Immunofluorescence signal intensity measurements as a semi-quantitative tool to assess sarcoglycan complex expression in muscle biopsy

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ABSTRACT

Sarcoglycanopathies are highly heterogeneous in terms of disease progression, muscular weakness, loss of ambulation and cardiac/respiratory involvement. Their clinical severity usually correlates with the residual protein amount, which makes protein quantification extremely relevant. Sarcoglycanopathy diagnosis is genetic, but skeletal muscle analysis - by both immunohistochemistry and Western blot (WB) - is still mandatory to establish the correct diagnostic process. Unfortunately, however, WB analysis cannot be performed if the bioptic specimen is scarce. This study provides a sensitive tool for semi-quantification of residual amount of sarcoglycans in patients affected by sarcoglycanopathies, based on immunofluorescence staining on skeletal muscle sections, image acquisition and software elaboration. We applied this method to eleven sarcoglycanopathies, seven Becker muscular dystrophies, as pathological control group, and four age-matched controls. Fluorescence data showed a significantly reduced expression of the mutated sarcoglycan in all patients when compared to their respective age-matched healthy controls, and a variable reduction of the other sarcoglycans. The reduction is due to the effect of gene mutation and not to the increasing age of controls. Fluorescence normalized data analyzed in relation to the age of onset of the disease, showed a negative correlation of α-sarcoglycan fluorescence signal *vs* fibrosis in patients with an early age of onset and a negative correlation between δ-sarcoglycan signal and fibrosis in both intermediate and late age of onset groups. The availability of a method that allows objective quantification of the sarcolemmal proteins, faster and less consuming than WB analysis and able to detect low residual sarcoglycan expression with great sensitivity, proves useful also in view of possible inferences on disease prognosis. The proposed method could be employed also to monitor the efficacy of therapeutic interventions and during clinical trials.

Key words: sarcoglycans; immunofluorescence; protein quantification; histology; fibrosis.

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Introduction

Limb girdle muscular dystrophies (LGMD) are a heterogeneous group of genetically inherited diseases. Sarcoglycanopathies belong to LGMD family. Sarcoglycans are four different cell membrane glycoproteins that form a tetrameric complex across the sarcolemma of skeletal muscle fibers and are encoded by four different genes *SGCA*, *SGCB*, *SGCG*, *SGCD*.[1-4] Correct assembly and translocation of the sarcoglycan complex to plasma membrane are required for preservation of sarcolemma stability during muscle contraction.[5,6] Sarcoglycanopathies are characterized by a heterogeneous clinical phenotype, which ranges from a severe Duchenne-like muscular dystrophy to forms with mild muscular involvement. The most frequent clinical phenotype includes progressive muscle weakness and atrophy, predominantly of the shoulder and pelvic girdles, with variable respiratory and cardiac involvement.[7]

The majority of sarcoglycanopathies is associated with missense mutations that generate substitution of single residue, causing the complete absence or the presence of only trace amounts of the protein along the cell membrane. A deficiency in a single component of the sarcoglycan protein complex leads to concomitant reduction or loss of the other sarcoglycans, and clinical severity usually correlates with the quantity of residual protein.[8]

Sarcoglycanopathy diagnosis is genetic, but muscle examination by immunohistochemistry and Western blot analysis are still mandatory for the correct diagnostic process. To date, there is still no specific treatment for these patients, but clinical trials aimed at restoring protein expression are underway.[9,10] A quantitative evaluation of sarcoglycan expression and the visualization of their correct membrane localization are fundamental targets for therapeutic strategies based on the partial or complete restoration of protein expression.

The aim of this study is to provide a tool for semi-quantification of residual amount of sarcoglycans combining immunofluorescence staining of muscle sections and image software elaboration. To this purpose, muscle sections from 11 patients with a genetically defined sarcoglycanopathy were analyzed for the expression of each sarcoglycan and for laminin-α2 by immunofluorescence and signal intensity quantification. Becker muscular dystrophy (BMD) patients, carrying dystrophin reduction, were included as pathological controls. Furthermore, control muscles, with unknown neuromuscular disorders were analyzed.

Materials and Methods

Patients

This study was performed on diagnostic muscle biopsy specimens from individuals with a suspected neuromuscular disorder and all of them had signed written informed consent before undergoing skeletal muscle biopsy. In detail, we examined skeletal muscle specimens from 11 genetically confirmed LGMD patients: 7 LGMDR3 (*SGCA* mutated) (average age 24±13.2 years), 2 LGMDR4 (*SGCB* mutated) (average age 9±4.2 years) and 2 LGMDR5 (*SGCG* mutated) (average age 6±2.8 years). All LGMDR4 and LGMDR5 patients were young, while LGMDR3 cohort includes both pediatric (three patients) and adult cases (four patients). Also, we included seven BMD specimens (from patients aged 39±11 years), as pathological control group, and four age-matched healthy control specimens (no abnormalities found at muscle biopsy). Table 1 summarizes the classes, the number of samples and the age range of both patients and controls. Study protocol and consent forms were approved by the local Ethics Committee.

Morphology

Routine haematoxylin and eosin (H&E) histology was performed on 8 µm-thick cryostat muscle sections. On each section, four randomly, non-overlapping, selected fields were photographed at 20x magnification, using optical microscope Leica DC200 equipped with camera and IM50 image analysis software (Leica Microsystems, Wetzlar, Germany). On H&E-stained muscle sections the fibrotic area (connective and adipose tissue) was quantified by color subtraction using Leica Application Suite 4.9.0 and ImageJ 1.53c (https://imagej.nih.gov/ij/download.html) software. Briefly on each H&E image a color deconvolution was applied. The resulting image was then converted to a binary image to obtain fibers colored in white and connective and/or adipose tissue colored in black.[11]

Immunohistochemistry

To process samples for a double immunofluorescence staining, skeletal muscle frozen sections (8 µm) were fixed with acetone for 3 min, washed three times with Phosphate Buffer Saline (PBS) and blocked with 1% bovine serum albumin in PBS for 30 min at room temperature (RT).

