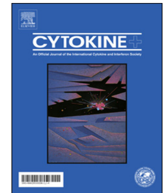
Cytokine 127 (2020) 154966

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine



Short communication

The myokine GDF-15 is a potential biomarker for myositis and associates with the protein aggregates of sporadic inclusion body myositis

Boel De Paepe[a,*], Fien Verhamme[b], Jan L. De Bleecker[a]

[a] Department of Neurology and Neuromuscular Reference Centre, Ghent University Hospital, Ghent, Belgium
[b] Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium

ARTICLE INFO

Keywords:
Biomarkers
Growth differentiation factor-15
Idiopathic inflammatory myopathy
Myositis
Sporadic inclusion body myositis

ABSTRACT

Background: The cytokine growth differentiation factor-15 (GDF-15) has been associated with inflammatory and mitochondrial disease, warranting exploration of its expression in myositis patients.
Methods: GDF-15 protein levels are evaluated in 35 idiopathic inflammatory myopathy (IIM) serum samples using enzyme-linked immunosorbent assays, comparing with levels in samples from healthy individuals and from patients with genetically confirmed hereditary muscular dystrophies and mitochondrial disorders. Muscle tissue expression of GDF-15 protein is evaluated using immunofluorescent staining and Western blotting.
Results: GDF-15 protein levels are significantly higher in IIM sera (625 ± 358 pg/ml) than in that of healthy controls (326 ± 204 pg/ml, $p = 0.01$). Western blotting confirms increased GDF-15 protein levels in IIM muscle. In skeletal muscle tissue of IIM patients, GDF-15 localizes mostly to small regenerating or denervated muscle fibres. In patients diagnosed with sporadic inclusion body myositis, GDF-15 co-localizes with the characteristic protein aggregates within affected muscle fibres.
Conclusions: We describe for the first time that GDF-15 is a myokine upregulated in myositis and present the cytokine as a potential diagnostic serum biomarker.

1. Introduction

Idiopathic inflammatory myopathies (IIM) also termed myositis are a heterogeneous group of rare muscle diseases subtyped based upon clinical characteristics and their associated skeletal muscle damages. Dermatomyositis (DM) patients develop complement-mediated blood vessel destruction, perimysial inflammation and perifascicular muscle fibre atrophy, and often display typical cutaneous manifestations. Polymyositis (PM) and sporadic inclusion body myositis (IBM) are characterized by invasion of nonnecrotic muscle fibres by auto-aggressive cytotoxic T-cells and macrophages, with inflammation building up mostly at endomysial sites. IBM muscle fibres additionally develop degenerative changes, with rimmed vacuoles and inclusions containing aggregates of ectopic proteins. In immune-mediated necrotizing myopathy (IMNM), necrosis of muscle fibres predominates over tissue inflammation. Additional subtypes based upon associated autoantibody profiles have recently been recognized, and subgroups of patients develop overlap myositis associated with other rheumatic diseases [1]. Biomarker development aimed at discriminating IIM from other muscle diseases and subtyping disease groups, remains a priority for these pathologies.

An altered mitochondrial phenotype has long been recognized in the subgroup of IBM patients, largely based upon the histological evidence of ragged-red fibres and cytochrome *c* oxidase deficient muscle fibres in patient biopsies [2]. In addition, hereditary muscular dystrophies may contain mitochondrial abnormalities [3], and prominent mitochondrial damage is observed in individuals with PM poorly responsive to immunosuppressive therapy, a disorder also termed PM with mitochondrial pathology [4]. As growth differentiation factor-15 (GDF-15) is involved in both mitochondrial [5] and inflammatory [6] regulation, we set out to investigate this cytokine in the IIM. GDF-15 is a member of the transforming growth factor-β (TGF-β) superfamily of cytokines with many aliases, and is also called prostate differentiation factor, macrophage inhibitory cytokine-1, placental bone morphogenetic protein, non-steroidal inflammatory drug-activated protein 1, and placental TGF-β. It is a versatile regulatory factor that behaves as a transcription factor in the cell's nucleus, and in the cytoplasm associates with mitochondria regulating the cell's energy homeostasis [7]. The secreted

