Available online at www.sciencedirect.com
ScienceDirect
Neuromuscular Disorders xxx (xxxx) xxx
www.elsevier.com/locate/nmd

Over three decades of natural history of limb girdle muscular dystrophy type R1/2A and R2/2B: Mathematical modelling of a multifactorial study

Antonella LoMauro[a,*], Sandra Gandossini[b], Annamaria Russo[b], Eleonora Diella[b], Cristina Pistininzi[b], Eraldo Marchi[b], Riccardo Pascuzzo[c], Simone Vantini[d], Andrea Aliverti[a], Maria Grazia D'Angelo[b]

[a] *Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Milano, Italy*
[b] *Scientific Institute IRCCS E. Medea, NeuroMuscular Unit, Bosisio Parini, LC, Italy*
[c] *Neuroradiology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy*
[d] *MOX-Department of Mathematics, Politecnico di Milano, Milano, Italy*

Received 16 September 2020; received in revised form 11 February 2021; accepted 23 February 2021
Available online xxx

Abstract

We aimed to describe the natural history of Limb Girdle Muscular Dystrophy type 2A and 2B over more than three decades by considering muscular strength, motor, cardiac and respiratory function. 428 visits of nineteen 2A and twenty 2B patients were retrospectively analysed through a regression model to create the curves of evolution with disease duration of muscle strength (through Medical Research Council grading), motor function measure scale (D1, D2 and D3 domains) and cardio-pulmonary function tests. Clinically relevant muscular and motor function alterations occurred after the first decade of disease, while mild respiratory function alterations started after the second, with preserved cardiac function. Although type 2A showed relatively stronger distal lower limb muscles, while type 2B started with relatively stronger upper limb muscles, the corresponding motor functions were similar, becoming severely compromised after 25 years of disease. This was the longest retrospective study in types 2A and 2B. It defined curves of disease evolution not only from a neuromuscular, but also from functional, cardiac, and respiratory points of view, to be used to evaluate how the natural progression is changed by therapies. Due to slow disease progression, it was not possible to identify time sensitive endpoints.

Keywords: LGMDR1; LGMD2A; LGMDR2; LGMD2B; Mathematical modelling; Natural history.

1. Introduction

Autosomal recessive limb girdle muscular dystrophies (LGMD2) are a group of genetically heterogeneous diseases typically characterised by progressive weakness and wasting of the shoulder and pelvic girdle muscles. The disease onset, clinical progression, cardiac and respiratory involvement can vary greatly among the different LGMD subtypes. The two most common forms of LGMD2/R in Italy are LGMDR1/LGMD2A and LGMDR2/LGMD2B [1].

Despite improved diagnostics and pathomechanistic insight, a curative therapy is currently lacking for any of these diseases [2–4]. Medical care consists of the symptomatic treatment of complications, aiming to improve life expectancy and quality.

Until now, efficacy of any therapy has not been proven, due to the therapeutic strategy itself but also to the lack of sensitive outcome measures [5]. Slow progression, that requires decades before showing important clinical changes, different pathogenic mechanisms and small number of patients have been the most relevant factors interfering with the development of clinical trials in young/adult onset LGMD2 forms and/or to define milestones and/or markers of disease progression.

Defining the natural history of a disease is an essential requisite to any therapeutic intervention. Indeed natural history data are crucial to show and prove if and how

* Corresponding author.
E-mail address: antonella.lomauro@polimi.it (A. LoMauro).

https://doi.org/10.1016/j.nmd.2021.02.018
0960-8966/

the progression of the disease is changed or halted by any therapy (pharmacological and/or rehabilitative). In spite of this, natural history studies for LGMD are still poor.

Mathematical modelling is increasingly recognised as an important tool to describe disease trends in order to predict disease evolution and to aid decision making in both health and clinical medicine [6,7].

Richard and co-workers performed an observational study of clinical manifestations and disease progression in 85 genetically confirmed LGMDR1/LGMD2A patients for up to 4 years. They showed a slowly progressive disorder, with symptoms onset in the first or second decade of life, characterized by a certain degree of variability related to gender and mutation type [8]. Angelini and co-authors described specific pathogenetic mechanisms to play a relevant role in the different clinical courses of patients with LGMDR1/LGMD2A and LGMDR2/LGMD2B [9].

The principal aim of our study was to describe through a mathematical model LGMDR1/LGMD2A and LGMDR2/LGMD2B disease evolutions over more than three decades by considering several aspects, namely muscular strength and motor function, cardiac, respiratory and swallowing.

The secondary aim was to define time sensitive endpoints in the natural history of the two considered dystrophies.

2. Patients and methods

2.1. Ethics statements

All the evaluations were performed following *standard care* guidelines for LGMD and all the retrospective data were anonymised. All patients or parents/legal tutors signed informed consent to anonymous data analysis, approved by the local ethics committee according to the declaration of Helsinki.

2.2. Patients

We included patients affected by LGMDR1/LGMD2A and LGMDR2/LGMD2B attending the Neuromuscular Unit of the Scientific Institute “E. Medea” in the last 30 years. The diagnosis of LGMDR1/LGMD2A was confirmed by two mutations in CAPN3 (calpain3) gene and decrease in muscular calpain 3 on western blot; the diagnosis of LGMDR2/LGMD2B was confirmed by two mutations in DYSF (dysferlin) gene and dysferlin protein defect on muscle biopsy [8,10,11]. Only LGMDR2/LGMD2B patients with limb girdle onset weakness were included in the study.

2.3. Clinical assessment

Clinically relevant information such as age of disease onset, disease duration, age of loss of ambulation, and family history were collected for each patient.

2.3.1. Manual muscle testing

The British Medical Research Council (MRC) scale was used to assess muscle strength [12][MRC *grades* 5 (*muscle contracts against maximal resistance*), 4 (*muscle contracts against resistance*), 3 (*muscle contracts and moves with no resistance*), 2 (*muscle contracts without movement*), 1 (*trace of muscle contraction*) *and* 0 (*lack of contraction*)].

