High prevalence of sporadic late-onset nemaline myopathy in a cohort of whole-exome sequencing negative myopathy patients

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Willem De Ridder[1,2,3], Peter De Jonghe[1,2,3], Volker Straub[4], Jonathan Baets[1,2,3]

[1] Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

[2] The Laboratory of Neuromuscular Pathology, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

[3] The Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, Antwerp, Belgium

[4] The John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle-upon-Tyne, United Kingdom

Correspondence to Prof. Dr. Baets, Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, Drie Eikenstraat 655, B-2650 Edegem, Belgium. Telephone number: +32-3-821.34.23. Fax number: +32-3-821.43.12. Email address: jonathan.baets@uantwerpen.be.

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Appendix: Supplementary figures and tables. (3 supplementary figures and 2 supplementary tables)

willem.deridder@uantwerpen.be Antwerp, Belgium

peter.dejonghe@uantwerpen.be Antwerp, Belgium

volker.straub@newcastle.ac.uk Newcastle-upon-Tyne, United Kingdom

jonathan.baets@uantwerpen.be Antwerp, Belgium

Abstract

Sporadic late-onset nemaline myopathy (SLONM) is an enigmatic, supposedly very rare, putatively immune-mediated late-onset myopathy, typically presenting with subacutely progressive limb-girdle muscular weakness (LGMW), yet slowly progressing cases have been described too. We systematically studied (para)clinical and histopathological findings in a cohort of 18 isolated yet suspected inherited myopathy patients, showing late-onset, slowly progressive LGMW, remaining unsolved after whole-exome sequencing (WES).

The presence of a monoclonal gammopathy of unknown significance (MGUS) and anti-HMGCR antibodies was determined. Biopsies were systematically re-evaluated and systematic immunohistochemical and electron microscopy studies were performed to particularly evaluate the presence of rods and/or inflammatory features.

Ten patients showed rods as core feature on muscle biopsy on re-evaluation, four of these had an IgG κ MGUS in blood. As such, these ten patients represented suspected slowly progressing SLONM patients, with auxiliary data supporting this diagnosis: 1) additional muscle biopsy features pointing towards Z-disk and myofibrillar pathology; 2) a common selective pattern of muscle involvement on MRI; 3) inflammatory features on muscle biopsy.

Findings in this proof-of-concept study highlight difficulties in reliably diagnosing slowly progressing SLONM and the probably underestimated prevalence of this entity in cohorts of WES negative myopathy patients, initially considered having an inherited myopathy.

Key words: SLONM, limb-girdle muscular weakness, MRI.

1. Introduction

Muscle disorders, most typically presenting with progressive proximal muscle weakness, comprise a heterogeneous group of acquired and inherited diseases affecting skeletal muscle (1). With the exception of sporadic inclusion-body myositis (sIBM), only few acquired muscle disorders present with slowly progressive muscle weakness and as such, an inherited muscle disorder (IMD) is typically suspected in case of this clinical presentation. A few other atypically presenting acquired muscle disorders might however also constitute relevant differential diagnoses. Recent literature suggested that myopathies with anti-HMGCR antibodies may present with slowly progressive muscle weakness (2). The same has been shown for an enigmatic, supposedly very rare, putatively immune-mediated late-onset myopathy, called sporadic late-onset nemaline myopathy (SLONM) (3). Contrary to the highly recognizable SLONM cases presenting with subacutely progressive and severe muscle weakness, the cases on the less fulminant side of the apparent spectrum are difficult to detect in light of the rather non-specific features, nemaline rods on muscle biopsy, with or without a monoclonal gammopathy of unknown significance (MGUS) in blood (3).

In this study, we present a case series of (para)clinically and histopathologically systematically characterized sporadic myopathy patients showing late-onset, slowly progressive limb-girdle muscular weakness (LGMW) who remained unsolved after whole-exome sequencing (WES). Our findings in this proof-of-concept study highlight the difficulties in reliably diagnosing slowly progressing SLONM and the probably underestimated prevalence of this sporadic entity in WES-unsolved myopathy patient cohorts.

