the low levels of pyruvate and adenosine triphosphate cause, in turn, a fuel shortage in voluntary muscles, as well as the secondary impairment of oxidative phosphorylation [2]. Therefore, McArdle's disease results in a pure myopathy characterized by intolerance to static or dynamic exercise, myalgia, muscle's contractures and high serum creatin kinase levels (hyperCKemia); furthermore, there is a reduced oxygen consumption during exercise and an unbalanced exercise-induced increase in heart rate. Symptoms occur in childhood in most patients, although the diagnosis may be delayed until 30 years of age [1]. To date, 183 mutations affecting *PYGM* gene are listed in Human Gene Mutation Da-

tabase Professional 2021.2 including missense and nonsense mutations, splicing mutations, small insertions and deletions (http://www.hgmd.cf.ac.uk). Herein, we report the case of a young male referred to our Neuromuscular Clinic for intolerance to static exercise and hyperCKemia who was diagnosed with McArdle's disease caused by a novel compound heterozygous mutation in *PYGM* gene.

Case report

In January 2020, a 17-year-old boy presented to our Neurologic Clinic at the University Hospital "Paolo Giaccone", Palermo, Italy, complaining of isolated muscle cramps and myalgia after brief and intense exercise. The past medical history was non-contributory, despite he reported the occurrence of vomiting, cephalalgia and black-colored urine following physical exercise in childhood. His parents were not consanguineous and they did not suffer from any neurological disease. The young boy exhibited an occasional laboratory analysis showing hyperCKemia (2937 U/L [normal value 26-192 U/L]) and elevated myoglobin (174,5 ng/ml [normal value less than 60 ng/ml]) and troponin (122.5 pg/ml [normal value less than 14 pg/ml]) serum levels. Other laboratory parameters (i.e., complete blood count, urinalysis, liver function, creatinine and urea) were within normal limits. The neurological examination showed mild motor weakness at biceps and triceps brachii bilaterally (grade 4 according to Medical Research Council) in absence of muscle wasting. Sensory function, deep tendon reflexes and coordination were normal. As a myopathy was suspected, the patient underwent to needle-electromyography of the proximal and distal muscles of the upper and lower limbs, which showed the presence of small motor unit potentials without spontaneous activity in overall tested muscles (Fig. 1). Subsequently, the patient underwent to monthly serum CK testing with the finding of dramatic hyperCKemia (11.375 U/l) in the days following vigorous physical exercise. However, serum CK in his parents was normal. In further examinations, the patient reported marked improvement in muscle fatigability few minutes after starting aerobic exercise. Hence, genetic testing for metabolic myopathies was performed with a high suspicion of McArdle's disease.

The proband and his parents signed the informed consent forms for diagnostic workup and genetic analysis. The documents were those currently in use at "P Giaccone" University Hospital, Palermo, Italy and at Association Oasi Maria SS, Troina, Enna, Italy. Genetic testing was performed via Next Generation Sequencing (NGS), which included a complete genetic panel for metabolic myopathies. The presence of pathogenetic or potentially pathogenetic variants was confirmed by Sanger

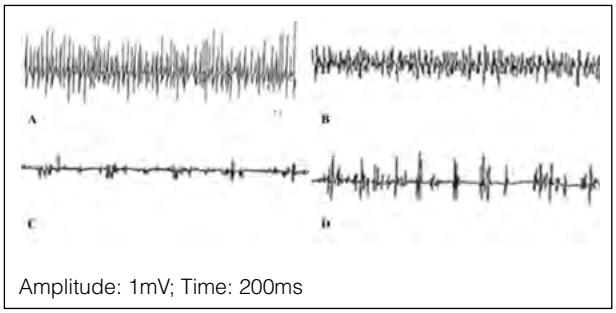

Figure 1. Needle-electromyography during maximal muscular contraction shows early motor unit recruitment as well as the presence of both small and high-amplitude motor units potentials at right biceps brachii (A); small and polyphasic motor unit potentials are showed at right deltoideus (B), right gastrocnemius (C) and right tibialis anterior (D)

sequencing. Variant analysis was performed employing bioinformatic prediction tools (i.e., Mutation Taster, Sift, Polyphen, ClinVar) and the pathogenicity classification was conducted according to the American guidelines College of Medical Genetics and Genomics (ACMG). NGS revealed the missense mutation c.1709G > A (p.R570Q) and the frameshift mutation c.2262delA (p.K754Nfs*49) in a novel compound heterozygous of the *PYGM* gene, confirming the diagnosis of McArdle's disease (Fig. 2).

The patient routinely underwent to urinalysis and laboratory testings (i.e., CK, urea, creatinine). He was recommended to avoid toxic drugs such as statin and isometric exercise in favor to mild aerobic exercise. Also, carbohydrates assumption before physical activity was recommended. At the scheduled follow-up visits after six, twelve and eighteen months from the diagnosis, the neurological examination was normal without disease progression although serum CK was persistently elevated (i.e., 2555 U/l, 2204 U/l and 3055 U/l respectively).

Discussion

We described a case of McArdle's disease caused by a novel compound heterozygous mutation in the *PYGM* gene consisting of the missense mutation p.Arg570Gln (p.R570Q;c.1709G > A) and the frameshift mutation p.Lys754AsnfsTer49 (p.K754NfsX49; c.2262delA), in the exons 14 and 18, respectively (Fig. 2). The familiar segregation analysis showed the presence of p.R579Q and p.K754NfsX49 mutations in the mother and in the father of the proband, respectively (Fig. 3). Both these mutations are known as pathogenetic, although their compound heterozygous has never been reported to date. The p.R570Q mutation was found to be in compound

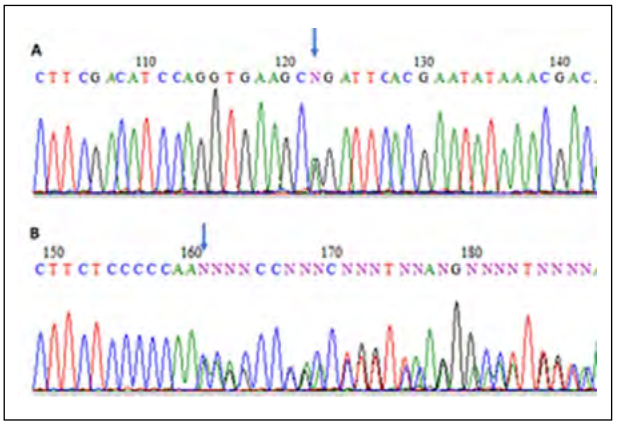

Figure 2. Electropherogram of the exon 14 region encompassing the c.1709A > G mutation (p.R570Q) in *PYGM* gene (A); Electropherogram showing the A deletion at nt2262 in the exon 18 resulting in a frameshift and a premature termination of the protein 47 amino acids downstream of the mutation (B).