A total of 14 muscles were examined on both sides, testing limb movement around the neck, shoulders, elbows, wrists, hips, knees, and ankles.

Next the following 7 anatomical districts, each including specific muscles, were defined: 1) upper limbs (including trapezious, deltoids, biceps, triceps, wrist flexors, wrist extensors and interosseous); 2) shoulder girdles (including trapezious and deltoid); 3) arms (including biceps and triceps); 4) wrists (including wrist flexors and wrist extensors); 5) lower limbs (including quadriceps, iliopsoas, thight abductor, tight adductor, tibialis anterior, tibialis posterior and gastrocnemius); 6) pelvic girdles (including quadriceps, iliopsoas, thight abductor and tight adductor) and 7) legs (including tibialis anterior, tibialis posterior and gastrocnemius) [13].

The mean MRC value between right and left sides was computed for each muscle of a patient. Then, for each anatomical district, we calculated the median MRC value of all the muscles included in that district.

Muscle contractions were considered not efficient for MRC values lower than 3.

The tests were performed by three operators during the 30-years follow-up period.

2.3.2. Motor function measure

From 2008, motor function was assessed using Motor Function Measure scale (MFM) that comprise 32 items divided into 3 domains: D1 (standing position and transfers), D2 (axial and proximal motor function) and D3 (distal motor function). A percentage result is assigned to each domain and to the overall test (MFM_{TOT}) [14]. Ambulation was regularly assessed through 6 min walking test (6MWT) from 2010. Motor function domains were considered compromised for values lower than 50.

Since 2015, the Performance of Upper Limb was evaluated through the PUL scale that includes 22 items with an entry item to define the starting functional level, and 21 items subdivided into 3 levels: shoulder (4 items), middle (9 items) and distal (8 items) [15–17].

MFM scale, 6MWT, and PUL were administered by the same operator.

2.3.3. Cardio-pulmonary function tests

Measurements of Forced Vital Capacity (FVC), Forced Expiratory Volume in one second, and Peak Expiratory Flow were performed with a flowmeter attached to a flanged rubber mouthpiece with the nose occluded. Subdivision of lung volumes (Functional Residual Capacity, Residual Volume, and Total Lung Capacity, TLC) was measured by the nitrogen washout technique (Vmax series 22, SensorMedics, Yorba

Linda, CA). FVC= 80% predicted and TLC= 80% predicted were the thresholds above which respiratory function was considered normal.

Mean nocturnal oxygen saturation (SpO_2) was measured using a digital pulse oximeter (Nonin, 8500 digital pulse oximeter Quitman, TX).

Cardiac evaluation was performed by two-dimensional and Doppler echocardiogram (the left ventricle ejection fraction (LVEF) was considered), electrocardiogram (ECG) and 24-hour ECG monitoring.

LVEF= 60% was the threshold above which cardiac function was considered normal.

Along the 30 considered years of follow-up, the same two pulmonologists and cardiologists evaluated the patients.

2.3.4. Swallowing

Swallowing was evaluated in dedicated sessions by two speech and language therapists. According to food texture preparation, swallowing was classified as regular, mild dysphagia, moderate dysphagia, severe dysphagia [18].

2.3.5. Statistical analysis: mathematical model

A previously developed regression model [19] was used to reconstruct the evolution over time of each of the following measurements: MRC values of the seven anatomical district, MFM values of the three domains and overall, FVC, TLC, and LVEF.

Briefly, the model computed the population mean curve for each measurement by maximum-likelihood estimation, based on the longitudinal data of each patient, separately for the two groups (LGMDR1/LGMD2A and LGMDR2/LGMD2B). The model took into account the dependency of the data belonging to the same patient by introducing a "random effect" that incorporated a subject-specific correction to the population mean curve. For each measurement, the significance of the random effect related to the subject was assessed with a likelihood ratio test [19].

The proposed model was implemented in R (version 3.2.3 R Core Team (2015). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/) with the package "lme4" [20] and then a dedicated shiny app, being an R package that makes it easy to build interactive web app, was developed requiring the patients' database as input and providing the corresponding maximum-likelihood estimation population mean curve and the 95% pointwise asymptotic confidence intervals of each parameter.

Data are presented as median (25^{th}–75^{th} percentile). Significance was determined by $p<0.05$.

3. Results

3.1. LGMDR1/LGMD2A

Nineteen patients (11 females) with confirmed diagnosis of LGMDR1/LGMD2A were included in the study for a total of 220 visits (10 median number of visit per patient; range 2–31 visits each); the median age at first evaluation at our center was 26.9 years, while the last evaluation was at a median age of 37.9 years. Median age at symptoms onset was 11.0 (8.0–19.0) years. Ten patients had very early onset with muscular weakness occurring < 12 years and 9 patients had juvenile or adult onset. The median age of loss of ambulation was 30.5 years in the overall LGMDR1/LGMD2A patients group; however patients with early onset weakness (7 patients out of 10) had a median age of loss of ambulation of 24.5 years; the ones with adult onset (3 subjects out of 9) lost ambulation at the median age of 38 years. Nine patients (5 females) were still ambulant at time of evaluation.

Clinical data of LGMDR1/LGMD2A patients are summarized in Table 1.

As shown in Fig. 1, overall lower limbs moderate weakness (*i.e.*: MRC≤3) started 18 years after disease onset; while girdle and legs impairment started respectively after 12 years and after 25 years.

Similarly, overall upper limbs moderate weakness started 17 years after disease onset and this trend was mirrored by those of the shoulder girdles and of the arms muscles, but not by the wrist that preserved its strength over the three decades (Fig. 2).

Fig. 3 shows that global motor function (MFM_{TOT}) started to become inefficient after 22 years of disease, with an extremely earlier impairment (after 12 years) of the score related to standing position and transfers (D1), while both D2 as well as D3 dimensions were always above 50%.