Sections were incubated, at the same times, with a rabbit primary polyclonal antibody anti-laminin-α2 (1:200 in PBS; PA5-49929; Thermo Fisher, Waltham, MA, USA), and one of the monoclonal antibodies anti-SG-α, SG-β, SG-γ and SGδ (all 1:50; Novocastra Leica Biosystem, Newcastle upon Tyne, UK), for 2 h at RT. Following three washing with PBS, sections were incubated with a mix of the secondary antibodies, goat anti-rabbit Alexa-488 for laminin-α2 (green channel) and goat anti-mouse Alexa-568 for SGs (red channel) (both 1:500; Thermo Fisher). Finally, after three washing with PBS, slides were mounted with anti-fading reagent Fluormount (Thermo Fisher).

Fluorescence intensity measurements

Immunolabelled sections were submitted to image analysis by using a Leica fluorescence microscope equipped with camera DFC420C (Leica). Leica Application Suite V4.6.2 software was used for image acquisition. Five non-overlapping fields at 20x magnification were photographed for each patient and control in the same set of acquisition conditions (image resolution, saturation, gain and exposure). Two images were acquired on the same field, one for laminin-α2 in the green channel (Led 480: Ex 480/40 bandwidth nm, Em 527/30 bandwidth nm) and one for sarcoglycan in the red channel (Led 530: Ex 546/12 bandwidth nm, Em 585/40 bandwidth nm). Quantification of fluorescence signal for laminin-α2 and for

Table 1. Classes, number and age range of both patients and controls.

Patients	Number of samples	Age range (years)
LGMDR3	7	24±13.2
Paediatric LGMDR3	3	7.6±4.0
Adult LGMDR3	4	28±7.8
LGMDR4	2	9±4.2
LGMDR5	2	6±2.8
Pathological control		
Becker muscular dystrophy	7	39±11
Healthy controls		
Paediatric	4	9.6±5.8
Adult	4	27.2±6.0

each sarcoglycan was performed using ImageJ 1.53c. Each RGB original 2592 x 1944 pixels image (Figure 1A) was first converted in 8-bit greyscale image before proceeding (Figure 1B). Then image was converted in a binary image as follows: Image => Adjust => Threshold => Dark Background (Figure 1C). In this image, only black and white pixel intensities remain, corresponding to cytoplasm and fiber membrane respectively. To start the analysis, the following settings were performed in the measurement setup: "set measurements" selecting area, area fraction, mean grey value and integrated density. The mean integrated density of each field was normalized by the number of fibers present in the field. Cell Counter plug-in of ImageJ software was used to count fibers in each field basing on the outlining of all viable myofibers *via* laminin-α2 staining (Figure 1 G-L).