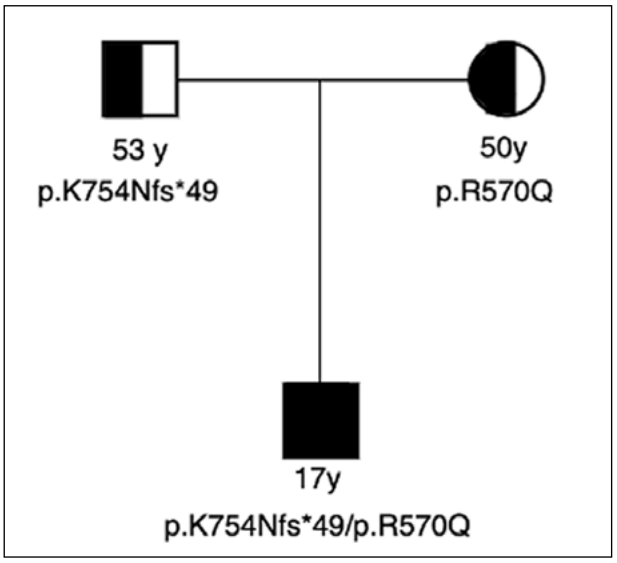

Figure 3. Familiar segregation analysis. The parents of the proband are healthy carriers for mutations; the proband was found to be compound heterozygous for p.R570Q and p.K754NfsX49 mutations in *PYGM* gene.

heterozygosis with the p.R50X in a Portuguese patient[3]. Moreover, the p.K754NfsX49 mutations was found to be homozygous or in compound heterozygous together with the p.R50X mutation [4]. The genotypes p.R50X/p.R50X and p.R50X/p.W798R account for about the 50% of *PYGM* genotypes undelying McArdle's disease; indeed, the p.R50X nonsense mutation in the exon 1 of *PYGM* gene, is the most frequent mutation in Caucasian and Brazilian patients, whereas it has not been found in Japan, where the most frequent is the p.F710del mutation [5]. The clinical heterogeneity (e.g., age at symptoms onset, disease severity, neurological impairment) and an unclear genotype-phenotype relationship make challenging the diagnosis of McArdle's disease. The complete absence of *PYGM* enzymatic activity and the absence of its gene-transcript in overall *PYGM* mutations have been reported as underlying factors of the lacking genotype-phenotype relationship [6]. However, Vissing et al. reported a little genotype-phenotype correlation in McArdle's disease, because of two patients affected by milder phenotype of McArdle's disease were found to be compound heterozygous for deep intronic mutations and other alleles with a subsequent residual *PYGM* enzymatic activity [7]. However, although we did not test the *PYGM* enzymatic activity, our case resembles a typical case of McArdle's disease. In this scenario, it is reasonable to collect a detailed medical history focusing on exercise intolerance, both static and dynamic as well as on vomiting episodes, cephalalgia and black colored urine following physical activity. Moreover, the "spontaneous second wind phenomenon" and the "glucose-induced second wind phenomenon" are highly specific and sensitive for McArdle's disease; the former refers to the marked improvement in muscle fatigability between 8 and 10 minutes after starting exercise, whereas the latter indicates the rapid improvement in exercise-tolerance after intravenous glucose infusion [8]. The biochemical mechanisms underlying the "glucose-induced second wind phenomenon" are strictly connected with the recommendation to assume carbohydrates before exercise. Moreover, the persistently elevated serum CK levels with episodic peaks following short-intensity exercise, as well as a normal level of serum lactate after forearm exercise, support the clinical suspicion of McArdle's disease even with a normal neurological examination [9]. Finally, the muscle biopsies of the vastus lateralis or the biceps brachialis might show subsarcolemmal or intermyofibrillar glycogen deposits driving toward the diagnosis of McArdle's disease [10]. However, given the availability of NGS with a genetic panel for metabolic myopathy and the stringent pandemic restrictions, genetic testing was the first choice for the diagnosis. Future studies are needed to better clarify the genotype-phenotype relationship in McArdle's disease.

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Authors' contributions

SI, AL and VDS diagnosed and followed up the patient. EB performed the genetic analyses. SI drafted the manuscript. SI, AL, VDS and FB designed the report and made the final revision. All authors reviewed and approved the final version of the manuscript.

Ethical consideration

The manuscript in part or in full has not been submitted or published anywhere. All procedures were in accordance with the standards of the bioethical committee and the Declaration of Helsinki.

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Peculiar histological and ultrastructural skeletal muscle alterations in a patient with CMV infection and autoimmune myositis: case evaluation and brief literature review

Michela Ripolone[1], Laura Napoli[1], Vittorio Mantero[2], Monica Sciacco[1], Simona Zanotti[1]

[1] Neuromuscular and Rare Disease Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [2] UOC Neurologia - Stroke Unit, Presidio "A. Manzoni", ASST Lecco, Italy

We report the case of a young woman with CMV infection, high level of creatine kinase and myopathy. Electromyography showed a myopathic pattern. Muscle biopsy showed a marked increase of NADH enzymatic activity in the central area of almost all type I fibres, few degenerative and necrotic fibres and scattered mononuclear cell infiltrates. Ultrastructural analysis showed a marked disarrangement of sarcomeric structure and large inclusions of thin filaments in some fibres, while immunohistochemistry evidenced alteration in desmin, actin and αB-crystallin protein signals. PCR for CMV detection on muscle sections was negative. Histological, immunological and ultrastructural evaluations were compatible with a necrotic inflammatory myopathy. The correlations between CMV liver infection and the myopathic pattern are discussed. This case underscores the need to consider CMV infection in the differential diagnosis of myopathy with undetermined aetiology, quickly providing directions for a targeted muscle pharmacological intervention.

Key words: CMV, muscle biopsy, myofibrillar disorganization, Z-band streaming

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Correspondence
Simona Zanotti
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Disease Unit, via F. Sforza 35, 20122 Milan, Italy. Tel. +39 02 55036504. Fax. +39 02 55053827. E-mail: simona.zanotti@policlinico.mi.it

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Introduction

Viral infections have been frequently reported in association with development of secondary myopathies characterized by different forms of muscle involvement that can vary from mild to severe inflammatory myopathy. Literature reported evidences of nemaline myopathy and myositis after human immunodeficiency infection (HIV) [1], myositis after infection by hepatitis B and C [2], Epstein-Barr virus [3], herpes simplex virus [4] and, less frequently, cytomegalovirus (CMV) [5]. Few cases of severe rhabdomyolysis in association with CMV infection [6,7], and a case of polymyositis associated with primary CMV infection were reported [5].

