Specific muscle biopsy findings in necrotizing autoimmune myopathy

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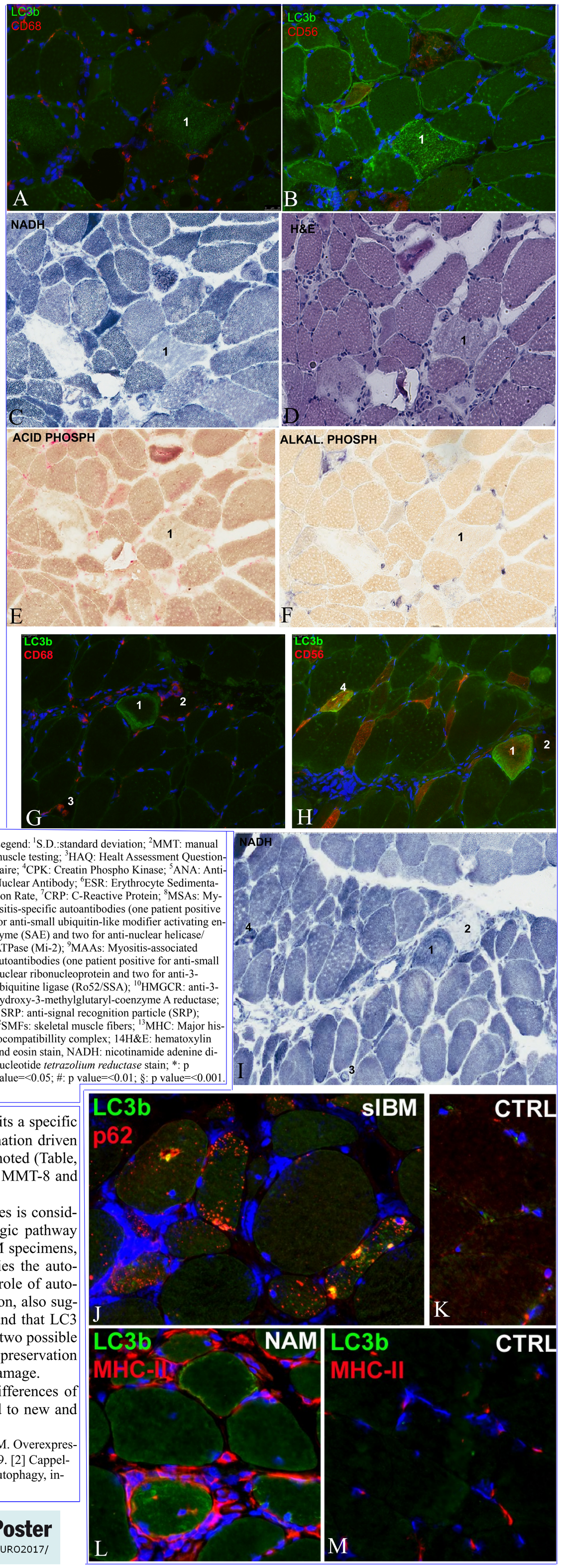
INTRODUCTION. Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of autoimmune muscular diseases that includes dermatomyositis (DM), polymyositis (PM), sporadic inclusion body myositis (sIBM), necrotizing autoimmune myopathy (NAM). Due to the rarity of these diseases, the aim of the study is: (a) to present the demographic, clinical, laboratory and therapeutic profiles of NAM patients compared to the other IIMs (Table 1), (b) to better understand the pathogenetic mechanisms exploring autophagic process and its relation with the immune system.

METHODS: 132 patients with banked sera, muscle biopsy specimens available for review, and a myopathy characterized by proximal muscle weakness, elevated CK levels, myopathic EMG findings, and/or myopathic features on muscle biopsy were enrolled in a longitudinal study from January 2015 through June 2017. The study has been carried out on 6 NAM, 4 DM, 5 PM, 3 sIBM patients and 6 age-matched controls, 3 of whom underwent orthopedic operations and other 3 were shown to be morphologically normal on routine staining after having performed all the other laboratory tests. Myopatologic findings and quantitative analyses on immunolabelled sections with membrane attack complex C5b-9, MHC I, MHC II, p62, SMI31, ubiquitin, autophagy marker LC3b, macrophage marker CD68, and CD56, reliable marker of regenerating skeletal muscle fibers (SMFs), have been correlated with clinical, laboratory, and therapeutic parameters.

Table: Clinical and laboratory features of patients.