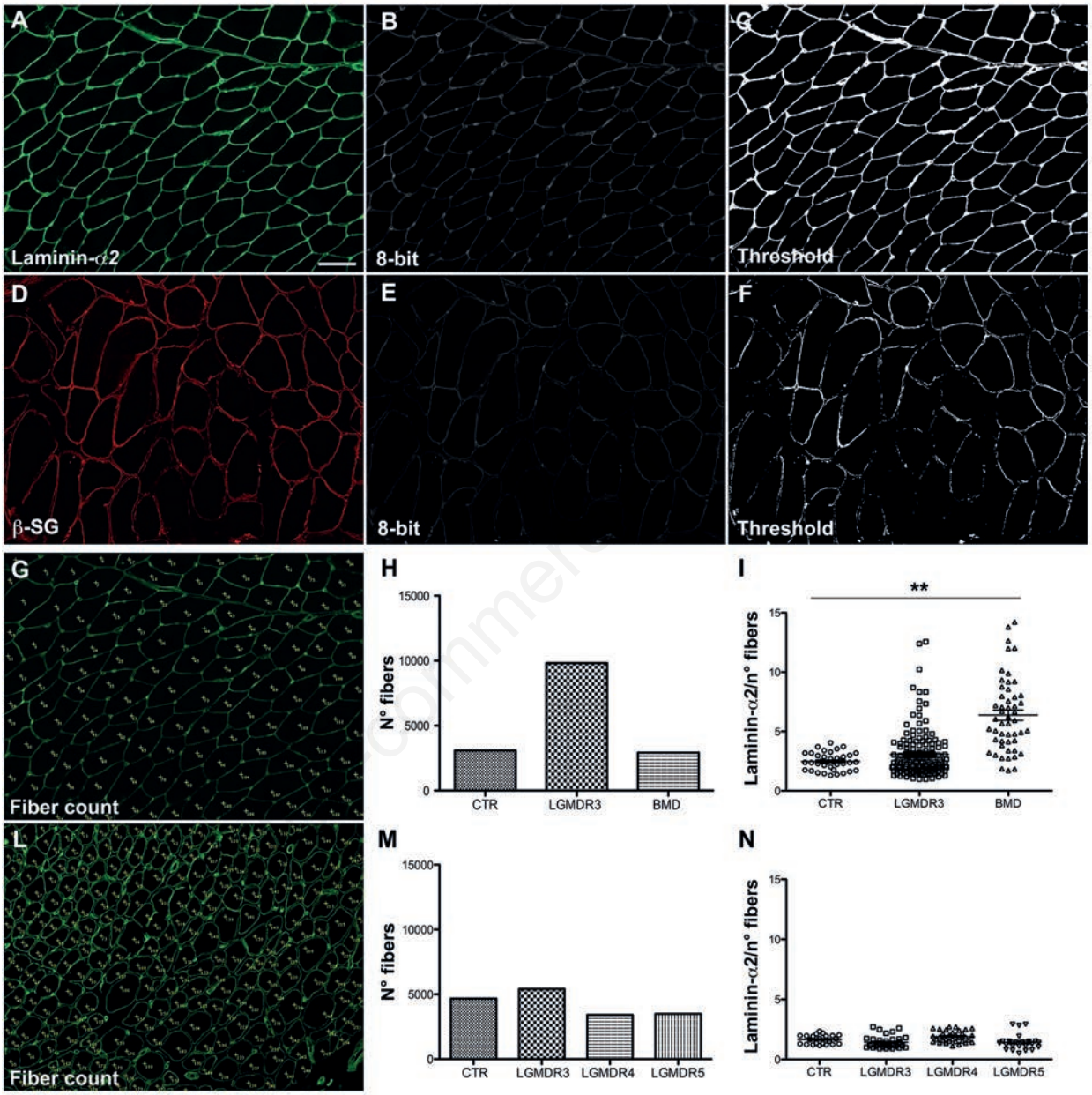

Figure 1. Example of protocol application. A) Representative channel green shows the fluorescent staining for laminin-α2 at 20x magnification; scale bar: 50 µm. B) Conversion on RGB image in 8-bit image. C) Threshold image. D) Representative channel red shows the fluorescent staining for β−SG. E) Conversion on RGB image in 8-bit image. F) Threshold image. G,L) Fiber count with ImageJ plug-in in adult (G) and in a pediatric (L) muscle section stained with laminin-α2. H) Histogram showing the number of counted fibers in adult healthy controls (n=4), in adult LGMDR3 patients (n=3) and in BMD pathological controls (n=7). M) Histogram showing the number of counted fibers in pediatric healthy controls (n=4) and in pediatric LGMDR3 (n=3), LGMDR4 (n=2) and LGMDR5 (n=2) patients. I) Dot plot of laminin-α2 fluorescence signal intensity normalized *vs* number of counted fibers in adult healthy controls (n=4), adult LGMDR3 patients (n=4) and in BMD pathological controls (n=7). N) Dot plot of laminin-α2 fluorescence signal intensity normalized *vs* number of counted fibers in pediatric healthy controls (n=4), and in LGMDR3 (n=3), LGMDR4 (n=2) and LGMDR5 (n=2) pediatric patients.

Western blot

The Western blot data available are those dating back to patients' examination at time of diagnosis, and further analyses were not possible due to the lack of muscle sample. In these patients, Western blot had been performed, in some cases, on all four proteins of the sarcoglycan complex and, in other cases, only on the mutated protein. For protein detection mouse monoclonal antibodies for α-SG, β-SG, γ-SG and δ-SG (1:50), all from Novocastra Laboratories, (Newcastle upon Tyne, UK) had been used. Mouse monoclonal antibody anti-Myosin heavy chain (1:200, Novocastra) had been used as loading control and for normalization of sarcoglycan bands. Western blot bands had been quantified by using Image J 1.46r software.

Data analysis

The signal of laminin-α2 antibody, staining the myofiber boundary, was used as sarcolemmal reference both as an indicator of individual muscle fibers integrity and as an internal standard to evaluate the efficiency of the immunofluorescence reaction. The laminin-α2 channel images were also used to count fibers in each muscle section. The image acquired in the red channel for each sarcoglycan was used only for quantification of signal intensity. The integrated density measured by ImageJ for each image was normalized for the corresponding number of fibers present in the same image. The data are expressed as mean ± standard error of the mean (SEM). The means were obtained by inserting the single value of normalized fluorescence intensity (integrated density/number of fibers) obtained from each of the five fields analyzed for laminin-α2 and for sarcoglycans. Scatter dot plots represent data dispersion for laminin-α2 and for SG proteins fluorescence signal. The Mann-Whitney U test was applied to compare the differences among groups. To analyze the relationship between the clinical aspects and the residual SG protein expression, a two-tailed Spearman correlation coefficient (r) was applied. The plot of Bland-Altman was used to identify the 95% limits of agreement and bias.[12] Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, LaJolla, CA, USA). Significant levels were set as $p \leq 0.001$ (**) and $p \leq 0.05$ (*). The entire method process is depicted in the block diagram of Figure 2.

Results

Immunofluorescence signal intensity quantification of laminin-α2 as internal standard

Data of laminin-α2 mean intensity normalized for fiber number showed a significant higher level in BMD muscles (6.373±0.4362) compared to age-matched controls (2.486±0.1181; $p<0.0001$), in agreement with what has been previously reported for spectrin and laminin-α2 in DMD and BMD muscle biopsies.[13] No significant variations for laminin-α2 mean intensity was observed in adult LGMDR3 muscle sections compared to age-matched controls (3.063±0.172 *vs* 2.486±0.1181; $p=0.0907$) (Figure 1I). Similarly, no significant variations were observed in pediatric patients, in particular, in LGMDR3 laminin-α2 mean intensity was 1.415±0.082 ($p=0.0518$), in LGMDR4 1.894±0.081 ($p=0.0542$) and LGMDR5 1.429±0.1389 ($p=0.1647$) compared to controls (1.659±0.080) (Figure 1N).