Herein, we describe the case of a young woman with hepatitis by primary CMV infection, muscle weakness, myalgia, oedema and increased serum creatine kinase (CK) levels associated with severe and marked structural alterations in skeletal muscle, whose symptoms improved after

immunomodulatory treatment (intravenous immunoglobulin followed by steroid).

Materials and methods

Case history

A 29-year-old female, with a medical history of asymptomatic SARS-CoV-2 infection (September 2020) and Raynaud's syndrome, was hospitalized in February 2021 because of a CMV hepatitis associated with asymmetrical upper limb muscle weakness. She reported a three-week history of asthenia, myalgia, local swelling, marked oedema in the upper limbs and low-grade fever without any dyspnoea or dysphagia. Needle electromyography (EMG) showed myopathic pattern in the proximal and distal muscles of the four limbs with abundant signs of denervation in the active phase. During hospitalization, haematological analyses showed a progressive macrocytic anemia (up to a Hb 8.8 g/dL, MCV 99.7), reduced blood cell count (leukocyte 3.200/μL, platelets 162.000/μL), and high levels of D-dimer, most likely attributable to a systemic inflammatory state. No myoglobinuria was detected.

Laboratory examinations showed an increase in CK (3371 U/L, n.v. = 26-192) and lactate dehydrogenase (LDH = 536 U/L, n.v. = 125-250) levels and a deranged liver function (ALT = 220 U/L and AST = 549 U/L). Renal function was normal. A full body CT-scan indicated a slightly enlarged spleen.

The serological viral panel for CMV, EBV, HCV, HIV showed positivity for CMV (anti-CMV IgG 80.00 U/mL, IgM 53.70 U/mL). RT-PCR investigation for CMV in skeletal muscle was negative. The screening for autoantibodies (ASMA, AMA, ANA, ANCA, ENA, anti CCP, anti ds-DNA, anti-beta2 glycoprotein and rheumatoid factor) was negative and so was the screening for autoimmune myositis (antibodies anti-PL-7, PL-12, SRP, Mi-2, EJ, MDA-5, TIF-g, SAE1, SAE2, NXP-2). There were no skin lesions suggesting dermatomyositis.

The patient was treated with intravenous immunoglobulins 0.4 mg/kg for 5 days and with steroid therapy (methylprednisolone 500 mg intravenous for 5 days followed by oral prednisone 50 mg daily) with progressive improvement of asthenia and normalization of CK levels (58 U/L).

At the follow-up two months after discharge, the patient reported an almost complete recovery, with normal walking also possible on toes and heels. Haematological analyses were within normal range except for platelets (141.000/μL). A control EMG three months after dismissal showed regression of spontaneous activity in both proximal and distal muscles of four limbs, only modest myopathic signs being evident in the right ileopsoas muscle.

Muscle biopsy

Skeletal muscle biopsy from left quadriceps was performed at the Neurologia-Stroke Unit of Lecco Hospital and sent to our Neuromuscular and Rare Disease Unit for histological, immunohistochemical and electron microscopy evaluations.

Muscle sections from patients without any detectable muscle diseases were used as normal controls while muscle sections from three patients with diagnosed inflammatory myopathy served as pathological controls (all patients had signed written informed consent when they had undergone muscle biopsy).

Tissue specimen was frozen in isopentane-cooled liquid nitrogen and processed according to standard techniques, as previously described [8]. For histological analysis, 8 μm-thick cryosections were picked and processed for routine staining with Haematoxylin and Eosin (H&E), Modified Gomori Trichrome (MGT), myosin ATPase (pH 9.4-4.6-4.3), cytochrome c oxidase (COX), succinate dehydrogenase (SDH), phosphatase acid (PA), NADH, Oil Red O, Periodic Acid Schiff (PAS) and Congo Red.

Electron microscopy

For ultrastructural examination, a small part of muscle sample was fixed in 2.5% glutaraldehyde (pH 7.4), post fixed in 2% osmium tetroxide and then, after dehydration in a graded series of ethanol, embedded in Epon's resin. Finally, ultrathin sections were stained with lead citrate and uranyl acetate and examined with Zeiss EM109 transmission electron microscope.

Immunofluorescence

Immunohistochemical staining was performed for evaluation of inflammatory component using the following mouse monoclonal antibodies: anti-HLA-ABC Antigen/RPE (1:10), anti-MAC (1:50), anti-CD4 (1:50), anti-CD8 (1:10) and anti-CD68 (1:50), all from Dako Agilent (Santa Clara, CA, USA). Mouse monoclonal anti-cytomegalovirus antibody (not diluted, clone CCH2+DDG9; Dako) was used to detect the presence of virus in muscle sections.

For muscle structural evaluation the following antibodies were tested: actin (1:500 rabbit polyclonal; ThermoFisher, Rockford, IL, USA), myotilin (1:10 mouse monoclonal; Novocastra Laboratories, Newcastle upon Tyne, UK), desmin (1:100 mouse monoclonal; Chemicon, Millipore, Billerica, MA, USA), αB-crystallin (1:100 rabbit polyclonal; Chemicon). Slides were then incubated with the appropriate secondary antibody conjugated to Alexa 488 or Alexa 568 (1:200, Invitrogen Life Technologies, Carlsbad, CA, USA).

Results

Light microscopy

The outstanding feature of muscle biopsy was the strong increase of NADH enzymatic activity in the central area of almost all type I fibres while subsarcolemmal areas of reduced enzymatic activity were appreciated in rare type I fibres (Fig. 1D). H&E and MGT stains showed a few degenerative and necrotic fibres and scattered mononuclear infiltration (Figs. 1A-B). No centronucleated fibres were observed. Fibres were normal in shape with a physiological variability in size. Endomysial and perimysial connective tissue was normal. Rare splitting fibres were also observed. ATP-ase staining showed