Forced vital capacity approached 80% of predicted values after 20 years from disease onset, however maintaining similar values thereafter. Total lung capacity, nocturnal oxygen saturation (mean values: 96 ± 0.86%), swallowing (data not shown) and cardiac functions were preserved throughout the considered period (Fig. 4).

3.2. LGMDR2/LGMD2B population

Twenty patients (10 females) with confirmed diagnosis of LGMDR2/LGMD2B and pelvic-girdle weakness as sign of disease onset were included in the study for a total of 208 visits (10 median number of visit per patient; range 4–26). The median age at first evaluation at our center was 38.1 years, while the median age at last evaluation was 52.5 years. Median age at symptoms onset was 20.0 years, with only 2 patients having disease onset < 12 years. The median age of loss of ambulation was 41.0 years for LGMDR2/LGMD2B patients. Eleven patients (7 females) were still ambulant at the last evaluation. One LGMDR2/LGMD2B patient died at 44.4 years due to lymphoma.

All the clinical data of LGMDR2/LGMD2B patients are summarised in Table 1.

Overall lower limbs moderate weakness (*i.e.*: MRC≤3) started 15 years after disease onset, and pelvic girdles and legs showed a similar behaviour (Fig. 1).

Overall upper limbs moderate weakness occurred 5 years later than the lower limbs and this was due by both the

Table 1
Clinical data of LGMDR1/LGMD2A and LGMDR2/LGMD2B patients.

	LGMBR1/LGMB2A			LGMBR2/LGMB2B		
n°	19			20		
gender	11 F	8 M		10 F	10 M	
ambulation at last evaluation	5			11		
	median	*25thp*	*75thp*	*median*	*25thp*	*75thp*
age of debut (age)	11.0	8.0	18.0	20.0	16.0	23.5
age at first evaluation (years)	26.9	18.1	34.9	38.2	26.7	42.2
age at last evaluation (years)	37.9	31.3	42.7	52.2	41.4	53.9
evaluation period (years)	11.5	3.7	15.5	12.3	7.1	14.7
disease duration (years)	22.2	19.1	28.5	26.0	23.7	30.6
L.O.A. age (years)	30.5	23.8	36.3	42.0	40.3	46.2

L.O.A.: loss of ambulation; 25th p: 25th percentile; 75th p: 75th percentile.

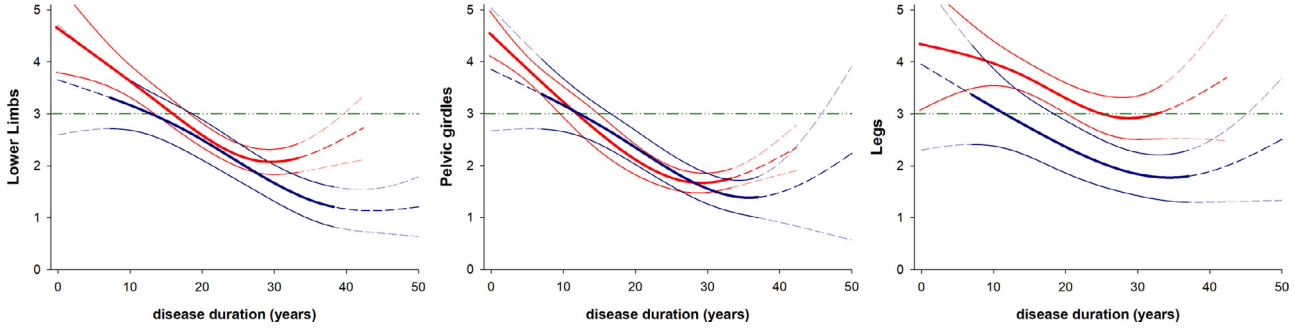

Fig. 1. Evolution over time of the population mean curve (thick line) and its 95% confidence intervals (thinner lines) of MRC score of lower limbs (left), pelvic girdles (middle) and legs (right) in LGMDR1/LGMD2A (red) and LGMDR2/LGMD2B (blue) patients. Dash-dotted green lines indicate the threshold when muscle contracts and moves with no resistance. Short-dashed lines represented the extremities of the database characterized by low number of visits/patients and "bizarre" behaviours due to outliers that may not represent the true clinical evolution, although mathematically valid.

shoulder girdles and the arms, while wrist weakness occurred after 30 years of disease (Fig. 2).

Global motor function (MFM_{TOT}) and D2 dimension started to become inefficient after the second decade of the disease. D1 dimension became lower than 50% after 15 years, while D3 dimension never reached that threshold (Fig. 3).

A restrictive pattern (i.e., FVC < 80% predicted and TLC < 80% predicted) occurred after 25 years from disease onset, however maintaining relatively good values thereafter. Two LGMDR2/LGMD2B patients were using non-invasive mechanical ventilation at the last evaluation at the age of 48 and 52 years (Fig. 4).

Nocturnal oxygen saturation (mean value: $95.7 \pm 1.9\%$), swallowing (data not shown) and cardiac functions (as indicated by the left ventricle ejection fraction and by the absence of arrhythmias) were preserved throughout the considered period (Fig. 4).

4. Discussion

To the best of our knowledge, this was the longest retrospective study focused on LGMDR1/LGMD2A and LGMDR2/LGMD2B that considers several aspects of the over more than three decades of disease duration.

A part from Richard et al. [8] as well as Angelini et al. [9], we did not to find other published observational clinical and natural history studies focused on LGMDR1/LGMD2A and LGMDR2/LGMD2B.

