CLINICAL DETERMINANTS OF DISEASE PROGRESSION IN PATIENTS WITH LIMB GIRDLE MUSCULAR DYSTROPHIES TYPE 2E

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Acronym: NeuroLGMD2E

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2. Abbreviations
6MWT = Six Minutes Walking Test
AHI = Apnea Hyponea Index
ALT= Alanina Aminotransferasi
ALP= Alkaline Phosopatase
AST= Aspartato Aminotransferase
BNP= Brain Natriuretic Peptide
CPK= Creatine phosphokinase
CPT= Lung Total Capacity
DMD= Duchenne Muscular Dystrophy
ECG= Elettrokardiogram
FC= Cardiac Frequency
FE= Ejection Fraction of left ventricle
FEF = Forced Expiratory Flux
FEV1= Forced Expiratory Volume
FVC = Forced Vital Capacità
GGT= Gamma Glutamiltransferase
GSGC= Gait Stair Gleason Chair
INR= International Normalised Ratio
LDH= Lactic Dehydrogenase
LGMD= Limb Girdle Muscular Dystrophy
MEF= Maximal Expiratory Flux
MEP= Maximal Expiratory Pressure
MFM= Motor Function Measure
MIP= Maximal Inspiratory Pressure
MMEF= Mean Maximal Expiratory Flux
MOC= Mineralometry Bone Computed
MVV= Maximal voluntary Ventilation
NSAA= North Star Ambulatory Assessment
ODI= Oxygen Desaturation Index
PEF= Peak of Expiratory Flux
PTH= Parathormone
QMT= Quantitative Muscle Testing
RM = Magnetic Resonance
RX= radiography
VIT D= Vitamin D
VR= Residual Volume

3. Responsibility (role of the study promoter and of researchers)
Main researcher: Prof. Yvan Torrente Collaborators: Dott.ssa Giulia Marchetti, graduated with Prof. Torrente and now attending the first year of school in medical genetics at the Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico. She will collect clinical charts and report clinical data to analyze in a excel file that will be used for the statistics. Prof. Giacomo Comi, Director of the Neuromuscular and rare Disease Unit
4. Emendamenti ed altre modifiche al protocollo Not relevant.
5. Timelines Planning: start of data collection: June 2020. End of data collection: September 2020 Final report: December 2020
6. Rational and background
LGMD2E is a rare type of a hereditary muscular dystrophy transmitted in an autosomic recessive manner. This disease is determined by the presence of mutations involving the Beta Sarcoglycan gene, coding for a protein which is involved in the stabilization of the glycoprotein complex

associated to dystrophin (1). The main role of beta Sarcoglycan only partly explains the severity of the clinical phenotype reported in these patients compared with other LGMD (2). LGMD 2E debuts in early childhood, involving skeletal muscles with a progressive centripetal trend that in last stages affects also respiratory and cardiac muscles (3). The early onset and the severity of the disease affecting cardiopulmonary apparatus, make this disease very close to the more famous and studied Duchenne Muscular Dystrophy (4). More than 60% of LGMD 2E patients suffer from heart failure (5), not less common is the involvement of respiratory system which insufficiency represents the main cause of death in these patients (6). Unfortunately, because of its rareness, literature is poor of data about this disorder and no natural history study have ever been reported until now. Lacking similar studies, therapeutical and clinical management is not easy and often based on studies investigating other subtypes of muscular dystrophies, often more benign.

Furthermore, we have little knowledge about possible genotype-phenotype correlations for this disease (7). We do believe that a natural history study might help us to improve our knowledge of this condition's evolution, leading to the design of specific guidelines for the follow up of these patients, both under the therapeutical and clinical point of view.

Guidelines will uniform the clinical approach to this disease, both on the National and the international scenario, and will improve the use of sanitary resources. Furthermore, with a phenotype-genotype correlation study, it will be possible to recognize those patients that are more at risk to develop a more severe disease.

7. Research query and objective

The lack of data on natural history of this rare disease and the need to define guidelines for the best follow up of these patients, compelled us to set up this study. In this study any investigation performed in patients affected by LGMD 2E will be considered with the aim to recognize the progression of the disease and those variables that are more useful and precise in describing its evolution. Primary objective of the study is to obtain more and better knowledge of this rare group of LGMD, describing its natural history through the analysis of clinical charts of the more affected patients possible.