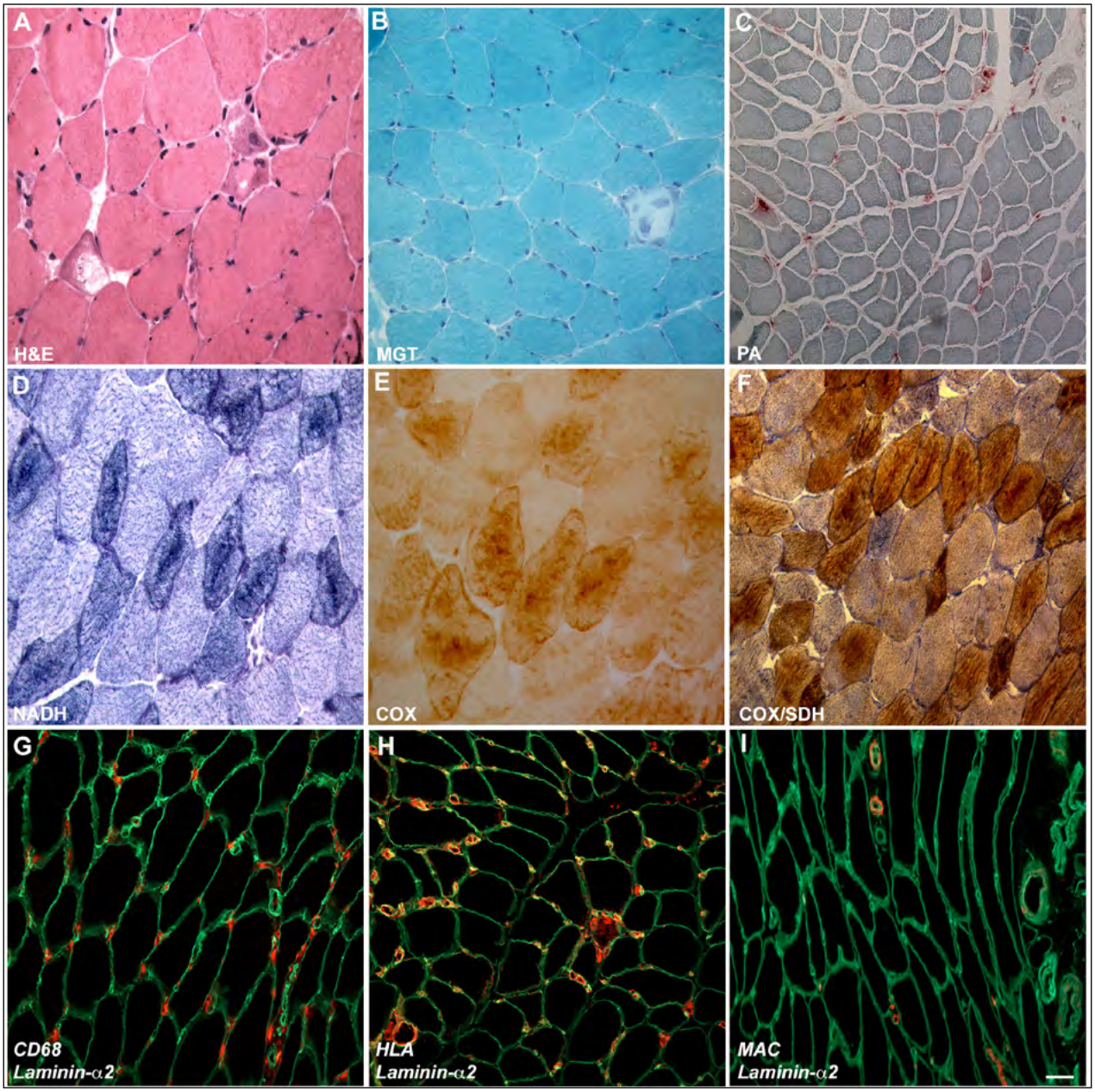

Figure 1. Light microscopy observations. H&E (A) and MGT (B) show few degenerative and necrotic fibres and normal connective tissue (magnification 400 x). Acid phosphate reaction (C) is positive in necrotic fibres and at interstitial level (magnification 200 x). NADH (D) signal is increased in type I fibres, COX (E) and COX-SDH (F) reactions show COX signal reduction in some scattered fibres with normal SDH activity (magnification 400 x). Immunohistochemistry shows CD68 (macrophage) positivity (G), HLA positivity at membrane level and in the cytoplasm of necrotic fibres (H) and MAC positivity in capillaries (I). Laminin-α2 (green) was used for membrane counterstaining (magnification 400 x).

a normal distribution of type I and type II fibres. Diffuse and moderate increase in acid phosphatase activity in necrotic fibres and in the interstitial tissue were detected (Fig. 1C). COX activity was reduced in scattered fibres with normal SDH (Figs. 1E-F). No changes in glycogen and lipid content were observed.

Electron microscopy

Ultrastructural examination showed alteration of sarcomeric structure in several muscle fibres. In particular, we observed three main types of structural changes. In a fair number of fibres, we found thin filamentous inclusions, localized either in the sarcoplasm or at subsarcolemmal level (Figs. 2B, and E). Also, we detected focal areas of myofibrillar disorganization in the centre of otherwise normal muscle fibres (Figs. 2A, C and F). Lastly, as shown in figure 2D, in some areas we observed a disruption of the Z-bands with longitudinal streaming of Z-band material along the sarcomere. Additional observations include a slight increase in lipid droplets (Fig. 2D) and rare lipofuscines (Fig. 2E). Nuclei showed a normal aspect with the exception of those located in the necrotic fibres.

Immunofluorescence

Infiltrating cells were predominantly CD68-positive (Fig. 1G), while rare positivity was observed for CD4 and CD8. Anti-MAC stained some capillaries and anti-HLA showed positivity at the sarcolemma of nearly all muscle fibres and in the cytoplasm of necrotic fibres. Sarcoplasmic HLA-positivity was occasionally observed in some non-necrotic fibres (Figs. 1H-I). No positivity was observed in muscle biopsy tissue from controls.

In addition, several other alterations in fluorescent signals were detected compared to control muscles. In particular, anti-desmin staining showed a discontinuous signal at membrane level as well as a diffuse cytoplasmic signal mostly localized in the central area of several fibres. This positivity was different from the vacuolar staining observed in diagnosed inflammatory myopathies. In control muscles desmin staining was localized only at membrane level (Fig. 3).

Anti-actin staining showed areas of strong positivity in a discrete number of fibres, mainly localized at subsarcolemmal level. No alterations were detectable in both control muscles and in inflammatory myopathy specimens (Fig. 3).

With αB-crystallin staining, many fibres showed a diffuse strong positivity in aggregate-like structures, in some other fibres, however, the staining was localized either in subsarcolemmal areas, or extended to the almost totality of the cytoplasm. This pattern is totally different from what we see in inflammatory myopathies (Fig. 3).