Guimaraes Costa et al. [21], recently retrospectively reviewed 16 years of medical records evaluating a large cohort of patients with LGMDR3/2D, R4/2E and R5/2C (alfa, beta and gamma sarcoglycanopathies) while Fayssoil et al. [22] focused their attention on 10 years of follow-up of a group of LGMDR5/2C and R3/2D patients (gamma and alfa sarcoglycanopathies). They respectively identified early disease onset as a predictor of early loss of ambulation, parallel progression of motor and respiratory function decline and impact of cardiac and respiratory function in mortality rate. Finally, 23 patients affected by LGMDR9/2I were studied at 1-year period and then re-enrolled at 6 years by Murphy et al. [23] through motor fuctional scales and muscle magnetic resonance showing an higher responsiveness to disease progression of fat fraction measurements than functional scales.

Winckler and collaborators performed a multicenter historical cohort study of a wide Brazilian population of patients with autosomal recessive forms of LGMD (370 patients), showing that also in Brasil, the most frequent LGMD subtypes were LGMDR1/LGMD2A and LGMDR2/LGMD2B [24] as reported by Magri et al. in the Italian population [1]. They have built survival curves for LGMD R3-R6/2C-F and R1/2A and R2/2B and

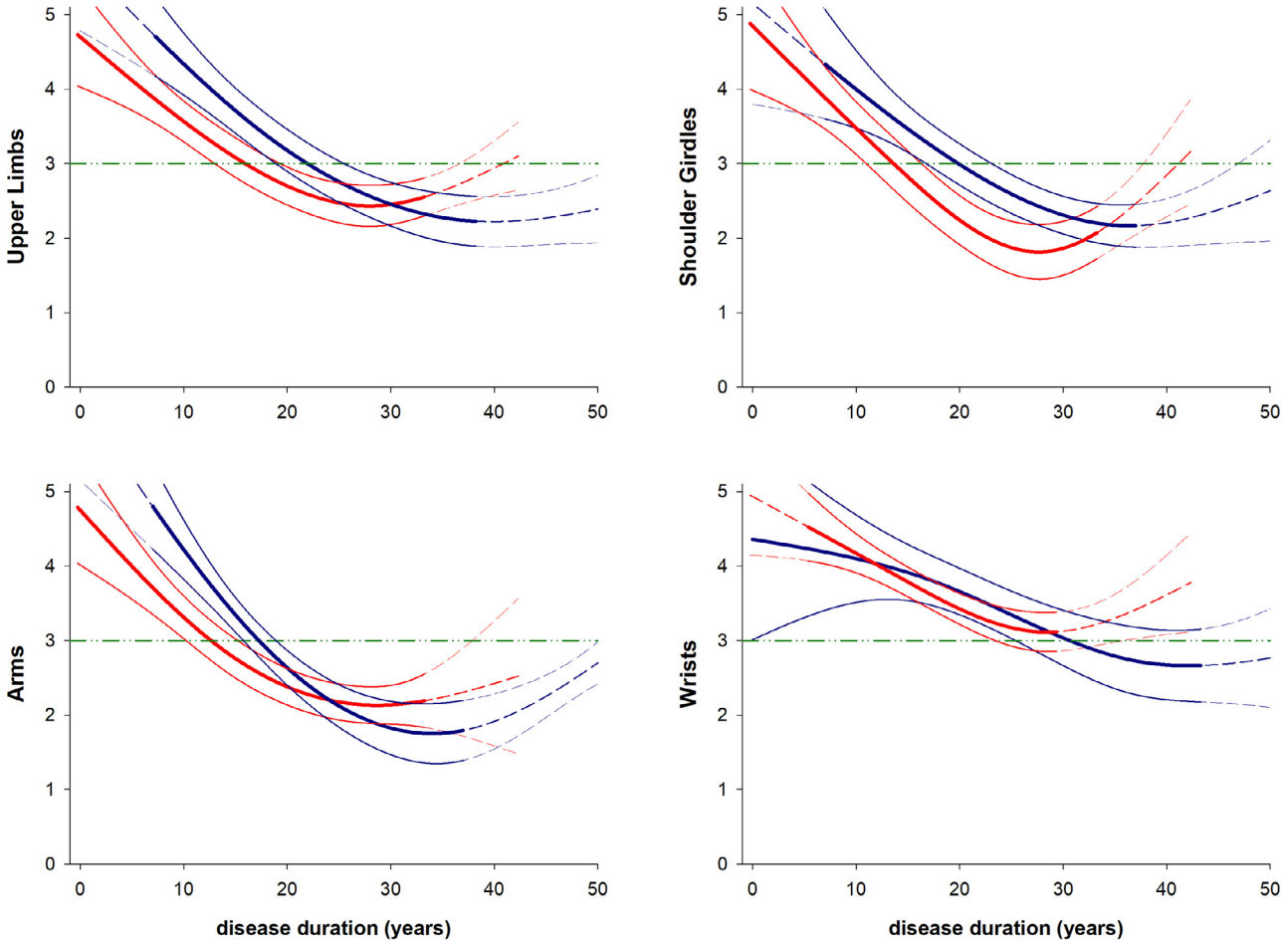

Fig. 2. Evolution over time of the population mean curve (thick line) and its 95% confidence intervals (thinner lines) of MRC score of upper limbs (top left), shoulder girdles (top right), arms (bottom left) and wrists (bottom right) in LGMDR1/LGMD2A (red) and LGMDR2/LGMD2B (blue) patients. Dash-dotted green lines indicate the threshold when muscle contracts and moves with no resistance. Short-dashed lines represented the extremities of the database characterized by low number of visits/patients and "bizarre" behaviours due to outliers that may not represent the true clinical evolution, although mathematically valid.

genotype-phenotype correlations, showing that females with LGMDR2/LGMD2B had less severe progression to handicap (aid in walking and wheelchair bounding) than males. In addition, they showed that LGMDR1/LGMD2A patients with truncating variants had earlier disease onset and more severe progression to handicap than patients without truncating variants, this in line with Richard et al. and Angelini et al. [8,9].