Abbreviations: DM, dermatomyositis; GDF-15, growth differentiation factor-15; IBM, sporadic inclusion body myositis; IIM, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotizing myopathy; PM, polymyositis

* Corresponding author at: Laboratory for Neuropathology, Route 1484, Building 10K12IE, Corneel Heymanslaan 10, 9000 Ghent, Belgium.
E-mail address: boel.depaepe@ugent.be (B. De Paepe).

https://doi.org/10.1016/j.cyto.2019.154966
Received 12 July 2019; Received in revised form 29 November 2019; Accepted 25 December 2019
1043-4666/ © 2019 Published by Elsevier Ltd.

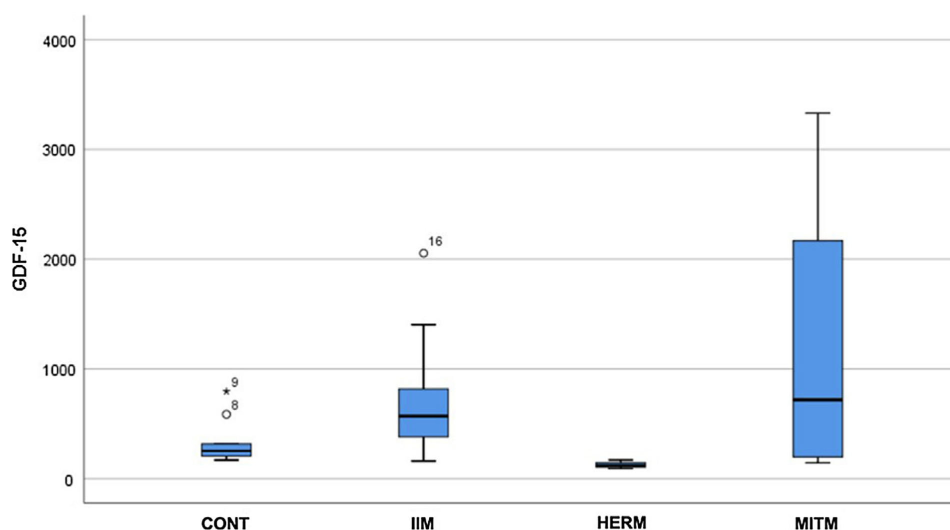


Fig. 1. Graphic representation of circulating growth differentiation factor-15 (GDF-15) expressed as pg per ml serum. Mean levels in healthy controls (CONT), patients diagnosed with idiopathic inflammatory myopathies (IIM), hereditary muscular dystrophies (HERM), and mitochondrial myopathy (MITM) are shown. Mean ages of subjects ± SD and the numbers of patients per group were: 33.7 ± 12.1n = 10 (CONT), 63.7 ± 10.5n = 35 (IIM), 43.0 ± 15.4n = 3 (HERM) and 40.8 ± 16.2 n = 5 (MITM).

disulfide bond-linked GDF-15 dimer possesses endocrine functions, controlling food intake and body weight [8].

2. Patients and methods

2.1. Patients

This retrospective study includes muscle biopsies and sera from an established cohort of 35 IIM patients clinically and myopathologically diagnosed with IMNM (n = 21), IBM (n = 10), PM (n = 3), and DM (n = 1) (Supplementary Table 1). Controls are ten commercial sera from healthy subjects aged between 22 and 41 years (Zenbio, Durham, NC) and sera from patients with hereditary muscle disease: Patients with limb girdle muscular dystrophy due to homozygous c.191dupA in the *ANO5* gene and c.1A > G in the *BVES* gene, and a patient with clinical IBM carrying a homozygous c.374G > A alteration in *VCP*. As positive controls, five patients with mitochondrial myopathy are included, in which disease is due to heteroplasmic m.3243A > G mutations leading either to chronic progressive external ophtalmoplegia, or mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; a homoplasmic m.11778G > A mutation causing Leber's hereditary optic neuropathy; and a nuclear *POLG* variant c.2864A > G (p.Tyr955Cys) causing chronic progressive external ophtalmoplegia.