To select clinical variables that are more useful to define the evolution of the disease and to identify any possible differences in clinical presentation that might be due to differences in genotype-phenotype correlation. Secondary objective of the study is to demonstrate the real impact of cardiopulmonary impairment on the prognosis of these patients.

Thank to these knowledges, to lead to a better therapeutic approach to this disease, stimulating the development of new drugs.

8. Methods

8.1 Study design

Retrospective observational monocentric study, aimed to investigate the natural history of a rare disease like the LGMD 2E

8.1.1 Primary endpoints

To describe the natural history of the LGMD 2E

To define of the clinical variables that more strongly relate with the evolution of the disease

To identify any possible correlation between variables

To identify any possible correlation between variables and clinical outcomes

To identify any possible correlation between phenotype and genotype

8.1.2 Secondary endpoints

To define the better follow up routine for LGMD 2E patients in view of possible genetic differences

To define those therapies that are able to slow the progression of the disease

To ameliorate the use of sanitary resources for the management of these chronic patients.

To gain new knowledges that could be used for the development and the management of future new therapies.

8.2 Setting

Patients followed by the ambulatory of neuromuscular disorders of Centro Dino Ferrari, Padiglione Monteggia of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Unit of Neurology), will be recruited during the ordinary follow up routine or will be reached out to participate to this study. Furthermore, thank to the sponsorship of Gruppo familiari beta sarcoglicanopatie (GFB), more patients that are in the network of this association will be reached out from the PI of the study and eventually included. Data will be provided by patients themselves during the visit at the room number 17 of Padiglione Monteggia of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Unit of Neurology). If the patients won't be able to move and will be interested in participating to the study a collaborator of the main researcher will reach the patients at his residence to recruit him. Main source of data will be the clinical chart of the patient and all his clinical documents assessing any operation, ascertain, visits performer through the years until this moment. Main centre will be the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Unit of Neurology). Data collection will last six months and later data will be studied.

8.2.1 Study population

Studied population. The association GFB (GRUPPO FAMILIARI BETA-SARCOGLICANOPATIE) is a group of families of patients affected by beta-sarcoglycanopathy and other muscular dystrophies. This association has been created by few families resident in Lombardia and now it includes people from all over Italy. The study will be sponsored by GFB that will inform its associated about this study. Patients interested in participating to the study will reach out the PI of the study to be eventually included.

Furthermore, patients affected by LGMD 2E followed at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Unit of Neurology) and that have previously signed an informed consent for the use of their data for scientific research will be included. Data from this second group of patients will already be available in databases created in our hospital for previous scientific researches.

8.2.2 Inclusion criteria

Will be included only those patients, both adults and minors, that have a genetical diagnosis of LGMD 2E and that will sign the specific informed consent for data management.

8.2.3 Exclusion criteria

Th lacking subscription of informed consent and or the lack of genetic diagnosis represent the only exclusion criteria.

8.2.4 Patient participation

Patients might be asked t ogive to their physician their clinical charts in order to evaluate the visits and investigations performed through the years, also in other hospitals.

8.3 Variables

Will be extract from the clinical charts of these patients, after the sign of the informed consent for the data management, only retrospective data (therefore all those data older than the day of the recruiting visit).

These data will be reported in a excel file specifically created. On this file data will be anonymized through a patient identification code that will be decrypted in another separate file. For this file will be considered the following data:

a) Dati anagrafici:

1. Anonymized name
2. Sex
3. Age

Form 2- DIAGNOSIS:

1) Age at onset

a) clinical presentation

b) clinical progression

2) CK at onset

3) EMG

4) Biopsy:
a) Histology
b) Immunohistochemistry
c) Western Blot
5) Genetic:
a) In frame del/dupl
b) Out of frame del/dupl
c) Missense
d) Nonsense