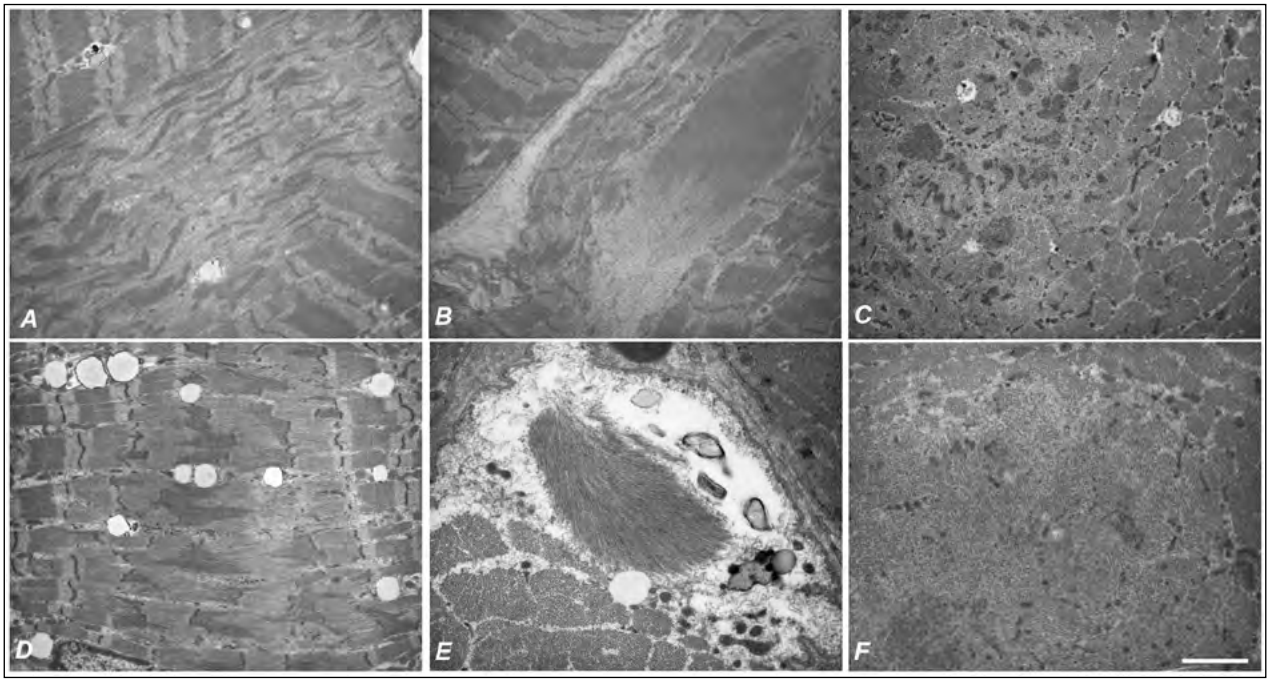

Figure 2. Ultrastructural observation. Central areas of myofibrillar disorganization (A, C, F). Inclusion of thin filaments in the sarcoplasm and in the subsarcolemmal region (B, E). Disruption of the Z-bands with longitudinal streaming of Z-band material, and a slight increase in lipid droplets (D). Scale bar: 2270 nm (A-D), 1420 nm (E, F).

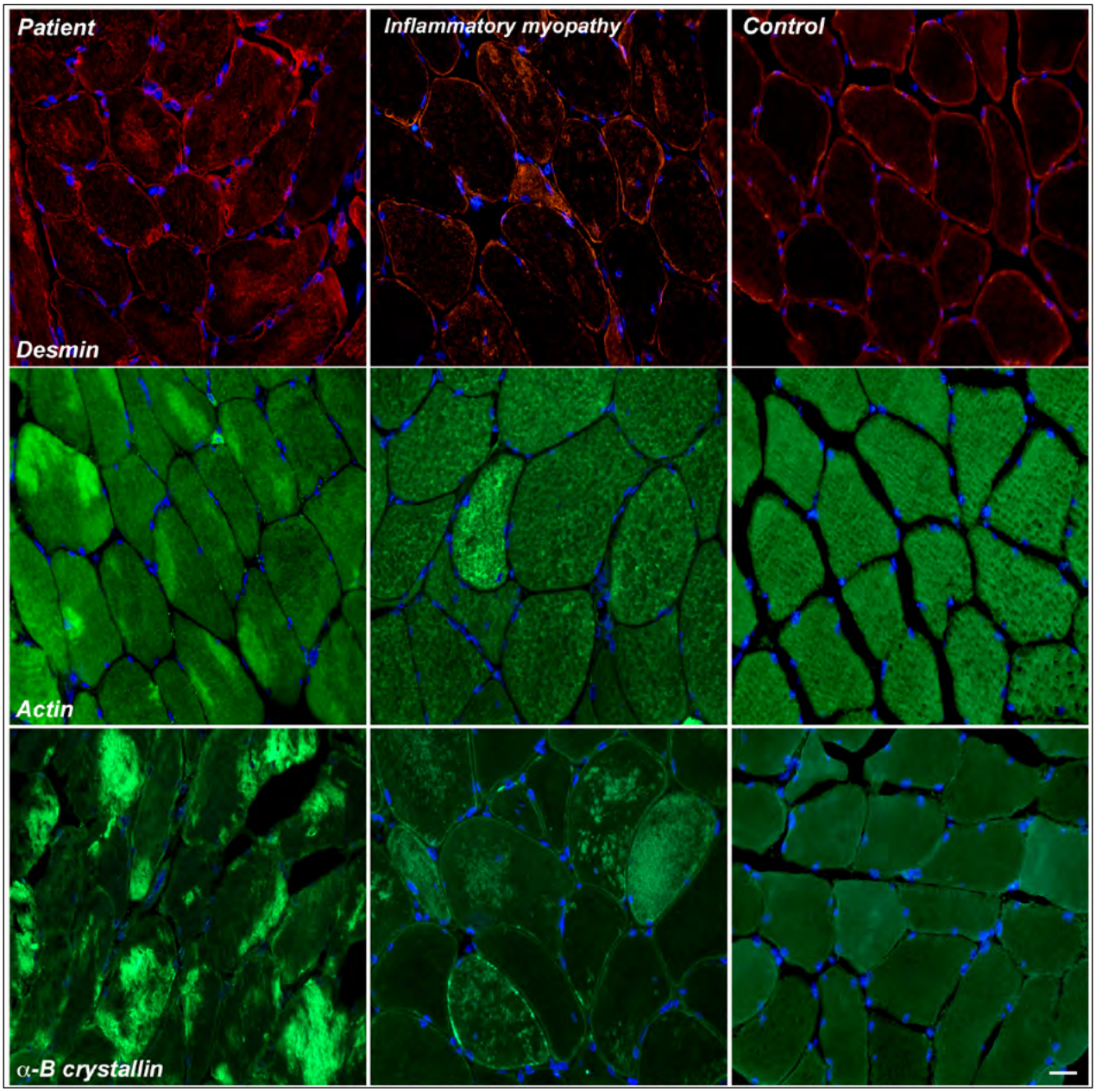

Figure 3. Desmin, actin and αB-crystallin staining in our patient, in diagnosed inflammatory myopathy and in control muscle. Nuclei were detected with DAPI. Magnification 400 x.