2.2. Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays are performed with the Human GDF-15 DuoSet ELISA kit from R&D Systems (Bio-Techne, Abingdon, UK) according to the manufacturer's specifications. Duplicates of sera diluted 1/10 and 1/20 (controls), 1/10 and 1/50 (patients), are loaded onto 96-well plates. Values are calculated as the mean of duplicates and of the two dilutions tested, and reported as mean ± SD. Shapiro-Wilk test ruled out normality of result values, hence the Mann-Whitney *U* test is used to investigate significance. Wilcoxon Signed Rank test is used to analyse a possible association of GDF-15 levels with clinical characteristics. All analyses are done with SPSS software version 25 (IBM, New York, NY).

2.3. Immunofluorescent staining

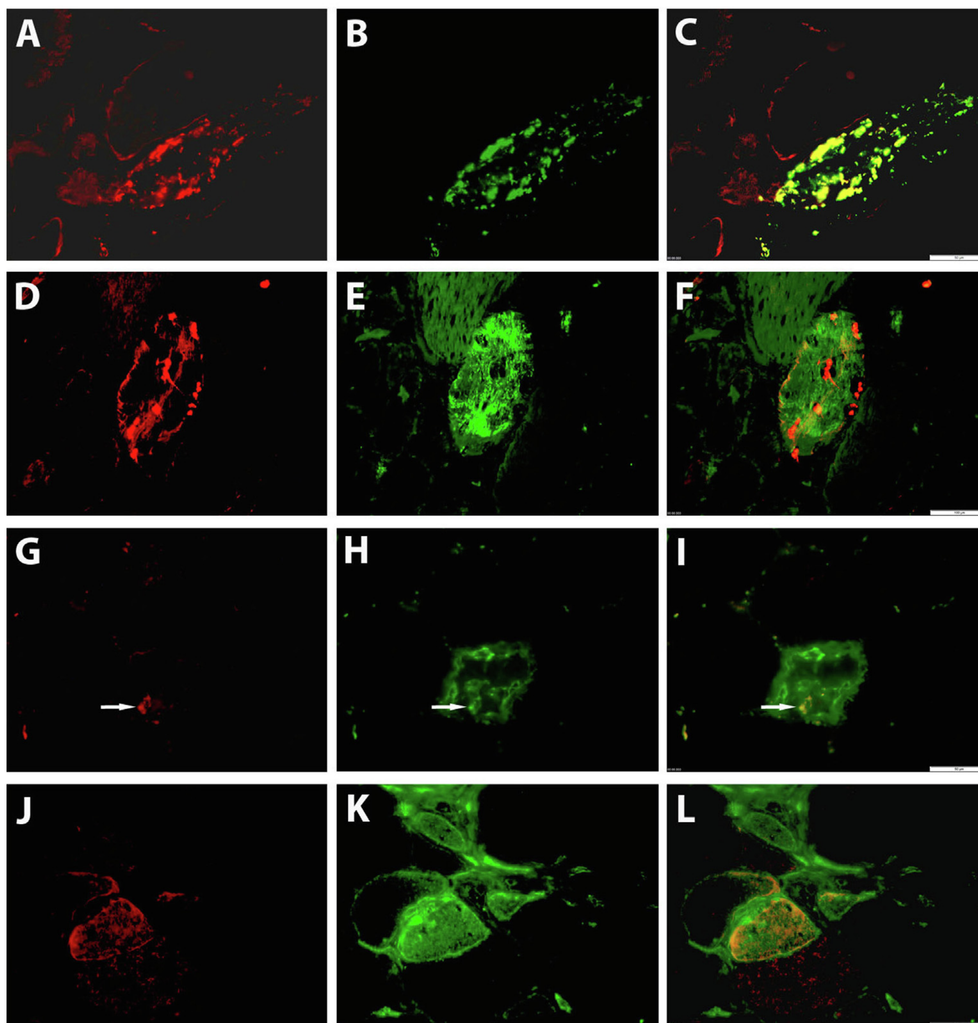
To visualize GDF-15 in frozen muscle sections, 4 µg/ml mouse monoclonal IgG_{2a} (clone H-2, Santa Cruz Biotechnology, Santa Cruz, CA) and 10 µg/ml goat polyclonal (B2413, LifeSpan BioSciences, Seattle, WA) anti-GDF-15 antibodies are used. Blocking and incubations with primary antibodies are carried out in phosphate buffered saline with 5% donkey serum, 10% heat-inactivated human serum and 2% bovine serum albumin (Millipore Sigma, St Louis, MO). Double staining is done with 0.7 µg/ml rabbit polyclonal anti-CD68 (H-255, Agilent, Santa Clara, CA), 1 µg/ml rabbit polyclonal anti-CD56 (Fisher Scientific, Waltham, MA), 1.3 µg/ml mouse monoclonal IgG_1 anti-p62 (BD Biosciences, San Jose, CA), 1.25 µg/ml rabbit polyclonal anti-LC3B (Abcam, Cambridge, UK), and 28.5 µg/ml rabbit anti-von Willebrand factor (Agilent). Fluorescent secondary antibodies are used labelled with CY3 (Jackson ImmunoResearch Laboratories, West Grove, PA) and AlexaFluor488 (Invitrogen, Carlsbad, CA). Slides are mounted with Fluoromount (Southern Biotech, Birmingham, AL) and analysed under a fluorescence microscope (Zeiss, Goettingen, Germany). Controls consist of the omission of primary antibody and the substitution by non-immune IgGs.