When they considered disease duration as the time variable, median disease duration at walking aid dependency occurred earlier for sarcoglycanopathies (9 years), but at similar durations in LGMDR1/LGMD2A and LGMDR2/LGMD2B (respectively 21 years and 20 years). Similarly, our two groups of patients lost ambulation after 19.5 years of disease duration in LGMDR1/LGMD2A and after 22 years in LGMDR2/LGMD2B.

Similarly, we analysed our patients data according to disease duration and we have shown that both dystrophies were characterized by slow progression with clinically relevant muscular and motor function alterations occurring after the first decade from disease onset, while mild respiratory function alterations started after the second. Instead, cardiac and swallowing functions were preserved.

Unlike Winkler, we analysed in detail muscles and motor functional scales showing that pelvic girdle weakness became clinically evident in both dystrophies at a similar disease onset; whereas legs showed clinically significant weakness only in LGMDR2/LGMD2B. Upper limbs were involved later in LGMDR2/LGMD2B compared to LGMDR1/LGMD2A.

More specifically, LGMDR1/LGMD2A showed relatively stronger distal lower limb muscles while LGMDR2/LGMD2B started with relatively stronger upper limb muscles. In both dystrophies, the impairment of the upper limbs seemed to be equally distributed between the shoulder girdles and the arms, with a relatively spared wrist. Similarly, the muscles of the lower girdles and of the legs started to become weak at the same time, but this occurred only in LGMDR2/LGMD2B. By contrast, there was a time delay of almost a decade in LGMDR1/LGMD2A, with the lower girdles muscles weakness starting in the first disease decade, while the legs muscles impaired in the late second disease decade.

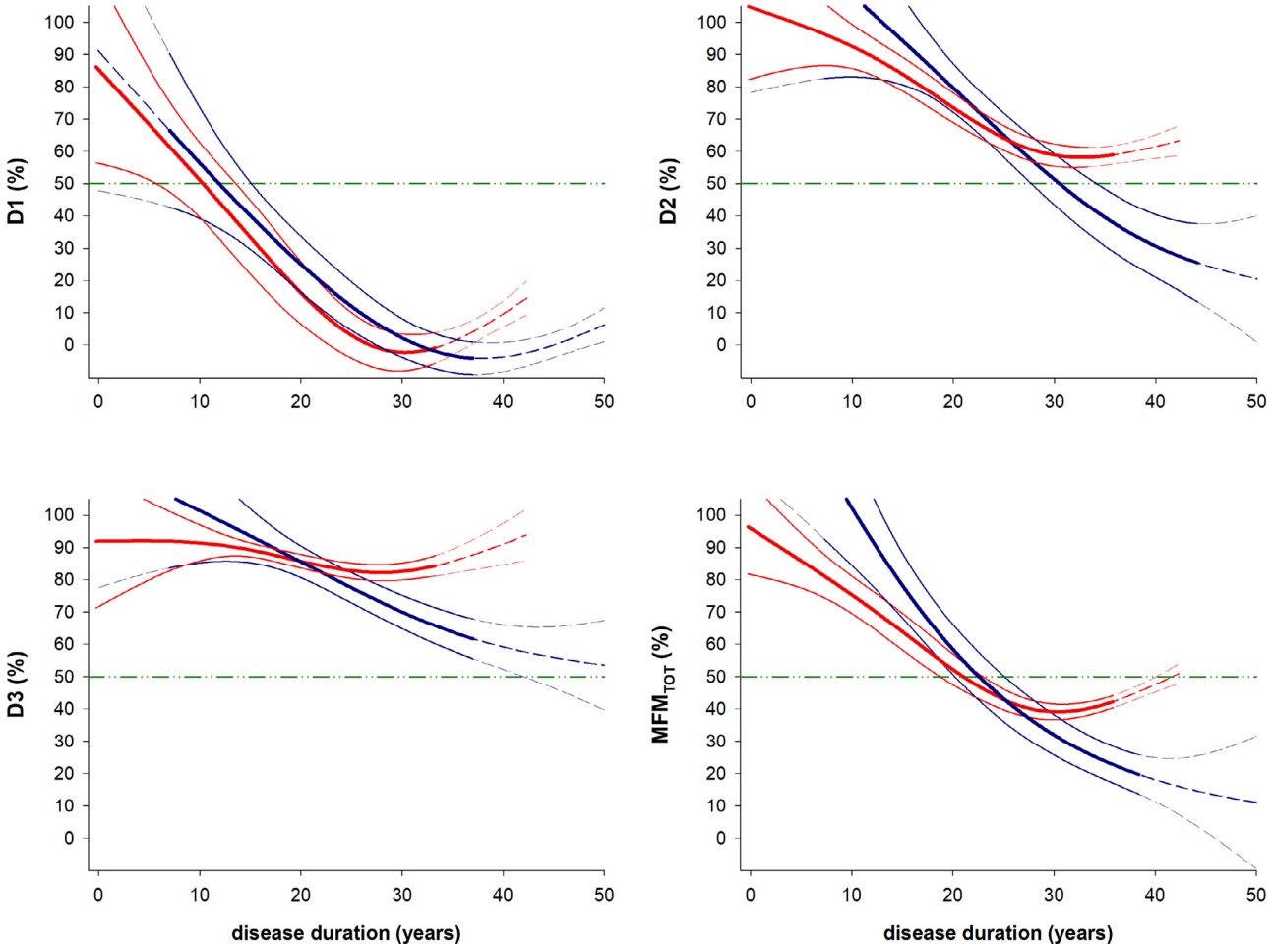

Fig. 3. Evolution over time of the population mean curve (thick line) and its 95% confidence intervals (thinner lines) of dimension D1 (top left), dimension D2 (top right), dimension D3 (bottom left) and MFM score of overall tests (bottom right) in LGMDR1/LGMD2A (red) and LGMDR2/LGMD2B (blue) patients. Dash-dotted green lines indicate the threshold below which the motor function was considered inefficient. Short-dashed lines represented the extremities of the database characterized by low number of visits/patients and "bizarre" behaviours due to outliers that may not represent the true clinical evolution, although mathematically valid.