Conclusions

We report the case of a young woman, hospitalized because of a CMV hepatitis and muscle weakness, discharged with a diagnosis of autoimmune myositis. Routine muscle histology was suggestive of a primary necrotizing inflammatory myopathy with peculiar central areas of increased NADH activity in type I fibres. Further investigation by ultrastructural analysis showed, in several fibres, large inclusions of thin filaments, focal areas of myofibrillar disorganization and disruption of the Z-bands with longitudinal streaming of Z-band material along the sarcomere. Immunofluorescence showed marked alterations in desmin, actin and αB-crystallin expression and localization in several fibres.

These findings are quite unique for a primary myositis and, to our knowledge, no cases of similar "secondary myopathies" have ever been associated with CMV hepatitis. A previous single case of muscle weakness, diffuse myalgia with rhabdomyolysis and acute respiratory pa-

ralysis probably associated with CMV infection was reported [7].

Although the presence of viral particles has not been confirmed in skeletal muscle by real-time PCR or immunohistochemistry, and the mechanism through which the virus could affect skeletal muscle is still unknown, we can hypothesize that the CMV infection has caused the observed alterations in skeletal muscle as an indirect host-derived effect. Indeed, besides the direct viral liver infection, indirect effects probably mediated by the immunological response can cause detrimental consequences including skeletal muscle alterations [9]. However, a possible direct viral muscle infection cannot be completely excluded. Indeed, viral count could have remained below threshold detection level due to methodological limits and/or very low (latent) viral activity when PCR was performed. Ultrastructural changes have been reported in different types of CMV-infected cells as direct effects: in human bone marrow fibroblasts, mitochondrial enlargement, production of dense bodies and cytoplasmic accumulation were observed [10]. During in vitro CMV infection, a rapid and progressive alteration of actin, microfilaments and cytoskeleton was observed in both human embryo and lung fibroblasts [11], however what happens in cells and tissues not directly invaded by the virus is still poorly understood.

The clear improvement of the electromyographic pattern following the acute phase, confirms the non-primary nature of the myopathy.

To summarize, several issues – clinical presentation, serological and neurophysiological evidence, skeletal muscle findings and progressive improvement of clinical and instrumental parameters after therapy – favour the hypothesis of an autoimmune/inflammatory myopathy. We could not demonstrate the presence of viral particles in skeletal muscle, but, as explained, an indirect host-derived effect is likely implicated, not to mention concomitance between liver infection and onset of myopathic symptoms.

The study of this case has an important implication for the medical internist approach towards primary CMV viral infection; indeed, the presence of symptoms induced by viral hepatitis could cause underestimation of severe effects on other tissues/organs including skeletal muscle.

Also, our report underscores the need to consider CMV infection in the differential diagnosis of myopathy with undetermined aetiology, providing directions for a targeted muscle pharmacological intervention.

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Conflict of interest statement

The Authors declare no conflict of interest

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Authors' contributions

MR and SZ wrote the manuscript, MR, LN, MS, SZ interpreted the results, revised the literature and revised the manuscript. VM performed clinical evaluation.

Ethical consideration

All procedures were in accordance with the standards of the bioethical committee and the Declaration of Helsinki.

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Temporary positive expiratory pressure (TPEP) as an alternative approach in the treatment of persistent atelectasis in a patient with Steinert disease: a case report

Antonietta Coppola, Anna Annunziata, Elena Sciarrillo, Salvatore Musella, Giuseppe Fiorentino

UOC Pathophysiology and Respiratory Rehabilitation, Intensive Care Department, Azienda Ospedaliera dei Colli, Naples, Italy

We describe the clinical case of a patient affected by Steinert disease with persistent dyspnea complicated by a complete obstructive atelectasis of left lower lung lobe. The atelectasis has been successfully treated using the TPEP machine, with resolution of radiological pattern and improvement of the symptoms.

Introduction

Patients with neuromuscular pathologies may present difficulties in the management of secretions and sometimes dysphagia may contribute to the stagnation of secretions, especially in the sloping regions of the chest [1,2]. Respiratory complications in neuromuscular diseases with ineffective cough are represented by the onset of pneumonia and impaired gas exchange, with acute respiratory failure. In patients with muscular dystrophy, the altered relationship of tension in muscle length and the reduction in muscle elasticity and plasticity result in impaired inspiratory muscle function and a decrease in vital capacity. In addition, loss of deep breathing further increases the risk of alveolar collapse, hypoventilation and atelectasis, while acute episodes of respiratory infection determine an increase in bronchial secretions. These alterations can cause reduced values of vital capacity and peak flow, which urgently need manual or mechanical assistance to cough. A personalized rehabilitation treatment, carried out by an experienced team, can lead to clinical resolution.

Case description

A 44-year-old man, never smoker, affected by Steinert disease was admitted to the respiratory care unit of Monaldi hospital for persistent dyspnea.

The patient reported fever about one month earlier and worsening of dyspnea in last time. Admitted to the emergency room, the nasopharyngeal swab was negative for SARS-CoV-2. Chest x-ray revealed left low-

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Correspondence
Anna Annunziata
Unit of Pathophysiology and Respiratory Rehabilitation, Intensive Care, Department, Azienda Ospedaliera dei Colli, via L. Bianchi, 80131 Naples, Italy.
E-mail: anna.annunziata@gmail.com

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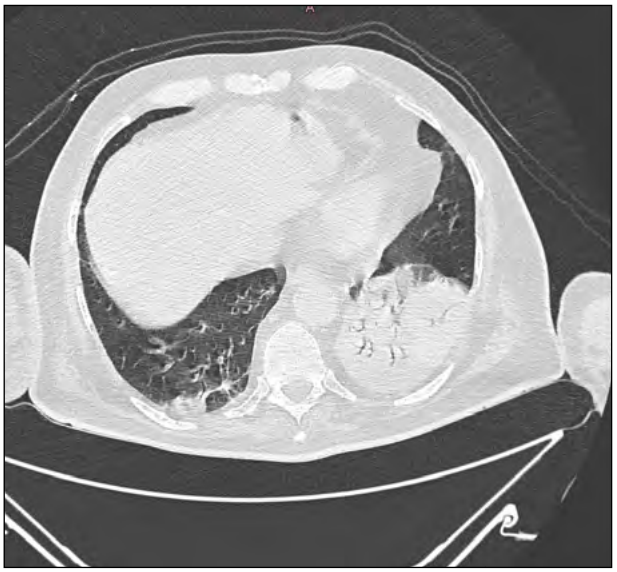

Figure 1. Persistent pulmonary atelectasis.

er lobe atelectasis. CT scan confirmed this finding and homolateral hemidiaphragm elevation (Fig. 1). Lung ultrasound confirmed the presence of a left pulmonary atelectasis which appeared as an area of pulmonary parenchyma with a tissue-like pattern and abolished lung sliding in the presence of lung pulse. Blood gas analysis revealed mild hypoxemia with a normal acid-basis balance. Functional respiratory tests showed a mild restrictive syndrome. Cough peak flow was 270 L/sec. Sputum microbiological examination, carried out on several samples, was negative.