2.4. Western blotting

Protein samples are prepared from muscle biopsies using a commercial extraction kit (Fisher Scientific). Proteins are separated on a 10% bis tris polyacrylamide gel and blotted onto a nitrocellulose membrane (Invitrogen). The membrane is incubated overnight with 0.2 µg/ml rabbit polyclonal anti-glyceraldehyde 3-phosphate dehydrogenase, tris-buffered saline with 2% milk added served as blocking and incubation solution. Chemiluminescence is generated after incubating 1 h with 1 µg/ml peroxidase-labelled goat anti-rabbit by adding the Clarity Max substrate (Biorad, Hercules, CA), visualized with the Chemidoc Imaging System and Image Lab 6.0 software (Biorad). The same membrane is then washed and incubated with 2 µg/ml mouse monoclonal IgG_{2a} anti-GDF-15 (clone H-2, SantaCruz Biotechnology) for 4 h at room temperature, followed by colorimetric visualization using the WesternBreeze anti-mouse kit (Invitrogen).

3. Results

Serum levels of GDF-15 are increased in IIM patients (Fig. 1). Compared to controls (326 ± 204 pg/ml), circulating GDF-15 levels are significantly higher in IIM patients (625 ± 358 pg/ml) according to the Mann-Whitney *U* test (p = 0.01), with levels not significantly different between IMNM (557 ± 368 pg/ml) and IBM (747 ± 326 pg/ml) subgroups (p = 0.1). GDF-15 levels do not correlate with CK values (p = 0.2), gender (p = 0.07), associated other autoimmune diseases (p = 0.3), autoantibody positivity (p = 0.9), malignancy (p = 0.5), or extensive endomysial inflammation (p = 0.07). GDF-15 levels in patients diagnosed with limb girdle muscular dystrophy and hereditary IBM (127 ± 41 pg/ml) are notably