Form 3- CLINICAL DATA AND TREATMENTS:
Hospitalization: Data – Cause – Frequency∗ Surgery: Data – Complications∗ Fractures: Data – Frequency – Location∗ Fell: Data – Frequency∗ Emergency Room Accesses: Data – Cause∗
APPARATUS
Skeletal-Muscle
1) Clinical tests:
NSAA∗ MFM∗ QMT∗ Brook Scale∗ Egen Klassification∗ Barthel Index∗ Walton and Gardner∗ & Medwin GSGC∗ Grip Strength (quantitative)∗ Handheld Dynamometry∗ Performance of the Upper Limb Module for DMD 2.0∗ 10m Walk: 1- normal, 2- slightly impaired- 3 moderately impaired- 4- impaired- 5 with device - 6 not walking but stands- 7 wheelchair 6MWT∗ time to rise from the floor∗ Climb 4 stairs (1 no help - 2 one hand on the leg – 3 two hands on the legs - 4 stand with help -5 Handrail - 6 hardly climbs- 7 Not able). 10m Run
Time to rise from a chair (1 Normal - 2 with difficulties -3∗ helping with one hand - 4 Helping with two hands - 5 other helps - 6 Impossible). Time to stand from the floor (1 Normal - 2 first rise the pelvis and then one hand on the floor - 3 First rise the pelvis and then two hands on the floor - 4 One hand on the leg - 5 Two hands on the legs - 6 Other help - 7 Impossible).
Neuromuscular evaluation (date): maximal voluntary strength: bilateral knees extension, Isometric contraction test∗, bilateral knee flexion, Workspace volume
2) Exams:
CK*Muscular RMN
3) Symptoms:
Loss of independent ambulation* Calf hypertrophy (Unilateral/Bilateral, if bilateral → Symmetrical/Asymmetrical)∗Calf atrophy : Unilateral/Bilateral, if bilateral → Symmetrical/Asymmetrical)∗ Tendon contractures*Scoliosis Scapular winging* Tiptoe gait pattern* Muscle pain* Muscular trophism* Musvular wekness (Symmetrical/Asymmetrical - Proximal/Distal - Progression: chronic/acute)* Muscle contractures (Unilateral/Bilateral - site∗
Cramps: Severity – Frequency)∗ Clubfoot (Unilateral/Bilateral)∗ Spine anomalies∗ Ability to walk on toes∗ Fasciculations∗ Percussion induced muscular contractions∗ Macroglossia (yes/no)* Dysphagia(yes/no, Data onset, Progression chronic/acute, Severity-> light/moderate/severe, frequent aspirations:yes/no)
4)Muscules involved: shoulder* arms* forearms* hand* spine∗ pelvic muscules* thigh* leg* feet
5)Wheelchair: yes/no; if yes → changed through the years – still using yes/no – how long∗-other ambulatory devices yes/no; if yes → name → still using yes/no – how long – has it been changed through the years?
6)Deambulation and motility: general comments∗ Electromyography∗

Cardiovascular
1)Evaluations (data): general conditions: using devices to support heart function (eg. pacemakers, ICD, etc.) - name∗ still using? – How long? - has it been changed through the years?
2)Conduction system anomalies:

EKG ∗ Holter EKG* Echocardiogram* events monitoring∗
3)Cardiomyopathy∗ Heart RM∗
4) Simptoms: basal rattling* Oedemas* slopes edemas ∗ Holter∗ Echocardiogram: Description* FE* Left ventricle telediastolic Volume* Left ventricle telediastolic diameter∗ Arythmies∗ Coronaropathy∗ Valve∗ Pulmonary hypertension∗ Palpitations* Syncope∗ almost syncope∗

Respiratory

1) Evaluations (data):
2) Respiratory support: Invasive/ Noninvasive∗ Cough assist∗ Cough peak∗ Overnight BiPAP ∗ Other∗
3) Respiratory status∗ Pulsoxymetry/polysomnography (mean SpO2 - SpO2 minimal - FC mean - AHI – ODI – %∗ MVV – MIP – MEP. Dispnoea: supine position– stress induced – at rest∗
4) Quality of sleep: daily somnolence∗ not restorative sleep∗ frequent overnight awakening∗ morning headaches* daily fatigue∗

Laboratory Tests

1)Imaging tests (data): RX thorax∗ RX spine* RM muscle∗
2) Blood tests: Glucose* Proteins* Hemoglobin* White blood cells* Neutrophils ∗INR* RATIO* CPK* LDH* Amilase* Cistatine C∗ AST * ALT * ALP * GGT * Bilirubin total/direct/ indirect∗ Creatinine∗ Electrolytes (Sodium – Potassium - Clorum – Calcium total/ionized)∗ Urine (macroscopic view - blood (Yes/No) – anomalies)∗
3) Bone status: MOC - PTH pg/mL - VIT D1,25 pmol/L-VIT D 25 nmol/L∗
4)Glycemic state: Insuline - Hb1aC∗ Pro BNP∗