2. Subjects and methods

78 **2.1. Patient selection and (para)clinical evaluation**

79 We studied 18 suspected IMD patients from the Antwerp University Hospital (UZA),
80 included in the MYO-SEQ project, showing a (biopsy proven) myopathy with late adult onset
81 (> 40 years) LGMW with or without a high creatine kinase (CK) level, that remained
82 genetically unsolved after: 1) directed molecular genetic testing prior to inclusion in MYO-
83 SEQ to exclude a dystrophinopathy, facioscapulohumeral dystrophy (FSHD),
84 oculopharyngeal muscular dystrophy (OPMD) or myotonic dystrophy type 1 or 2 in case of
85 clinical suspicion; 2) WES data analysis as described previously (4). An overview of the
86 complete cohort is provided in Supplementary Table 1: 37 out of all 65 UZA cases (56.9%)
87 included in MYO-SEQ were genetically solved after WES. Of 43 patients without family
88 history however, 26 (60.5%) remained without a genetic diagnosis, 18 of these showed late
89 adult onset LGMW. For these patients, we systematically re-evaluated or completed all
90 (para)clinical, radiological, histopathological and lab features that could lead to the
91 alternative diagnosis of a previously unrecognized acquired myopathy. Muscle strength was
92 evaluated by manual muscle testing (MRC scale). The presence of an MGUS was evaluated
93 based on serum protein electrophoresis and immunofixation and a free light chain (FLC)
94 assay. Serum samples were screened for anti-HMGCR autoantibodies.
95
96 **2.2. Muscle biopsies**
97 Muscle biopsies of quadriceps, anterior tibial or deltoid muscles were obtained for all patients
98 and analysed following standard histological and immunohistochemical (IHC) light
99 microscopy and electron microscopy (EM) protocols. Biopsies were systematically re-
100 evaluated and systematic IHC studies were performed for: 1) myotilin, alpha-actinin and
101 desmin to evaluate the presence of rods and/or desmin aggregates; 2) MHC-1, MHC-II, CD8,
102 CD68 and C5b9 (MAC) to evaluate inflammatory muscle biopsy features.

2.3. Muscle MRI studies

Muscle MRI was performed on a 1.5T MRI platform at the UZA. Cross-sections at shoulder, abdominal, pelvic, thigh and calf levels were assessed on T1-weighted images to evaluate patterns of muscle involvement. Fatty replacement of muscle was graded according to the Mercuri scale (5). For multiple patients, longitudinal follow-up MRI studies were performed spanning multiple years of disease progression.

3. Results

After systematic documentation of all relevant (para)clinical, radiological, histopathological and lab features, the cohort of 18 patients showing late adult onset LGMW was divided in two subgroups based on the presence (Table 1) or absence (Supplementary Table 2) of rod-like aggregates on muscle biopsy (on Gomori, IHC for myotilin or α-actinin and/or on EM), as this is the core feature of SLONM. These rods can be easily overlooked on routinely performed Gomori stainings if not highly abundant and were best identified on IHC stainings for myotilin.

Ten patients showing rods on muscle biopsy (patient 1-10), all presented a rather non-specific pattern of muscle weakness at first sight, with predominantly proximal muscle weakness, more pronounced in lower than in upper limbs, though with marked weakness of gluteus maximus in six patients, marked paraspinal weakness in five and periscapular weakness in three. All patients showed slowly progressive weakness, for patient 1-7 and patient 10 leading to important walking difficulties and the need of ambulatory aids (Table 1). CK levels ranged from normal to moderately increased levels. Four of these patients showed an IgG κ MGUS, with polyclonal increased κ and λ chains in two and an increased κ/λ ratio of 2.41 in one patient. One patient (patient 3) showed a normal protein electrophoresis, yet had

markedly increased κ and λ chains on the FLC assay. Rods represented the key muscle biopsy feature, yet the following additional features were also frequently documented: 1) cytoplasmic bodies for five patients; 2) rimmed vacuoles for four patients; 3) the presence of numerous lobulated fibres for six patients; 4) hyaline inclusions on haematoxylin and eosin (H&E) stainings resembling spheroid bodies for three patients (representative images are shown in Figure 1A-F; additional illustrative muscle biopsy images are provided in Supplementary Figure 1). Patient 7 showed atypical inclusions on Gomori staining, which were partly immunoreactive to α-actinin, myotilin, desmin, nebulin and SERCA-2-ATPase (Supplementary Figure 1, H-K).