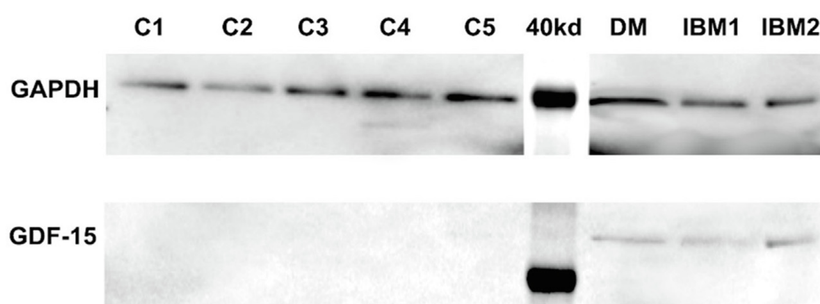


Fig. 2. Expression of growth differentiation factor-15 (GDF-15) in skeletal muscle tissues. **Immunofluorescence panel.** Sporadic inclusion body myositis. A: Strong granular staining for GDF-15 (CY3, red) is observed in a muscle fibre, B: Staining for the autophagic marker p62 (AlexaFluor488, green) shows the inclusions present in the muscle fibre. C: Presence of GDF-15 in the inclusions is shown in the combination image. D: A muscle fibre is strongly positive for GDF-15 (CY3, red). E: The muscle fibre is also positive for autophagic marker LC3B (AlexaFluor488, green). F: A double fluorescence combination image is shown. Immune-mediated necrotizing myopathy. G: A single cell stains for GDF-15 (CY3, red). H: Staining for CD56 (AlexaFluor488, green) identifies activated immune cells. I: The combination image shows the single GDF-15 positive immune cell invading a necrotic muscle fibre (arrow in all three images). Polymyositis. J: Muscle fibres stain for GDF-15 (CY3, red) in the sarcoplasm and on the fibre's edges. K: Staining with CD56 (AlexaFluor488, green) identifies regenerating or denervated muscle fibres. L: Double fluorescence combination image. Scale bar = 50 μm. **Western blotting panel.** A protein band for GDF-15 can only be detected in muscle samples from patients diagnosed with dermatomyositis (DM) and sporadic inclusion body myositis (IBM), while the protein could not be detected in five healthy control samples (C). Protein bands of the housekeeping protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) detected on the same membrane are given to evaluate equal loading.

lower. Sera from patients with mitochondrial disease display highest mean values (1312 ± 1393 pg/ml) and largest variations between individual samples, as has been reported [5].

Only low constitutive GDF-15 staining is present in healthy skeletal muscle, with rare muscle fibres displaying low sarcoplasmic expression and discontinuous staining along the muscle fibre edges. Staining increases in IIM muscle, with the sarcoplasm of small fibres often positive, most of which are CD56 positive regenerating or denervated muscle fibres (Fig. 2J–L). In IBM samples, subsets of fibres contain strong granular GDF-15 staining, which co-localizes with the autophagic markers p62/Sequestosome-1 (Fig. 2A–C) and LC3B (Fig. 2D–F). The vast majority of inflammatory cells are negative, with only occasional CD56 positive activated cytotoxic muscle fibre-invading cells staining positive (Fig. 2G–I), representing natural killer cells, cytotoxic T-cells, or M1 type macrophages [9]. Immunostaining patterns are similar for both antibodies used. Western blotting confirms GDF-15 expression in IIM muscle, with expression levels in control samples being below the detection limit (Fig. 2).

4. Discussion

Growth factors and cytokines are key factors in the pathogenesis of

the IIM [10] and in this light serum levels have been proposed as a means to monitor disease activity in DM. For GDF-15, it has been established that systemic levels vary in response to activity and stress, elevating after exercise [11] and in response to disease and organ injury [12]. Most notable increases have been observed in mitochondrial myopathy [5]. We now report for the first time a significant increase of GDF-15 levels in serum of IIM patients, with muscle representing a probable source. GDF-15 levels are increased in CD56 positive muscle fibres undergoing active regeneration or denervation, processes that are frequent in IIM muscle. We put forward circulating GDF-15 levels as a convenient potential biomarker to identify IIM patients. Although mitochondrial dysfunction is generally considered more pronounced in IBM compared to IMNM, GDF-15 serum levels were not significantly different between the two patient subgroups. Our observations would, however, need to be confirmed in larger patient cohorts. IIM are rare diseases, and existing international collaborations [13] can make it possible to further test the diagnostic potential of GDF-15 in the future. Also, our control group's average age was lower than that of IIM patients (33.7 versus 63.7 years of age), which could require checking in an aged-matched control group in the future to fully rule out an age effect.