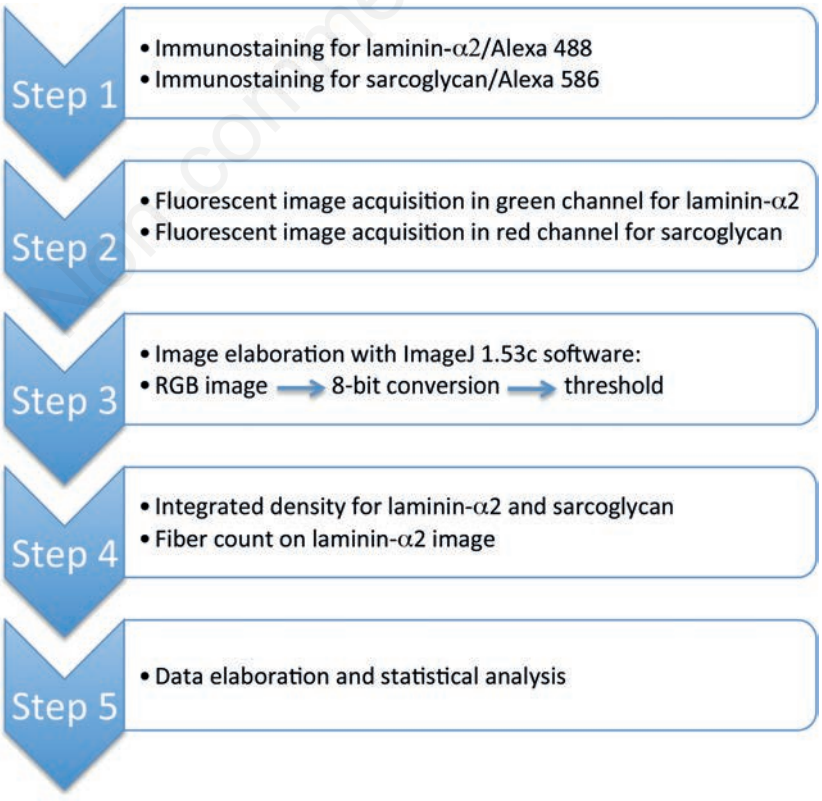

Figure 2. Schematic block diagram summarizing the steps of proposed method.

Immunofluorescence signal intensity quantification of SGC complex component in patient groups

Data showed a reduction of normalized fluorescence signal related to each sarcoglycan compared to respective healthy controls. The results obtained, in both adult and pediatric patients are in agreement with reported data that the reduced expression of a mutated sarcoglycan causes a variable reduction also in the expression of the other components of the complex.[14] In LGMDR5 we detected a more marked reduction of α-SG (0.2069±0.03797 *vs* 3.082±0.3669, p<0.0001) (Figure 3A), β-SG (0.1433±0.03997 *vs* 1.713±0.1104 p<0.0001) (Figure 3B) and γ-SG (0.035±0.00933 *vs* 0.9386±0.1084, p<0.0001) (Figure 3C) and a less evident δ-SG reduction (0.8515±0.2749 *vs* 1.418±0.131, p=0.0053) (Figure 3D). In LGMDR3 we observed a similar residual expression of all sarcoglycans as follows: α-SG (0.4665±0.086 *vs* 3.082±0.3669, p<0.0001) (Figure 2A), β-SG (0.6597±0.1134 *vs* 1.713±0.1104, p<0.0001) (Figure 3B), γ-SG (0.4572±0.1153 *vs* 0.9386±0.1084, p=0.0013) (Figure 3C) and δ-SG (0.4026±0.0796 *vs* 1.418±0.131, p>0.0001) (Figure 3D). In LGMDR4 we observed a larger residual amount for α-SG (0.323±0.05959 *vs* 3.082±0.3669, p<0.0001) (Figure 3A) and a lower residual amount for β-SG (0.155±0.0307 *vs* 1.713±0.1104, p<0.0001) (Figure 3B), γ-SG (0.174±0.0438 *vs* 0.9386±0.1084, p<0.0001) (Figure 3C) and δ-SG (0.085±0.01384 *vs* 1.418±0.131, p<0.0001) (Figure 3D). In BMD patients we detected a significant reduction in α-SG (1.647±0.2646 *vs* 3.082±0.2646, p=0.0024) (Figure 3A) and β-SG (0.758±0.0379 *vs* 1.713±0.1104, p=0.0001) (Figure 3B), conversely, a significant increase was observed in γ-SG (2.178±0.3479 *vs* 0.9386±0.1084, p=0.0015) (Figure 3C) and δ-SG (2.293±0.3004 *vs* 1.418±0.131, p=0.0146) (Figure 3D).

Immunofluorescence signal intensity quantification of SGC complex component in relation to age of onset of disease

Normalized fluorescence signal intensity for each sarcoglycan was also analyzed in relation to the age of onset of the disease. To this aim, patients were subdivided into three groups: a group with early onset, namely before the age of 10 years, a group with intermediate onset, between 10 and 18 years of age, and a group with later onset, more precisely after 18 years of age. Data collected showed a more marked reduction of the fluorescence signal intensity for all sarcoglycans in early onset patients (Figure 4). α-SG fluorescence signal intensity was significantly reduced in early onset patients compared to both the 10-18 years onset group (0.1435±0.0248 *vs* 0.2967±0.038, p=0.00011) and to the later onset group (0.143±0.0248 *vs* 0.7646±0.1468, p<0.0001) (Figure 4A). Similar results were obtained for β-SG whose signal intensity in early onset patients was reduced compared to both the intermediate (0.140±0.02421 *vs* 0.3795±0.05728, p=0.0005) and the later onset (0.140±0.02421 *vs* 0.9458±0.2002, p=0.0003) groups (Figure 4B). γ-SG intensity in early onset patients was decreased compared to both intermediate onset (0.06091±0.01075 *vs* 0.5353±0.1951, p=0.0056)