For two out of eight patients (patient 11-18) for whom no rods were detected on muscle biopsy, a specific lab or muscle biopsy feature oriented towards an alternative diagnosis (details see Supplementary Table 2): 1) patient 11 had positive anti-HMGCR antibodies, fitting the diagnosis of a HMGCR-related immune-mediated necrotizing myopathy with selective muscle fibre necrosis; 2) patient 12 showed striking tubular aggregates on muscle biopsy, of currently unknown aetiology. None of the 18 patients manifested significant signs or symptoms of bulbar, cardiac or respiratory involvement.

A set of IHC stainings was performed to evaluate the presence of inflammatory features and inflammatory cells on muscle biopsies of all patients, except for patient 2, 17 and 18 for whom the remaining amount of muscle material was insufficient. For five out of nine patients showing rods on muscle biopsy, diffuse or patchy sarcolemmal MHC-I upregulation was noted and for four patients inflammatory infiltrates, mainly consisting of CD68-positive macrophages. These inflammatory muscle biopsy features were not detected for any of the patients not showing rods on muscle biopsy.

On muscle MRI, these suspected SLONM patients showed a very similar pattern of selective muscle involvement, with early involvement of vastus intermedius, adductor magnus, biceps

femoris (caput longus) and soleus muscles (representative images see Figure 1G-L; an extensive overview of muscle MRI studies is provided in Supplementary Figure 2). Over years of disease progression, MRI studies mainly showed further selective involvement of posterior thigh muscles and lateral gastrocnemius muscles as well as progressive gluteal and paraspinal muscle involvement. Strikingly, for all patients sartorius and gracilis muscles remain selectively preserved throughout disease progression. Patient 8 however showed a slightly different MRI pattern with predominant anterior lower leg involvement. Patient 9 showed only mild, yet also selective involvement of adductor magnus, biceps femoris (caput longus) and soleus muscles, for patient 7 this similar pattern of involvement was markedly asymmetric and for patient 6 more patchy.

An overview of MRI images of patients showing no rods on muscle biopsy (patient 11-18) is provided in Supplementary Figure 3.

Table 1. Clinical and histopathological details of patients showing rods on muscle biopsy

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Gender	F	F	M	M	M	F	M	M	M	F
AAO, y	62	50	60	52	55	57	60	45	57	70
Rods on muscle biopsy	+	+	+	+	+	+	+	+	+	+
MGUS	IgG κ	-	-	IgG κ	-	IgG κ	-	-	-	IgG κ
Free κ/λ chains	κ chains increased, κ/λ ratio 2.41	Normal	κ and λ chains increased	Normal	Normal	κ and λ chains increased	Normal	Normal	Normal	NI
Age at last examination, y	76	75	72	68	70	70	79	47	64	77
Maximal motor capability / ambulatory aids	Bilateral support, 400 m	Bilateral support, short distances	Wheelchair outside	Bilateral support, 50 m	Bilateral support, 50 m	Wheelchair outside	Bilateral support, short distances	None, difficulties taking stairs	None	Bilateral support, short distances
UL / LL	LL	LL > UL	LL > UL	LL > UL	LL > UL	LL > UL	LL > UL	LL > UL	LL	LL > UL
Proximal / distal predominant	Proximal > distal	Proximal	Proximal	Proximal > distal; distal only in LL	Proximal > distal; distal only in LL	Proximal > distal; distal only in LL	Proximal	Proximal > distal; distal only in LL	Proximal	Proximal
Marked weakness M. gluteus maximus[a]	+	+	+	+	NI	+	+	Mild	-	NI
Marked paraspinal weakness[b]	+	+	+	-	NI	+	+	Mild	-	NI
Other		Periscapular					Periscapular	Periscapular		
CK (U/l)	250	232	405 - 1135	185	499	352	119	1080-2258	124	55
Cardiac investigations	Normal	Normal	Normal	Normal	NI	Ischemic heart disease	NI	Normal	Normal	Normal
anti-HMGCR antibodies	NI	Negative	Negative	Negative	Negative	Negative	Negative	Negative	NI	NI
Biopsied muscle	1. Anterior tibial; 2. Quadriceps	Deltoid	Quadriceps	Anterior tibial	Quadriceps	Quadriceps	Anterior tibial	Quadriceps	Quadriceps	Quadriceps