Sub-classification of the different IIM groups remains highly relevant for guiding therapeutic decisions and for offering founded prognoses. In this respect, autoantibody profiling is a recent addition to the diagnostic arsenal which already proved to be of primordial importance [14]. In addition to its powers for disease subtyping, serotyping offers also important indicators to aid patient follow-up. The presence of anti-MDA5 autoantibodies for instance, associates with severe interstitial lung disease, while anti-NXP-2 and anti-TIF-1γ on the other hand associate with malignancy. Analysing serum parameters has therefore firmly gained its place in the diagnostic workup of IIM, with blood samples being a convenient, low-invasive and expanding source of diagnostic information. Accurate and early subtyping of IBM is also very important for prognosis and guiding therapy, as in most instances this disease subtype is irresponsive to immunosuppressive therapies. Histological markers of IBM are available today, and concern components of the cytoplasmic inclusions that are present in muscle fibers, namely TAR DNA-binding protein 43 (TDP-43) and p62/Sequestosome-1 [15]. It has been recommended to combine markers to reach both high sensitivity and specificity, and we now propose GDF-15 as an additional aggregate-associated factor with biomarker potential.

5. Conclusions

We propose GDF-15 testing as possible addition to the diagnostic workup of IIM, with potential to reduce the diagnostic delay and to advance clinical care for this rare yet heterogeneous group of muscle disorders.

Ethics approval and consent to participate

The study was approved by the Hospital's local Ethics Committee. Sampling adhered to ethical standards and privacy regulations, all patients consented to participate to the study of which procedures had been approved by the Ghent University Hospital Ethics Committee (B670201836756, B670201938779). Written informed consent was obtained from all patients.

Authors' contributions

BDP was responsible for study concept and design. JDB enrolled patients in the study and arranged funding. BDP and FV carried out the experiments and analysed the data. BDP carried out statistical analysis and was responsible for data interpretation. BDP and JDB prepared and revised the manuscript. All authors read and approved the final version of the manuscript.

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.

Acknowledgements

This work was supported through charity donations received by the fund for research into neuromuscular diseases UGent GE461, managed by Jan De Bleecker.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.154966.

References

[1] I.E. Lundberg, M. de Visser, V.P. Werth, Classification of myositis, Nat. Rev. Rheumatol. 14 (2018) 269–278, https://doi.org/10.1038/nrrheum.2018.41.

[2] A. Oldfors, A.R. Moslemi, L. Jonasson, M. Ohlsson, G. Kollberg, C. Lindberg, Mitochondrial abnormalities in inclusion-body myositis, Neurology 66 (2006) S49–S55, https://doi.org/10.1212/01.wnl.0000192127.63013.8d.

[3] F. Liu, J. Lou, D. Zhao, W. Li, Y. Zhao, X. Sun, C. Yan, Dysferlinopathy: mitochondrial abnormalities in human skeletal muscle, Int. J. Neurosci. 126 (2016) 499–509, https://doi.org/10.3109/00207454.2015.1034801.

[4] T. Siepmann, M. Tesch, F. Krause, B.M. Illigens, G. Stoltenburg-Didinger, Polymyositis with cytochrome C oxidase negative fibers-a pathological and clinical challenge, Ann. Diagn. Pathol. 17 (2013) 183–186, https://doi.org/10.1016/j.anndiagpath.2012.10.004.