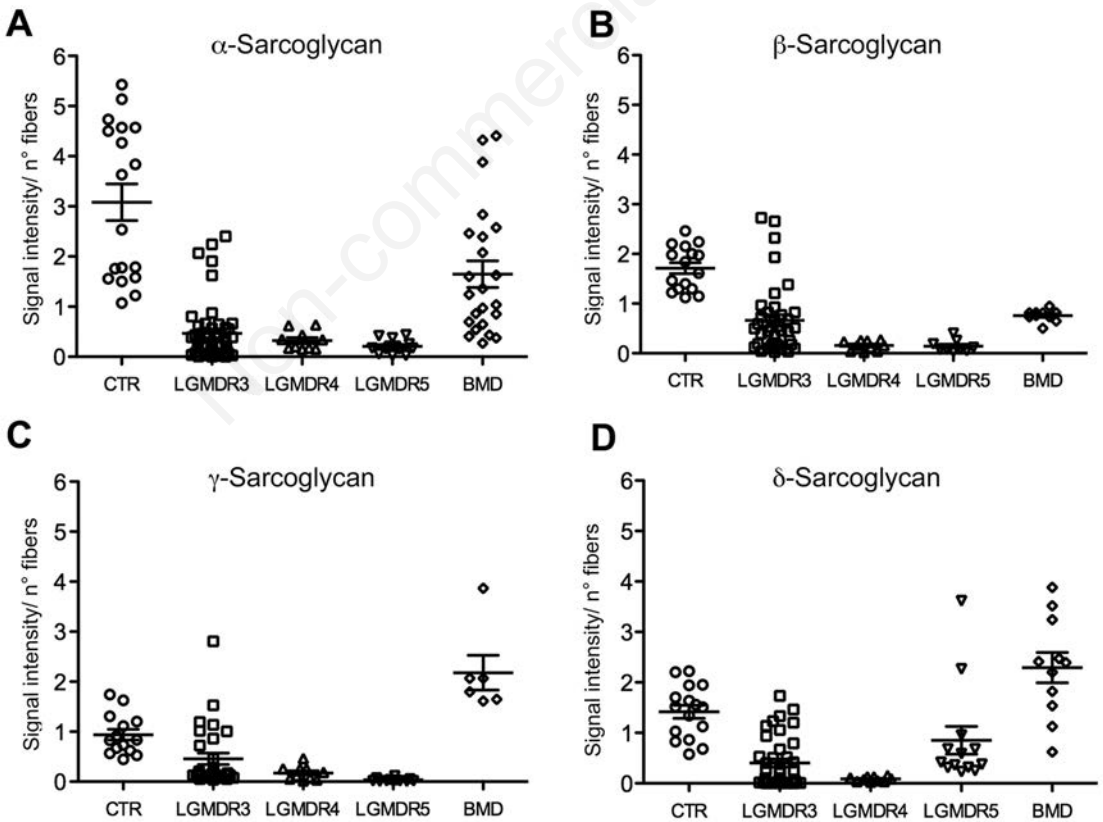

Figure 3. Comparative analysis of sarcoglycans expression in sarcoglycanopathies, Becker muscular dystrophy (BMD) and controls. Dot plot of fluorescence signal intensity of sarcoglycans normalized *vs* number of counted fibers in patients, healthy controls and BMD pathological controls. A) α-sarcoglycan, B) β-sarcoglycan, C) γ-sarcoglycan and D) δ-sarcoglycan.

and late onset (0.06091±0.01075 *vs* 0.4321±0.1126, p=0.0002) groups, no significant difference was observed between intermediate and late onset groups (0.5353±0.1951 *vs* 0.4321±0.1126, p=0.6566) (Figure 4C). For δ-SG fluorescence signal was significantly reduced in the early onset group (0.2894±0.05287) only compared to the late onset group (0.5964±0.151, p=0.0199), no significant difference being observed with the intermediate onset group (0.3885±0.0972, p=0.3361) (Figure 4D). Normalized fluorescence signal intensity for laminin-α2 showed no significant difference among the groups analyzed (Figure 4E).