Table 1. Clinical and histopathological details of patients showing rods on muscle biopsy (continued)

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Age at muscle biopsy, y	1. 62; 2. 75	63	65	59	60	64	79	47	59	76
Cytoplasmic bodies	+	-	+	-	+	-	-	++	+	-
Rimmed vacuoles	+	+	+	-	-	-	-	+	-	-
Lobulated fibers (numerous)	+	-	+	+	+	-	+	-	+	-
Cores	+	-	-	-	-	-	-	-	+	-
Desmin IR areas in muscle fibers (on IHC)	+	+	-	-	-	-	+	-	-	-
Spheroid bodies	+	+	-	-	-	-	-	+	-	-
Necrosis	-	-	-	-	+	-	-	+	-	-
Fatty infiltration	Mild	Mild	-	+	+	-	-	-	-	-
Endomysial fibrosis	Mild	Mild	Mild	+	+	Mild	Mild	-	-	-
Fiber type predominance	Type 1	Type 1	-	Type 1	Type 1	Type 2	Type 1	Type 2	-	-
Sarcolemmal MHC-I upregulation	Diffusely +	-	-	Patchy +	Patchy +	-	Patchy +	-	-	Patchy +
Sarcolemmal MHC-II upregulation	-	NI	-	-	-	-	-	-	-	-
Inflammatory infiltrates (endomysial)	-	NI	Small infiltrates	+	+	-	-	+	-	-
CD68-positive cells	+	NI	+	+	+	-	+	+	-	-
CD8-positive cells	-	NI	-	-	-	-	-	-	-	-
MAC (C5b9)-labelling of non-necrotic muscle fibers	-	NI	-	-	-	-	-	-	-	-

F, female; M, male; AAO, age at onset; y, years; MGUS, monoclonal gammopathy of unknown significance; NI, not investigated; UL, upper limbs; LL, lower limbs; CK, creatinine kinase; IR, immunoreactive; IHC, immunohistochemistry; MHC, major histocompatibility complex; MAC, membrane attack complex

a MRC 3/5 or less.

b The xiphoid process cannot be lifted from the bed

4. Discussion

We report on a cohort of 18 WES unsolved(para)clinically and histopathologically systematically characterized myopathy patients who had no family history of muscle disease, presenting with late-onset, slowly progressive LGMW. Our findings in this cohort strongly suggested enrichment of isolated cases of LGMW with rods as core muscle biopsy feature (10 out of 18 patients), with four of these patients having an IgG κ MGUS and one markedly increased (polyclonal) κ and λ chains, which is also indicative of an inflammatory disorder (6). As such, ten patients represented likely (slowly progressing) SLONM cases, as based upon an exhaustive interpretation of the available diagnostic criteria. We capitalized on findings in this homogeneous cohort in search for additional features supporting this putative diagnosis.