[5] S.G. Kalko, S. Paco, C. Jou, M.A. Rodriguez, M. Meznaric, M. Rogac, et al., Transcriptomic profiling of TK2 deficient human skeletal muscle suggests a role for p53 signalling pathway and identifies growth and differentiation factor-15 as a potential novel biomarker for mitochondrial myopathies, BMC Genom. 15 (2014) e91, https://doi.org/10.1186/1471-2164-15-91.

[6] F.M. Verhamme, L.J.M. Seys, E.G. De Smet, S. Provoost, W. Janssens, D. Elewaut, G.F. Joos, G.G. Brusselle, K.R. Bracke, Elevated GDF-15 contributes to pulmonary inflammation upon cigarette smoke exposure, Mucosal Immunol. 10 (6) (2017) 1400–1411 http://www.nature.com/articles/mi20173https://doi.org/10.1038/mi.2017.3.

[7] H.K. Chung, D. Ryu, K.S. Kim, J.Y. Chang, Y.K. Kim, H.S. Yi, et al., Growth differentiation factor 15 is a myomitokine governing systemic energy homeostasis, J. Cell. Biol. 216 (2016) 149–165, https://doi.org/10.1083/jcb.201607110.

[8] V.W.W. Tsai, L. Macia, H. Johnen, T. Kuffner, R. Manadhar, S. Beck Jorgensen, TGF-b superfamily cytokine MIC-1/GDF15 is a physiological appetite and body weight regulator, PLoS One 8 (2013) e55174, https://doi.org/10.1371/journal.pone.0055174.

[9] H.H. Van Acker, A. Capsomidis, E.L. Smits, V.F. Van Tendeloo, CD56 in the immune system: more than a marker for cytotoxicity? Front. Immunol. 8 (2017) e892, https://doi.org/10.3389/fimmu.2017.00892.

[10] Lucie Andrés Cerezo, Hana Hulejová, Barbora Šumová, Tereza Kropáčková, Olga Kryštůfková, Martin Klein, Heřman F. Mann, Josef Zámečník, Ondřej Pecha, Karel Pavelka, Jiří Vencovský, Ladislav Šenolt, Pro-inflammatory S100A11 is elevated in inflammatory myopathies and reflects disease activity and extramuscular manifestations in myositis, Cytokine 116 (2019) 13–20 https://linkinghub.elsevier.com/retrieve/pii/S1043466619300110https://doi.org/10.1016/j.cyto.2018.12.023.

[11] M. Kleinert, C. Clemmensen, K.A. Sjoberg, C.S. Carl, J.F. Jeppesen, J.F.P. Wojtaszewski, et al., Exercise increases circulating GDF-15 in humans, Molec. Metab. 9 (2018) 187–191, https://doi.org/10.1016/j.molmet.2017.12.016.

[12] T.A. Zimmers, X. Jin, E.C. Hsiao, S.A. McGrath, A.F. Esquela, L.G. Koniaris, Growth differentiation factor-15/macrophage inhibitory cytokine-1 induction after kidney and lung injury, Shock 23 (2005) 543–548, https://doi.org/10.1016/j.jss.2004.07.200.

[13] J.B. Lilleker, J. Vencovsky, G. Wang, L.R. Wedderburn, L.P. Diederichsen, J. Schmidt, et al., The EuroMyositis registry: an international collaborative tool to facilitate myositis research, Ann. Rheum. Dis. 77 (2018) 30–39, https://doi.org/10.1136/annrheumdis-2017-211868.

[14] S.G. Gofrit, H. Yonath, M. Lidar, Y. Shoenfeld, S. Kivity, The clinical phenotype of patients positive for antibodies to myositis and myositis-related disorders, Clin. Rheumatol. 37 (2018) 1257–1263, https://doi.org/10.1007/s10067-018-4032-3.

[15] O. Dubourg, J. Wanschitz, T. Maisonobe, A. Behin, Y. Allenbach, S. Herson, O. Benveniste, Diagnostic value of markers of muscle degeneration in sporadic inclusion body myositis, Acta Myol. 30 (2011) 103–108.