The patient had been treated with various antibiotics (amikacin, vancomycin, piperacillin tazobactam, fluoroquinolones) and chest physiotherapy. It was decided a different respiratory physiotherapy approach, with a recruitment maneuver using the insufflation phase of a cough machine, associated with manually assisted cough and mechanical in-exsufflation therapy. Despite further 20 days of antibiotic therapy, the patient had no benefit: low grade fever (37.5°C max) and dyspnea persisted. As the patient and his family did not give consensus to a bronchoscopy to remove secretions, we opted for an active breathing assisted technique through the Temporary Positive Expiratory Pressure (TPEP®) device, twice daily for seven days. Using visual targets, inspiratory and expiratory resistors, the "I/E mode" program guide the patient to gradually inhale more deeply, to hold the breath for a while and to exhale slowly with an open glottis and an oscillating expiratory counter-flow.

After a brief training session, the initial settings included an inspiratory target of 4 cmH_2O sustained for a mean of 5 seconds, a 3 sec tele-inspiratory pause and an expiratory target of 10 cmH_2O sustained for about 6 sec. This program lasted at least 20 minutes every session, in the right and left lateral position.

In order to not increasing the work of breathing, flow-dependent resistances were gradually adjusted to make the patient's work as easy as possible. Every session ended with 10 minutes of breathing with the "TPEP mode" program, that allows a guided slow and deep expiration with an open glottis while providing a temporized oscillating PEP of 1 cmH_2O during most of the expiration. This second phase was aimed to enhance the expiratory flows and secretion removal. After trials for two days, no symptoms or atelectasis extension were recorded.

At the seventh day, we observed an improvement of gas exchange and the resolution of atelectasis at lung ultrasound. Patient underwent a thoracic TC scan that confirmed the resolution of the atelectasis (Fig. 2).

Discussion

Lobar atelectasis is a common problem caused by a variety of mechanisms including resorption, airway obstruction, hypoventilation, and compressive atelectasis from abdominal distension and adhesive atelectasis due to increased surface tension.

In literature there is a lack of evidence-based studies to guide the management of this common problem. Treatment modalities that have been described include chest physiotherapy, bronchodilators, and the use of positive end-expiratory pressure [3].

Chest physiotherapy is the first-line therapy for atelectasis due to airway obstruction. If physiotherapy fails, further radiological examination of the chest may be helpful to identify airway obstruction and to determine whether proximal lobar or distal bronchi are involved. Bronchoscopy to aspire secretions is useful in the management of atelectasis when less invasive solutions fail [4].

In this case report, the patient had a lower left lobe atelectasis mainly due to presence of blood cot and se-

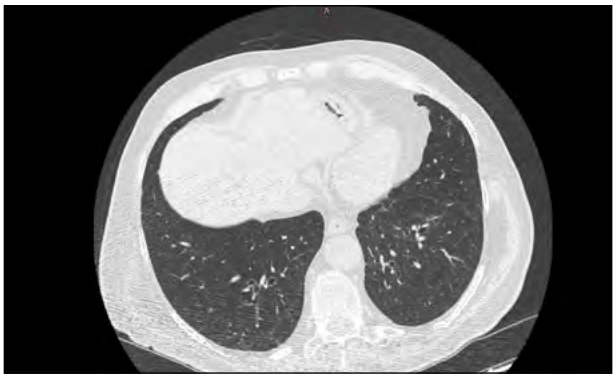

Figure 2. After TPEP therapy.

cretion retention. As the first approach with mechanical assisted lung recruitment fails, we decide for a bronchoscopy aimed to reduce the obstruction, but the patient refused it. Thus, we opted for an active device-assisted recruitment.

Temporary Positive Expiratory Pressure (TPEP) is a well-known patented technology to mechanically deliver a low positive expiratory pressure at the mouth during spontaneous breathing and it is usually used to assist patients with chronic obstructive pulmonary disease for airway clearance. The I/E mode was recently added to allow the treatment of deep lung in cooperative patients. The use of positive expiratory pressure to improve lung volumes is well documented in the literature; in general the physiotherapists use this strategy in patients with low lung volumes to improve ventilation and gas exchanges.

The use of PEP and T-PEP also improve dyspnea and quality of life and speed up the improvement of bronchial encumbrance [3,4]. Preliminary results show that an expiratory pressure ≤ 1cm H_2O applied for a fraction of the expiratory phase, may improve the distribution of alveolar ventilation [5].

The use of TPEP is documented in chronic respiratory diseases, including COPD, asthma and Cystic Fibrosis; it has been shown that symptoms and pulmonary function testing (including reduction in air-trapping) improved after 2 weeks of treatment [6]. Currently, no studies are available about the use of this device for the treatment of massive atelectasis, neither in the treatment of Steinert patients.

Nevertheless, we know that lung alterations in neuromuscular diseases are unusual and require extra-caution with the use of positive pressure. TPEP uses positive expiratory pressures several times lower than the commonly used devices and, when used in the I/E mode, pressures are used as a target driving the patient breath by breath, thus preventing excessive mechanical stress on the bronchial tree and lung parenchyma.

This clinical case was challenging because no results were obtained with the usual recruitment strategies (long-term low pressure mechanical insufflation), while with the active "I/E mode" strategy we obtained a complete resolution of a massive atelectasis, with improvement of gas exchanges and resolution of hypoxemia.

This experience encourages the use of the TPEP device in neuromuscular patients with persistent lung involvement. Clinical diffusion of the I/E mode is still limited, although there is a growing interest in identifying the potential benefits of this technology in a wide range of respiratory conditions, and its role in pulmonary rehabilitation programs for neuromuscular diseases.