In literature, SLONM is considered to constitute a clinical spectrum encompassing subacute SLONM patients, representing a highly recognizable clinical and histopathological entity, as well as patients presenting with slowly progressive LGMW (3). For the latter, diagnosis still relies on the two core features that are not necessarily completely specific: 1) nemaline rods are evidently typically observed in inherited nemaline rod myopathies, yet can be observed in other myopathies such as in advanced stage of muscular dystrophies (7, 8); (9)2) an MGUS is present in serum of approximately 3-5% of healthy subjects over 70 years (10). This means that slowly progressing SLONM is to an important extent a diagnosis of exclusion. Rigorous WES data analysis did not yield any candidate variant in a known myopathy gene, which implies that this cohort underwent systematic testing of all known myopathy genes, contrasting with most reported cases that were not investigated this thoroughly, at least genetically (3).

Unlike in HMGCR-myopathy, where the diagnosis can be made based on a single lab test (as in patient 11 of the current study), our current understanding of the enigmatic SLONM entity

197 does not allow us to easily diagnose earlier unidentified cases retrospectively. Clearly, there
198 is a pressing need for additional criteria supporting a probable diagnosis of slowly
199 progressing SLONM, which should be determined in patient cohorts, which are as
200 homogeneous as possible. Clinically, the pattern of muscle weakness appears to be rather
201 non-specific at first sight (3). All suspected SLONM patients in this cohort show LGMW,
202 though for most with marked gluteal and paraspinal muscle weakness. This pattern is
203 corroborated by muscle MRI studies showing a selective pattern, which strikingly resembles
204 a pattern described in a cohort of subacute SLONM cases (11). Myofibrillar disintegration
205 and rimmed vacuoles constitute frequently described additional histopathological findings,
206 features typically also observed in myofibrillar myopathies, for which pathology is primarily
207 located at the Z-disk; cores and lobulated fibres suggest myofibrillar disorganisation too (3,
208 12). On muscle biopsies of the suspected SLONM patients of this cohort, numerous
209 cytoplasmic bodies and hyaline inclusions containing abnormal myofibrillar material
210 resembling spheroid bodies or atypical caps (patient 7) are also frequently observed, which
211 might further suggest a histopathological spectrum of (slowly progressing) SLONM, relevant
212 with regard to the putatively immune-mediated pathomechanisms which appear to primarily
213 target sarcomeres (3).
214 This enigmatic entity is thought to represent an atypical immune-mediated myopathy. This is
215 mainly implied by the marked enrichment of the prevalence of a MGUS in SLONM cohorts
216 (+-53%), the fragmentary documentation of inflammatory features on muscle biopsy and the
217 favourable outcome after stem cell therapy in a few cases (3). Most of the suspected SLONM
218 cases show at least some inflammatory features on muscle biopsy, evident by sarcolemmal
219 MHC-I upregulation or the presence of small endomysial inflammatory infiltrates, which is
220 suggestive, though not a proof of immune-mediated pathomechanisms.
221

Based on the current study, we strongly advocate an active and prospective search for slowly progressing SLONM cases in large genetically well-studied patient cohorts, such as WES unsolved myopathy cohorts, in search for better biomarkers, pathomechanistic insights and therapeutic strategies. The studies in the current literature are likely biased towards patients showing the most conspicuous rods (due to referral bias), probably resulting in an underestimation of the histopathologic spectrum of this potentially treatable disease (3, 13). Particularly severely affected SLONM patients having an MGUS have been exposed to rather aggressive immune therapies such as autologous peripheral blood stem cell therapy, besides treatments with different immunosuppressants, intravenous immunoglobulines or plasmapheresis, with varying success (3, 13) and clearly more systematic prospective studies are necessary to ascertain what the role is of such treatments in SLONM.

Based on this proof-of-concept study, we propose that a probable SLONM diagnosis is initially based on the identification of rods on muscle biopsy of a sporadic late onset myopathy patient, with or without identification of an MGUS but could be substantiated by supportive criteria based on: 1) negative results of WES analysis; 2) muscle MRI imaging (showing a suggestive pattern); 3) findings of an MGUS on protein electrophoresis or increased κ and λ chains on a FLC assay; 4) serological exclusion of HMGCR-myopathy; 5) additional biopsy features such as cytoplasmic bodies and hyaline inclusions; 6) presence of endomysial inflammatory infiltrates and/or sarcolemmal MHC-I upregulation.