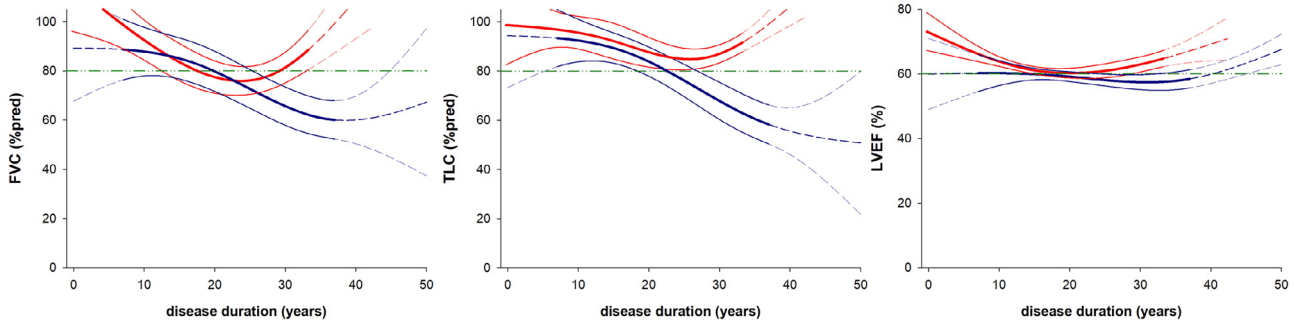

Fig. 4. Evolution over time of the population mean curve (thick line) and its 95% confidence intervals (thinner lines) of forced vital capacity (left), total lung capacity (middle) and left ventricle ejection fraction (right) in LGMDR1/LGMD2A (red) and LGMDR2/LGMD2B (blue) patients. Light grey areas indicate the interval where the two groups are significantly different ($p<0.05$). Dash-dotted green lines indicate the threshold for the specific parameters of pulmonary (FVC>80% pred and TLC%>80% pred) and cardiac (LVEF> 60%) function to be considered normal. Short-dashed lines represented the extremities of the database characterized by low number of visits/patients and "bizarre" behaviours due to outliers that may not represent the true clinical evolution, although mathematically valid.

In both dystrophies and at similar timing after the first decade from disease onset, the D1 domain of the MFM scale, representing standing position and transfers, started to become lower than 50%. The D2 domain (axial and proximal motor function) as the D3 domain (distal motor function) never decreased below 50% even after the whole follow-up time. However, LGMDR2/LGMD2B patients showed a tendency towards a D2 decay below 50%.

The total MFM domain started to show functional impairment after the second decade of disease duration.

Notably, there was an important correspondence between lower limbs muscular strength impairment and the corresponding motor function: both started to become severely compromised after 12 and 15 years of disease in LGMDR1/LGMD2A and LGMDR2/LGMD2B, respectively. By contrast, the progress of D2 domain seemed not to mirror the progress of upper limbs muscular strength impairment.

Such apparent discrepancy between upper limbs muscles weakness with the corresponding motor function can be explained by compensatory strategies adopted by patients.

The most important strength of the present study was the description of more than 30 years of natural history of the two most common LGMD in Italy, assessed through non-invasive and routinely performed evaluations. In addition, the high number of visits collected from the same patient over such a long period was implemented into a mathematical model that took into account the data dependency within the same patient. A mathematical model describes a system by using mathematical concepts and language in order to facilitate proper explanation of the system itself or to study the effects of different components and to make predictions on patterns of behaviour [25]. This would represent an extremely relevant tool for better care of patients and for clinical trial design. Indeed, the determinants of successful drug and/or of gene modulating therapies include good understanding not only of the phenotype, but also of the natural history of the disease. In addition, the mathematical model has the potential to define clinically relevant threshold outcome measures which help in the assessment of drug response.

In this direction, the results of our study pointed out some important considerations: 1) the relevance of population homogeneity, 2) the crucial role of data arrangement/representation and 3) the relative weakness of muscular grading and motor function scales in identifying specific outcome measure.

Some of the clinical trials, as well as other studies tended to pool together different neuromuscular diseases, presumably to increase the number of patients [26,27]. The inhomogeneity of the studied groups is an important bottleneck, as it could introduce important bias that might strongly affect the results and their interpretation. Not only the inhomogeneity of the population can introduce bias, but also the way of arranging and presenting the data.

For example, our mathematical model allowed also to compare different populations (e.g., different diseases, different timing of a particular treatment) among each other. In order to show the potential and the critical issues of a mathematical model, we have compared the two considered dystrophies, aware that this was not clinically appropriate and for this reason, it was not reported in the result section.

In our first analytical approach we represented data as function of patients' age and the results were dramatically different with LGMDR1/LGMD2A being systematically worse than LGMDR2/LGMD2B around the age of 30 years old (see online supplement reporting some exemplificative parameters plotted as function of patients' age). These results were however affected by the fact that we were comparing patients at different stages of the disease because of the different onset age. In other words, comparing a 25 years old LGMDR2/LGMD2B patient with a LGMDR1/LGMD2A peer implied to compare a patient at the beginning of the disease with a patient with more than almost a decade of disease progression difference. This could not be ignored.

Re-organizing our results according to disease duration was therefore a sort of data normalization by removing the bias induced by patients' age and/or different disease onset. This was just an example, but it further confirms that functional homogeneity is essential to recruit patients, as it might potentially reduce unexplained variation in patient outcomes to impact on clinical trial design and analysis [28].

Although our database was relatively large, the number of considered patients was too low to allow further possible patients' subgrouping according to LGMDR1/LGMD2A onset (early vs late) [9], to genetic background [8] or to ambulation/wheelchair bound conditions. In addition, few data were available on 6 min walking test and Performance of Upper Limb [29,30] and for this reason they were not reported. Of note, the low number of visits/patients at the extremities of the database (i.e.: very old patients or very young LGMDR2/LGMD2B) was the reason for some "bizarre" behaviours, like improving trends after 35 years of disease. In the majority of the cases, such curve rise was due to tests performed on one or two outlier patients, who were in a relatively better condition compared to the rest of the population. The model outcome at the extremities, therefore, was valid from the mathematical point of view, but not from the clinical point of view according to the general physiopathology of the disease.