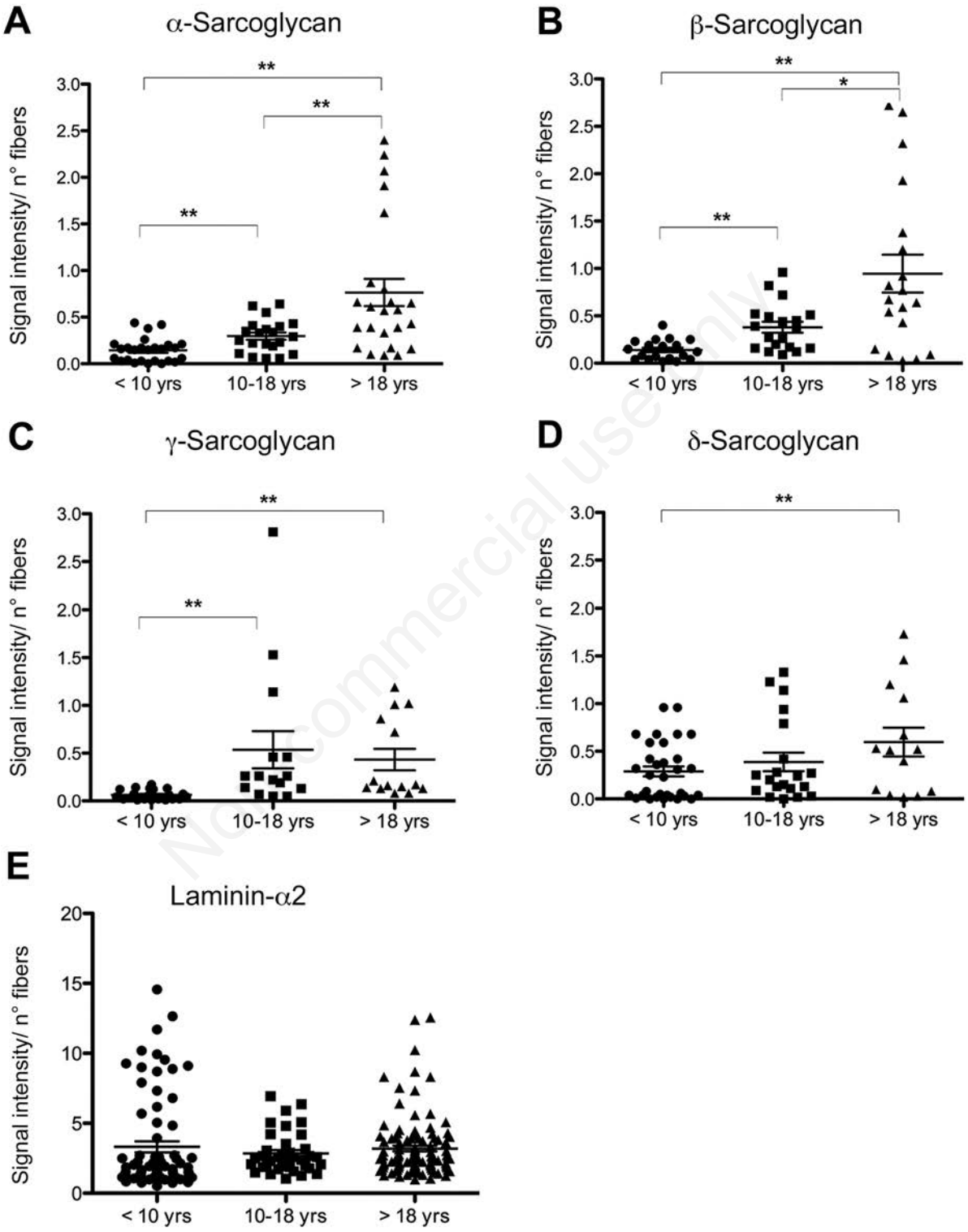

Figure 4. Dot plot of normalized fluorescence signal intensity of A) α-SG, B) β-SG, C) γ-SG, and D) δ-SG in the three groups of patients. E) Dot plot of normalized fluorescence signal intensity of laminin-α2 in the same patient's group. **p≤0.001; *p≤0.05.

Histological evaluation of fibrosis

Histological evaluations of fibrosis were made on H&E sections in relation to the age of onset of the disease. Deposition of adipose and connective tissue resulted more marked in patients with an early age of onset compared to the other two groups (Figure 5 A,B). In detail, mean fibrotic area was 33.80 $\mu m^2 \pm 3.211$ μm^2 in patients with the earliest age of onset (*vs* age-matched controls 10.53 $\mu m^2 \pm 0.559$ μm^2, $p<0.0001$), 13.51 $\mu m^2 \pm 0.820$ μm^2 in the 10-18 years group (*vs* 9.215 $\mu m^2 \pm 0.316$ μm^2, $p=0.0005$) and 12.48 $\mu m^2 \pm 1.364$ μm^2 in the late onset group (*vs* 9.985 $\mu m^2 \pm 0.5705$ μm^2, $p=0.0595$). Fibrotic area in patients with the lowest age of onset was significantly higher compared to both the intermediate ($p=0.0001$) and the late onset ($p=0.0001$) groups (Figure 5A).

Correlation of fluorescence signal intensity with histological aspects in relation to age of onset of the disease

The statistical analysis showed negative correlations between the residual expression of different sarcoglycans and the fibro-adipose tissue deposition in patients. Specifically, the residual normalized fluorescence intensity of α-SG negatively correlated with fibrosis in patients with the earliest disease onset age (r Spearman=-0.4567, $p=0.0429$, $n=20$) (Figure 5C). Normalized fluorescence intensity of δ-SG negatively correlated with fibrosis in both the intermediate (10-18 years) (r Spearman=-0.6202, $p=0.0136$, $n=15$) (Figure 5D) and the later (>18 years) (r Spearman=-0.8418, $p=0.0002$, $n=14$) age of onset groups (Figure 5E).

Immunohistochemistry and Western blot data comparison

To assess the level of agreement between immunofluorescence (Figure 6A) and Western blot (Figure 6B), data (obtained from the two protocols on the same patients), were plotted with a regression line (Figure 6C) and generated a Bland-Altman plot of difference (Figure 6D). This comparative analysis between immunofluorescence and densitometric data available from Western blot was limited to a small number of samples. Available Western blot data were associated with previously performed experiments, and given the scarcity or the complete absence of muscle specimens it was not possible to generate new data. The level of bias was -0.00517, and the upper and lower limits of agreement were 0.3899