Autoimmune disease

Name∗
Treatment∗

Therapy

1) Name - posology – data – rationale
2) Prescriptions∗
3) Pain therapy∗
4) Steroids∗

8.4 Data sources

Data used to collect variables will include any clinical document provided by patients or available in our hospital thank to the server of the Unit of Neurology. Only official clinical charts will be considered. Clinical outcomes will be defined as the age of loss of ambulation, the age of introduction of cardiological supporting drugs and the age of introduction of respiratory supporting devices/therapies. These ages will be directly obtained from the clinical reports (on report it is reported the age of the patient) of indirectly (according to the data of the report that describes the first introduction of the drug/devices or that firstly reports the loss of the ambulation)

8.5 Cohort dimension

Expected cohort dimension is of 33 patients that is the number of patients followed at the Neurological Unit in the last 10 years. Statistical analysis will be based on generalized linear regression models, suitable both for continuous variables both for independent ones. Variables with a nonlinear distribution will be used only in logarithmic scale. Only data corresponding to yearly visit (at least two data in two years) will be used for the linear correlation analysis and in multivariate correlation models. Furthermore, to verify the accuracy of clinical variables in

describing the evolution of the dystrophy, variables will be coupled and compared by a bivariate analysis.

8.6 Data management

Data will be managed on a specific database created in a excel file, defended by a password. The file won't include any personal data and patients will be identified through a pseudonym that could be decrypted only through the access to a second file, defended by a different password.

8.7 Data analysis

Analysis will be performer using generalized linear regression models suitable both for continuous variables both for independent ones. Variables with a nonlinear distribution will be transformed in logarithmic scale before the analysis to be performed. For any analysis data will be divided according to the age of the patient at the moment of the evaluation. Data will be analyzed with linear correlation both compared to the variable age both in bivariate models and multivariate models. Results obtained will be adjusted for confounding factors not related to muscular dystrophy.

Data of included patients will be globally studied and the divide in two groups homogenous for genotype.

8.7.1 Primary endpoints: analysis

-To obtain a variables distribution according to patients' age and to verify possible correlations with the disease's progression.

-To study possible correlation between variables identified.

-To evaluate possible connections with clinical outcomes

8.7.2 Secondary endpoints: analysis

-Identify those variables that should be kept in consideration during the follow up of these patients

-To identify the variables that better describe disease progression to start a promptly therapeutic diagnosis

-To possibly identify useless analysis and examinations in these patients

8.8 Quality control

Data quality will be assessed by the reliability of clinical charts examined that will be divided according to type of assessment and examination performed in national clinical centres and/or private hospitals.

Thereafter reliability of data according to patients' history and to normal range values will be evaluated. Statistical analysis will enable to validate also the reliability of these data thanks to the use of programs of bi-multivariate analysis, biased for factors that do not relate to dystrophy.

8.9 Limitation to the study

The limitations to this study relied in the rareness of this condition and therefore in the risk to have so few data that we won't be able to reach a statistical significance. Whenever few patients will participate to the study, we won't be able to get enough data to perform trustable analysis.

Another limitation is represented by the little knowledge we have nowadays about the best clinical variables to study and about the natural history of this condition. Therefore, we will analyze variables that will eventually prove to be not reliable.

Furthermore, the lack of data about the natural history of this disease won't allow us to provide reliable information about the real impact of therapies on the disease progression.

9. Protection of included patients

This study will be performer according to Good clinical practice standards and to ethical principles derived from the Helsinki declaration and to standing law for observational studies. The observational study and the linked documentation will be presented to Ethical Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. The study will start only once received the required authorizations according to the Centre's procedures. The ethical committee

will also approve any change to the study protocol and recruiting modalities to involve patients in the study, according to local laws.

9.1 Information sheet and data treatment consent

The present study will follow the law number law number 101 of August 10 2018 regarding the standing UE regulation 2016/679 of European Parliament and Council of April 27 2016, describing the protection of persons under the point of view of personal data protection and free circulation of data. Stands the duty to collect informed consent to data treatment of included patients in any case whenever it can be possible to inform patients adequately whenever they arrive to the Hospital during control visits.

9.2 Assurance

As the study is an observational one, no insurance policies are needed beyond those already provided for common clinical practice.

10. Plan of scientific divulgation and popularization

The PI of the study will write a final report and publish the results at the end of the study through the publication o fan article or through scientific communication at National or International congresses. Data will be published anonymized and will be presented in digest mode.

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