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7. Conflicts of interests

The authors report no financial conflicts of interests.

8. References

[1] Jackson CE. A clinical approach to muscle diseases. Seminars in neurology. 2008;28(2):228-40.

[2] Mohassel P, Landon-Cardinal O, Foley AR, Donkervoort S, Pak KS, Wahl C, et al. Anti-HMGCR myopathy may resemble limb-girdle muscular dystrophy. Neurology(R) neuroimmunology & neuroinflammation. 2019;6(1):e523.

[3] Schnitzler LJ, Schreckenbach T, Nadaj-Pakleza A, Stenzel W, Rushing EJ, Van Damme P, et al. Sporadic late-onset nemaline myopathy: clinico-pathological characteristics and review of 76 cases. Orphanet journal of rare diseases. 2017;12(1):86.

[4] Peric S, Glumac JN, Topf A, Savic-Pavicevic D, Phillips L, Johnson K, et al. A novel recessive TTN founder variant is a common cause of distal myopathy in the Serbian population. European journal of human genetics : EJHG. 2017;25(5):572-81.

[5] Mercuri E, Pichiecchio A, Allsop J, Messina S, Pane M, Muntoni F. Muscle MRI in inherited neuromuscular disorders: past, present, and future. Journal of magnetic resonance imaging : JMRI. 2007;25(2):433-40.

[6] Brebner JA, Stockley RA. Polyclonal free light chains: a biomarker of inflammatory disease or treatment target? F1000 medicine reports. 2013;5:4.

[7] Uruha A, Benveniste O. Sporadic late-onset nemaline myopathy with monoclonal gammopathy of undetermined significance. Current opinion in neurology. 2017;30(5):457-63.

[8] Jungbluth H, Treves S, Zorzato F, Sarkozy A, Ochala J, Sewry C, et al. Congenital myopathies: disorders of excitation-contraction coupling and muscle contraction. Nature reviews Neurology. 2018;14(3):151-67.

[9] Chahin N, Selcen D, Engel AG. Sporadic late onset nemaline myopathy. Neurology. 2005;65(8):1158-64.

[10] Mouhieddine TH, Weeks LD, Ghobrial IM. Monoclonal gammopathy of undetermined significance. Blood. 2019;133(23):2484-94.

[11] Monforte M, Primiano G, Silvestri G, Mirabella M, Luigetti M, Cuccagna C, et al. Sporadic late-onset nemaline myopathy: clinical, pathology and imaging findings in a single center cohort. Journal of neurology. 2018;265(3):542-51.

[12] Selcen D, Engel AG. Myofibrillar myopathies. Handbook of clinical neurology. 2011;101:143-54.

[13] Naddaf E, Milone M, Kansagra A, Buadi F, Kourelis T. Sporadic late-onset nemaline myopathy: Clinical spectrum, survival, and treatment outcomes. Neurology. 2019;93(3):e298-e305.

9. Figure legend

Figure 1. Representative images of muscle histopathology and muscle MRI studies in ten patients suspect of SLONM.

(A-F) Representative muscle histopathology images. (A) Myotilin positive rods in a muscle fiber on biopsy of patient 5. (B) Multiple atrophic fibers containing myotilin positive rods on muscle biopsy of patient 4. (C) Atrophic fiber showing nemaline rods (arrow) on electron microscopy on muscle biopsy of patient 6. (D) Cytoplasmic bodies on gomori trichrome staining for patient 3. (E) Spheroid bodies (arrow) on H&E staining and (F) sarcolemmal upregulation of MHC-I on muscle biopsy of patient 1. (G-L) Selection of muscle MR images at thigh and calf level, shown for: (G-H) patient 1, at age 64 years (G) and 75 years (H); (I) patient 3 at age 65 years; (J-K) patient 2, at age 63 years and 76 years; (L) patient 5, at 76 years. SLONM, sporadic late-onset nemaline myopathy