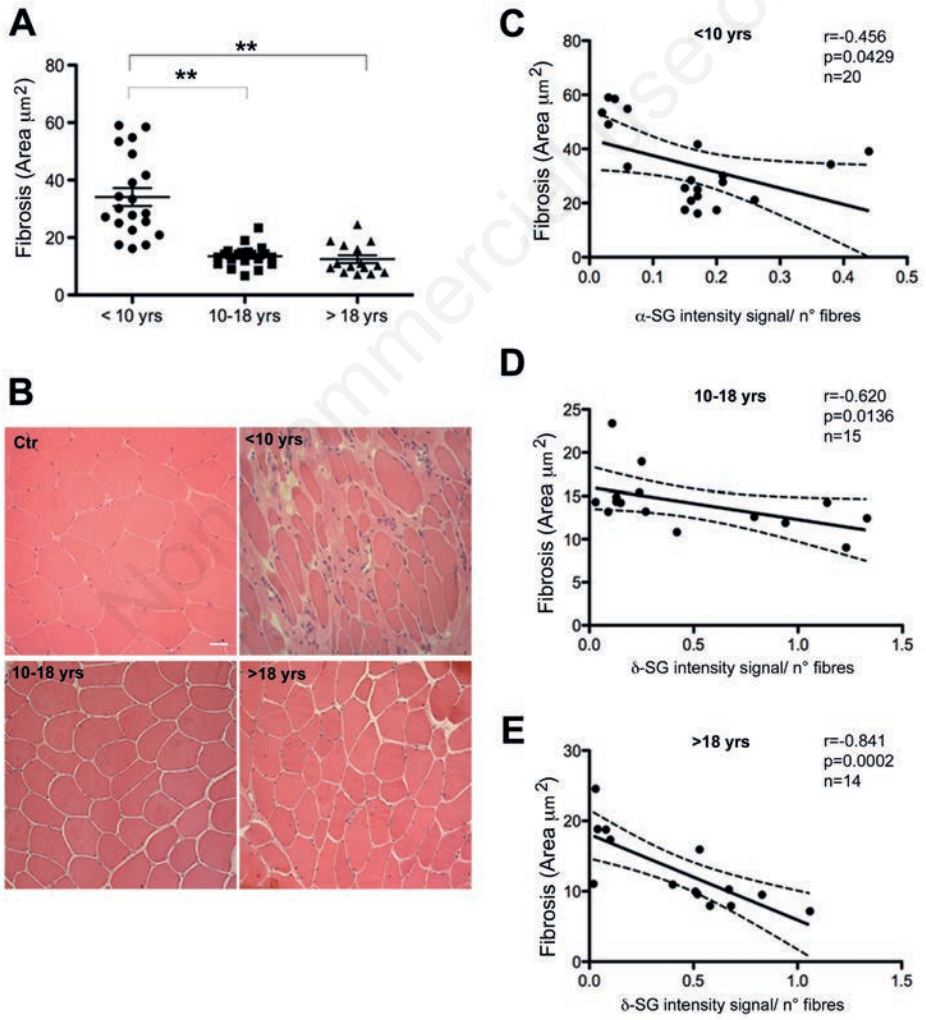

Figure 5. A) Dot plot of fibrosis in patients. B) H&E representative images of fibro-fatty tissue deposition in the three groups of patients and control muscle; correlation analysis of residual normalized signal intensity of sarcoglycans and fibrosis; scale bar: 50 µm. C) α-sarcoglycan negative correlation with fibrosis in patients with earlier age of onset. D) δ-sarcoglycan negative correlation with fibrosis in patients with 10-18 years as age of onset. E) δ-sarcoglycan negative correlations with fibrosis in patients with later age of onset.