Mathematical models in medicine are widely accepted tools that can answer otherwise unanswerable questions and expand the knowledge from actual study data. However, their outcome must be also critically reviewed and validated together with the medical science community according to their clinical experience [31].

Secondly and similarly to Angelini et al. and Richard et al. [8,9], because almost all the parameters in both groups of patients had a slowly linear trend, unfortunately it was not possible to define milestones or to identify time sensitive endpoints of the diseases (the second aim of the present study).

This conclusion, however, disagrees with one published work [32], that showed how MFM_{TOT}, MFM D1 domain, and adapted North Star Ambulatory Assessment significantly changed after 6 or 12 months in 193 LGMDR2/LGMD2B

patients. The authors found a median change of one point for all the scales that is statistically but not functionally significant [32]. Once again, it is extremely important to critically comment the statistical results according to the clinic.

Finally, functional scaling showed to be inadequate to provide insights on the muscular involvement. For example, the strength and the function of the thight anterior compartment did not differ between the two considered LGMDs. However, in a subgroup of the same patients at an advanced stage of the disease, Arrigoni et al. reported higher levels of structural muscle degeneration of the thight anterior compartment in LGMDR2/LGMD2B than LGMDR1/LGMD2A patients on magnetic resonance imaging [33]. Similar conclusions were reported by other authors [34,35]. A combination of functional and structural parameters, therefore, seems to be the best approach in clinical trial designing and in the possible anticipation of therapeutic and/or rehabilitative support.

5. Conclusion

Our work has the important merit to define curves of disease evolution of LGMDR1/LGMD2A and LGMDR2/LGMD2B that could be essential to evaluate how the natural progression is changed or halted by a therapy not only from a muscular, but also from functional, cardiac and respiratory points of view.

Mathematical modelling is a new fundamental ally for the clinicians who still need to critically evaluate its results according to their experience.

Financial support

This study was supported by the Italian Ministry of Health (Ricerca Corrente 2020 PI: dr Maria Grazia D'Angelo).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors acknowledge Prof G.P. Comi and Dr F. Magri for their support in clinical diagnosis of patients, and Dr M.T. Bassi for her support in research activity and funding support.

Special thanks to all the patients and the families for their collaboration and to AICa3 onlus association (Associazione Italiana Calpaina 3) for the diffusion of the study project to patients and families.

References

[1] Magri F, Nigro V, Angelini C, Mongini T, Mora M, Moroni I, et al. The Italian LGMD registry: relative frequency, clinical features, and differential diagnosis. Muscle Nerve 2017;55(1):55–68. doi:10.1002/mus.25192.
[2] Magri F, Brajkovic S, Govoni A, Brusa R, Comi GP. Revised genetic classification of limb girdle muscular dystrophies. Curr Mol Med 2014;14:934–43. doi:10.2174/1566524014666141010130244.
[3] Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol 2014;33:1–12.
[4] Vissing J. Limb girdle muscular dystrophies. Curr Opin Neurol 2016;29:635–41. doi:10.1097/WCO.0000000000000375.
[5] Straub V, Bertoli M. Where do we stand in trial readiness for autosomal recessive limb girdle muscular dystrophies? Neuromuscul Disord 2016;26:111–25. doi:10.1016/j.nmd.2015.11.012.
[6] Weinstein MC, O'Brien B, Hornberger J, Jackson J, Johannesson M, McCabe C, et al. Principles of good practice for decision analytic modeling in health-care evaluation: report of the ISPOR task force on good research practices - modeling studies. Value Heal 2003;6:9–17. doi:10.1046/j.1524-4733.2003.00234.x.
[7] Easterbrook PJ, Doherty MC, Perriëns JH, Barcarolo JL, Hirnschall GO. The role of mathematical modelling in the development of recommendations in the 2013 WHO consolidated antiretroviral therapy guidelines. AIDS 2014;28. doi:10.1097/QAD.0000000000000111.
[8] Richard I, Hogrel JY, Stockholm D, Payan CAM, Fougerousse F, et al. Natural history of LGMD2A for delineating outcome measures in clinical trials. Ann Clin Transl Neurol 2016;3:248–65. doi:10.1002/acn3.287.
[9] Angelini C, Nardetto L, Borsato C, Padoan R, Fanin M, Nascimbeni AC, et al. The clinical course of calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B). Neurol Res 2010;32:41–6. doi:10.1179/174313209X380847.
[10] Fanin M, Fulizio L, Nascimbeni AC, Spinazzi M, Piluso G, Ventriglia VM, et al. Molecular diagnosis in LGMD2A: mutation analysis of protein testing? Hum Mutat 2004;24:52–62. doi:10.1002/humu.20058.
[11] Kawabe K, Goto K, Nishino I, Angelini C, Hayashi YK. Dysferlin mutation analysis in a group of Italian patients with limb-girdle muscular dystrophy and Miyoshi myopathy. Eur J Neurol 2004;11:657–61. doi:10.1111/j.1468-1331.2004.00755.x.
[12] Aids to the examination of the peripheral nervous system (memorandum n° 45). Bailiere_Tindall 1976.
[13] O'Brien M. Medical research council of the UK. Aids to the examination of the peripheral nervous system. 5th Edition. Saunders Ltd; 2010.
[14] Bérard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure scale for neuromuscular diseases. Construction and validation study. Neuromuscul Disord 2005;15:463–70. doi:10.1016/j.nmd.2005.03.004.
[15] Pane M, Fanelli L, Mazzone ES, Olivieri G, D'Amico A, Messina S, et al. Benefits of glucocorticoids in non-ambulant boys/men with Duchenne muscular dystrophy: a multicentric longitudinal study using the performance of upper limb test. Neuromuscul Disord 2015;25:749–53. doi:10.1016/j.nmd.2015.07.009.
[16] Pane M, Coratti G, Brogna C, Mazzone ES, Mayhew A, Fanelli L, et al. Upper limb function in Duchenne muscular dystrophy: 24 month longitudinal data. PLoS One 2018;13. doi:10.1371/journal.pone.0199223.
[17] Gandolla M, Antonietti A, Longatelli V, Pedrocchi A. The effectiveness of wearable upper limb assistive devices in degenerative neuromuscular diseases: a systematic review and meta-analysis. Front Bioeng Biotechnol 2020;7. doi:10.3389/fbioe.2019.00450.
[18] Tilton AH, Miller MD, Khoshoo V. Nutrition and swallowing in pediatric neuromuscular patients. Semin Pediatr Neurol 1998;5:106–15.
[19] LoMauro A, Romei M, Gandossini S, Pascuzzo R, Vantini S, D'Angelo MG, et al. Evolution of respiratory function in Duchenne