Conclusions

The multidisciplinary team is necessary to guarantee a comprehensive diagnostic approach, early recognition of complications, and personalized therapy for these patients with multiple frailties. In this clinical case we have successfully used TPEP® and I/E mode for the treatment of low left lobe obstructive atelectasis in a patient affected by Steinert disease, achieving rapid resolution of atelectasis. This experience encourages the use of this device in the treatment of atelectasis as a viable option for other passive or invasive methods; moreover its role in the rehabilitation of lung inhomogeneities in neuromuscular patients.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Authors' contributions

AC: conceptualization, data collection, draft writing, final version writing; AA: conceptualization, reading, final version writing; SM: data collection; ES: data collection; GF: supervision

Ethical consideration

This study was approved by the Institutional Ethics Committee of University of study of Campania "L. Vanvitelli" (protocol number 372/2019). The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from patient for study participation and data publication.

References

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NEWS FROM AROUND THE WORLD

AIM

In the period between January and March 2022, the Association promoted and sponsored a masterclass on Congenital Myopathies which took place on 4th Febraury 2022 in virtual edition and involved neurologists, pediatricians, child neuropsychiatrists, neonatologists and geneticists.

The Association has given the patronage for the fifth virtual "Annual Day of the Neuromuscular Diseases" which took place on March 12, 2022 – simultaneously in 16 different Italian cities, representative of Expert Centers in Neuromuscular Diseases and belonging to the Italian Association of Myology and the Italian Association of Peripheral Nervous System (*www.giornatamalattieneuromuscolari.it*). In this context AIM sponsored a research grant for a project on Neuromuscular Diseases.

Prof. Olimpia Musumeci
Secretary of Italian Association of Myology

MSM

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

WMS

The 27th International Annual Congress of the World Muscle Society will take place 11th-15th October 2022, in Halifax, Nova Scotia, Canada. The congress venue is the Halifax Convention Centre at 1650 Argyle Street, in the heart of this Atlantic seaport. Electronic presentations and posters will be made available via the Society's platform as well as apps for delegates wishing to participate virtually. Hard work is being done to ensure that the registration process is as flexible as possible in these ever-changing and uncertain times.

To learn more, please visit the congress website: *https://www.wms2021.com*

FORTHCOMING MEETINGS

2022

March 11-13

260th ENMC Workshop: Congenital Myasthenic syndromes. Information: website: *https://www.enmc.org*

March 25-27

258th ENMC Workshop: Leigh syndrome. Information: website: *https://www.enmc.org*

April 28-May 02

14th European Paediatric Neurology Society Congress, Glasgow, UK. Information: website: *www.epns.org*

May 13-15

263rd ENMC Workshop: Focus on female carriers of dystrophinopathy: refining recommendations for prevention, diagnosis, surveillance and treatment. Information: website: *https://www.enmc.org*

June 10-12

257th ENMC Workshop: The 3rd ENMC workshop on Dystroglycan and the Dystroglycanopathies. Information: website: *https://www.enmc.org*

June 15-17

TREAT-NMD Conference 2022. Vancouver Convention Centre 1055 Canada Pl, Vancouver, BC V6C 0C3, Canada. Information: website: *https://treat-nmd-conference.org*

June 17-19

261st ENMC Workshop. Management of safety issues arising following AAV gene therapy. Information: website: *https://www.enmc.org*

June 24-26

253rd ENMC workshop. Skeletal muscle laminopathies – natural history and clinical trial readiness. Information: website: *https://www.enmc.org*

June 25-28

8th EAN Congress. Vienna, Austria. Information: website: *https://www.ean.org*

July 5-9

17th International Congress on NeuroMuscular Diseases (ICNMD). Brussel, Belgio. Information: website: *www.icnmd.org*

September 13-15

7th Congress of Myology. Nice Acropolis, France. Information: website: Institut de Myologie *https://www.institut-myologie.org*

September 15-17

Mitochondrial Medicine Meeting. Nice Acropolis, France. Information: website: Institut de Myologie *https://www.institut-myologie.org*

October 11-15

27th Congress of World Muscle Society. Halifax, Canada. Information: website: *https://worldmusclesociety.org*

2023

January 9-11

12th World Gene Convention. Sapporo, Japan. Information: website: *https://www.bitcongress.com/WGC2023*

July 1-4

9th EAN Congress. Budapest, Hungary. Information: website: *https://www.ean.org*

October 3-7

28th Congress of World Muscle Society. Charleston, USA. Information: website: *https://worldmusclesociety.org*

2024

June 29 - July 2

10th EAN Congress. Helsinki, Finland. Information: website: *https://www.ean.org*

October 8-12

29th Congress of World Muscle Society. Prague, Czech Republic. Information: website: *https://worldmusclesociety.org*

2025

October 7-11

30th Congress of World Muscle Society. Vienna, Austria. Information: website: *https://worldmusclesociety.org*

For application or renewal to MSM

MEDITERRANEAN SOCIETY OF MYOLOGY* (MSM)

APPLICATION/RENEWAL FORM

Application/Renewal for **1yr** ☐ **2 yrs** ☐

Prof. Luisa Politano, Cardiomiologia e Genetica Medica, Primo Policlinico, piazza Miraglia, 80138 Napoli, Italy
Fax: 39 081 5665101 E-mail: actamyologica@gmail.com • luisa.politano@unicampania.it
Fax or Mail to the above address. Type or print.

Name _____ Degree(s) _____
Last First

Department _____ _____

Institution _____

Street Address _____

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Tel (_____) _____ Fax (_____) _____
Area code Area code

* Amount payable: 1 year Euro 100
2 years Euro 180

❑ I enclose copy of the bank transfer to:

Bank name: Intesa San Paolo
Bank address: via Toledo 177/178
Account holder: MSM-Mediterranean Society of Myology
IBAN code: IT36 F030 6909 6061 0000 0160 879
BIC/SWIFT code (for foreign countries): BCITITMM

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.

Lectura. 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 name must be included with each reference** (when available). If not available, indicate the PMID number.

Examples of the correct format for citation of references:

Journal articles: Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230-238. doi.org/10.14639/0392-100X-1583

Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.

Please check each item of the following checklist before mailing:

- Three-six index terms, short title for running head (no more than 40 letter spaces) on the title page.
  Name(s) of the author(s) in full, name(s) of institution(s) in the original language, address for correspondence with email address on the second page.
- Summary (maximum 250 words).
- References, tables and figures cited consecutively as they appear in the text.
- Figures submitted actual size for publication (i.e., 1 column wide or 2 columns wide).
- Copyright assignment and authorship responsibility signed (with date) by all Authors.
- References prepared according to instructions.
- English style.
- Patients in photographs not recognisable.