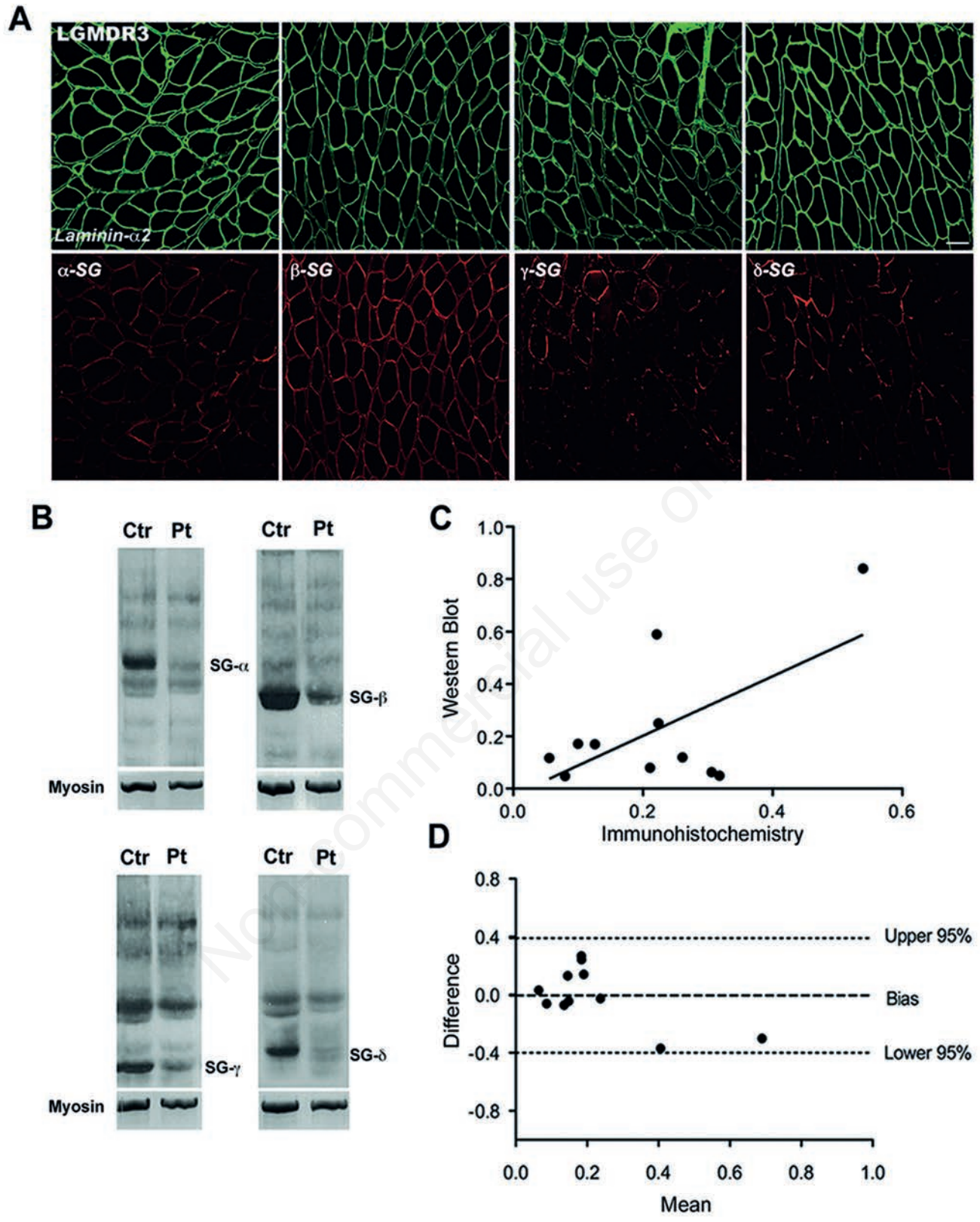

Figure 6. A) Representative immunofluorescence images of laminin-α2 and sarcoglycans in LGMDR3 patient; scale bar: 50 µm. B) Representative Western blot for sarcoglycans in one LGMDR3 patient. Myosin was use as internal standard. C) Regression line of single immunohistochemistry and Western blot date. D) Bland-Altman plot.

and -0.4003 respectively. Even if our sample size is small, the scatter of data points lays relatively close to the bias line therefore suggesting that the difference between the two methods was acceptable.

Discussion

Previous studies have been focused on developing a rapid and sensitive tool for immunofluorescent quantification of sarcolemmal proteins. This necessity originally arose from the need to quantify dystrophin in Duchenne and Becker muscular dystrophy patients subjected to pharmacological strategies, which aimed at partially restoring dystrophin expression.[13,15-18] However, this need is common also for sarcoglycanopathies for which pharmacological approaches using adenoviral vectors for restoring protein expression have been also proposed.[9,10]

So far, analyses of fluorescence signals of sarcolemma proteins have been part of a standard diagnostic procedure of limb-girdle dystrophies, providing an operator-dependent qualitative assessment. In the diagnostic procedure, immunofluorescence is frequently associated to Western blot analysis to provide complementary information. Indeed, immunofluorescence staining allows to simultaneously evaluation of protein expression and its correct location at the sarcolemma whereas Western blot provides a semi-quantitative protein evaluation. Also, a genetic mutation in one of the four sarcoglycan genes causes the reduction or complete absence of the corresponding protein, but, in addition, can have variable effects on the expression of the other components of the sarcoglycan complex.

Sarcoglycanopathies are highly heterogeneous in terms of disease progression, muscular weakness, loss of ambulation, cardiac and respiratory involvement. Furthermore, a great variability has been observed in both residual sarcoglycan expression and histological aspects. In sarcoglycanopathies, a correlation between phenotype and protein residual expression, evaluated through Western blot, has been reported.[19] However, Western blot analysis does not allow to evaluate the correct membrane localization and to discriminate between small variations of residual amount.

The availability of a method that allows objective quantification of the sarcolemma proteins - faster, more sensitive and less consuming than Western blot analysis - can be extremely useful to better define patient prognosis, expected disease evolution and, in case of clinical trials, efficacy of pharmacological treatment. Indeed, in most studies, one of the primary endpoints, along with the clinical improvement, is the restoration of the mutated protein expression along the sarcolemma.[9,10] Furthermore, even in very large, recently collected SG cohort, muscle SG expression was expressed using very approximate and broad qualitative terms.[20]

In this study, we have semi-quantified the fluorescence signal related to each sarcoglycan in muscle biopsies from patients affected with a sarcoglycanopathy. The results were compared to those obtained in either pediatric or adult healthy age-matched controls, and in BMD patients, representing the pathological control group. In the latter we observed a significant reduction of α-SG and β-SG and an increase of γ-SG and δ-SG.

Analysis of laminin-α2 revealed a similar expression in both patients and healthy age-matched controls. Semi-quantification of mean normalized intensity fluorescence signal in patients showed significant differences in sarcoglycan residual expression in the different sarcoglycanopathies. The residual expression of the mutated protein was more markedly reduced in LGMDR5 followed by LGMDR4 and by LGMDR3. The residual expression of the four proteins was higher in LGMDR3 compared to the other two forms, and more similar between LGMDR4 and LGMDR5. This trend correlates with patient clinical severity in agreement with the observation that in sarcoglycanopathies the residual expression of complex proteins influences disease progression and severity.[8] In addition, quantifications showed that the protocol is extremely sensitive, able to detect also very low levels of sarcoglycans. Statistical analysis of our data meant to identify possible correlations with some clinical aspects did not yield significant results probably due to the high clinical variability of sarcoglycanopathies and to the small number of analyzed samples, the latter condition however justified by the rare nature of these conditions; for this reason, the characterization of a larger case series would imply the contribution by other specialized neuromuscular centers to help better define the highlighted correlations.

Some interesting results were obtained when the mean intensity fluorescence signal was evaluated against patients' age of onset of the disease. Indeed, patients with the lowest age of onset showed the lowest residual expression of all sarcoglycans, whereas the highest residual fluorescence levels were detected in the late onset group. These data were statistically evaluated to search for possible correlations with histological features. In detail, a negative correlation between residual signal intensity of α-SG and fibrosis was found in the early age of onset group, whereas in both the intermediate and late age of onset groups a negative correlation was found between signal intensity of δ-SG and fibrosis.

Our protocol proves extremely accurate in detecting the expression and correct sarcolemmal location of sarcoglycan complex, even when at trace levels. In this regard, it turns out to be a valuable tool to study the natural history of sarcoglycan patients and to be employed in clinical / therapeutical trials aimed at restoring protein expression. The identification of any correlations between residual protein levels and clinical/histological features will enhance our knowledge in a group of diseases with a great and unpredictable clinical variability.

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