muscular dystrophy from childhood to adulthood. Eur Respir J 2018;51:1701418. doi:10.1183/13993003.01418-2017.

[20] Bates D, Mächler M, Bolker B. Fitting linear mixed-effects models using lme4. J Stat Soft 2015;67:1–48. doi:10.18637/jss.v067.i01.

[21] Guimarães-Costa R, Fernández-Eulate G, Wahbi K, Leturcq F, Malfatti E, Behin A, et al. Clinical correlations and long-term follow-up in 100 patients with sarcoglycanopathies. Eur J Neurol 2020. doi:10.1111/ene.14592.

[22] Fayssoil A, Ogna A, Chaffaut C, Chevret S, Guimarães-Costa R, Leturcq F, et al. Natural history of cardiac and respiratory involvement, prognosis and predictive factors for long-term survival in adult patients with limb girdle muscular dystrophies type 2C and 2D. PLoS One 2016;11:e0153095. doi:10.1371/journal.pone.0153095.

[23] Murphy AP, Morrow J, Dahlqvist JR, Stojkovic T, Willis TA, Sinclair CDJ, et al. Natural history of limb girdle muscular dystrophy R9 over 6 years: searching for trial endpoints. Ann Clin Transl Neurol 2019;6:1033–45. doi:10.1002/acn3.774.

[24] Winckler PB, da Silva AMS, Coimbra-Neto AR, Carvalho E, Cavalcanti EBU, Sobreira CFR, et al. Clinicogenetic lessons from 370 patients with autosomal recessive limb-girdle muscular dystrophy. Clin Genet 2019;96:341–53. doi:10.1111/cge.13597.

[25] Abramowitz M, Stegun I. Handbook of mathematical functions with formulas, graphs, and mathematical tables; 1972. National Bureau of Standards (U.S.).

[26] D'Angelo MG, Gandossini S, Boneschi FM, Sciorati C, Bonato S, Brighina E, et al. Nitric oxide donor and non steroidal anti inflammatory drugs as a therapy for muscular dystrophies: evidence from a safety study with pilot efficacy measures in adult dystrophic patients. Pharmacol Res 2012;65:472–9. doi:10.1016/j.phrs.2012.01.006.

[27] Walter MC, Lochmüller H, Reilich P, Klopstock T, Huber R, Hartard M, et al. Creatine monohydrate in muscular dystrophies: a double-blind, placebo-controlled clinical study. Neurology 2000;54:1848–50. doi:10.1212/wnl.54.9.1848.

[28] Mercuri E, Signorovitch JE, Swallow E, Song J, Ward SJ, Pane M, et al. Categorizing natural history trajectories of ambulatory function measured by the 6-minute walk distance in patients with Duchenne muscular dystrophy. Neuromuscul Disord 2016;26:576–83. doi:10.1016/j.nmd.2016.05.016.

[29] Mayhew A, Mazzone ES, Eagle M, Duong T, Ash M, Decostre V, et al. Development of the performance of the upper limb module for Duchenne muscular dystrophy. Dev Med Child Neurol 2013;55:1038–45. doi:10.1111/dmcn.12213.

[30] Pane M, Mazzone ES, Sivo S, Fanelli L, De Sanctis R, D'Amico A, et al. The 6min walk test and performance of upper limb in ambulant duchenne muscular dystrophy boys. PLoS Curr 2014;6. doi:10.1371/currents.md.a93d9904d57dcb08936f2ea89bca6fe6.

[31] Chambers RB. The role of mathematical modeling in medical research: "research without patients? Ochsner J 2000;2:218–23.

[32] Moore U, Jacobs M, James MK, Mayhew AG, Fernandez-Torron R, Feng J, et al. Assessment of disease progression in dysferlinopathy: a 1-year cohort study. Neurology 2019;92:E461–74. doi:10.1212/WNL.0000000000006858.

[33] Arrigoni F, De Luca A, Velardo D, Magri F, Gandossini S, Russo A, et al. Multiparametric quantitative MRI assessment of thigh muscles in limb-girdle muscular dystrophy 2A and 2B. Muscle Nerve 2018;58:550–8. doi:10.1002/mus.26189.

[34] Diaz-Manera J, Fernandez-Torron R, LLauger J, James MK, Mayhew A, Smith FE, et al. Muscle MRI in patients with dysferlinopathy: pattern recognition and implications for clinical trials. J Neurol Neurosurg Psychiatry 2018;89:1071–81. doi:10.1136/jnnp-2017-317488.

[35] Barp A, Laforet P, Bello L, Tasca G, Vissing J, Monforte M, et al. European muscle MRI study in limb girdle muscular dystrophy type R1/2A (LGMDR1/LGMD2A). J Neurol 2020;267:45–56. doi:10.1007/s00415-019-09539-y.