	Necrotizing autoimmune myopathy	Adult dermatomyositis	Polimyositis	Sporadic inclusion body myositis	Age-matched controls
Patient number	6	4	5	3	6
Demographic information					
Average age at onset of disease (years±[1]S.D.)	62.17±13.27	64.25±10.9	59±11.02	51.3±13.6	47.16±20.28
Sex: n. of female (%)	5 (83.3)	3 (75)	4 (80)	2 (66.7)	3 (50)
Race, % white	100	100	100	100	100
Diagnostic delay (months)	4.33±4.13	5±2.94	51.2±73.95	6.7±5.5	ND
Follow up (months)	13.8±10.8	10.8±7.2	40.2±9.4	22.7±22.7	ND
Deceased patients (%)	1 (16.7)	0	0	0	0
Clinical features					
% of proximal muscle weakness on exam	100	100	100	100	0
Rash (%)	3 (50)*	4 (100)#,§	0	0	0
Mechanic's hands sign (%)	0	0	0	0	0
Episodic fever (%)	4 (66.7)	1 (25)	0	2 (66.7)	ND
Gottron's sign (%)	3 (50)*	100#,§	0	0	ND
Myalgias at onset (%)	5 (83.3)	3 (75)	3 (60)	2 (66.7)	ND
Arthralgias (%)	1 (16.7)	0	0	0	ND
Dysphagia (%)	3 (50)	100	4 (80)	1 (33.3)	ND
Raynaud's phenomenon (%)	3 (50)	0	2 (40)	1 (33.3)	ND
Concomitant malignancy (%)	1 (16.7)	1 (25)	20	0	0
Thyroiditis(%)	0	2 (50)	2 (40)	2 (66.7)	0
Interstitial lung disease (%)	0	2 (50)	1 (20)	1 (33.3)	0
Hepatopathy (all causes) (%)	2 (33.3)	1 (25)	1 (20)	2 (66.7)	14.3
Mean onset [2]MMT total score	3.53±0.43	3.37±0.59	4.02±0.92	4.16±0.57	ND
Mean onset MMT-8 score	67.5±9.7	64±2.16	69±6.2	64.5±19.1	ND
Mean onset [3]HAQ score	2.5±0.65	2.49±0.17	1.47±1.14	1.45±1.33	ND
Therapeutic information					
Steroid users at biopsy time (%)	5 (83.3)	3 (75)	1 (20)	2 (66.7)	0
Steroid users at last follow up(%)	1 (16.7)	100	4 (80)	2 (66.7)	0
Average number of immunosuppressive drug at last follow up	1.2±0.75	1.5±0.57	0.4±0.54	0.67±0.57	0
Average number of immunosuppressive therapeutic switches per patient	0.83±0.4	1±0.81	1±1	0.67±1.15	ND
Immunoglobulin treated patients (%)	1 (16.7)	2 (50)	4 (80)	1 (33.3)	0
Rituximab treated patients (%)	2 (33.3)	0	0	1 (33.3)	0
Laboratory findings					
Onset [4]CPK, mean UI/L	4232±1535#,§	1498±1332	1087±935	373±527	135±78
Post-treatment CPK, mean UI/L	131±79	133±72	131±39	148±102	ND
Number of patients positive for [5]ANA (>1:160)	83±40.8	100	40±54.7	33.3±57.7	ND
Onset [6]ESR, mean mm/h	25.3±18.4	34.2±11.9	24.4±18.1	48.7±40.7	ND
Onset [7]CRP, mean mg/dL	4.98±3.1	10.35±5.45	3.84±3.6	11.6±8.9	ND
Number of patients positive for [8]MSAs (%)	3 (50)	1 (25)	0	1 (33.3)	ND
Number of patients positive for anti-[9]MAAs(%)	0	1 (25)	0	1 (33.3)	ND
Number of patients positive for anti-[10]HMGCR (%)	1 (16.7)	0	0	0	ND
Number of patients positive for anti-[11]SRP (%)	2 (33.3)	0	0	0	ND
Biopsy finding					
‰ of C5b-9$^+$ necrotic [12]SMFs	26.6±12.2§	0.9±1.8	2.1±2.7	ND	0
‰ of [13]MHC-I$^+$ SMFs	3.6±2.5	2.9±2.8	2.7±1.4	ND	0
‰ of MHC-II$^+$ SMFs	18.6±4.5	0.7±1.9	0.5±2.1	20.4±6.8	0
‰ of p62$^+$ SMFs	2.8±2.9	0.6±1.3	0	21.8±3.8#	0
‰ of SMI31$^+$ SMFs	5.2±2.7	0	1±2.2	41.6±3.7§	0
‰ of ubiquitin$^+$ SMFs	19.5±10*,#	0.5±1.1	8.5±12.1	ND	0
‰ of autophagy marker LC3b$^+$ SMFs	60.1±28.6§	0.1±0.03	7.1±11.1	51.1±23.98#	0
‰ of LC3b$^+$/CD56$^+$ regenerating SMFs (basophilic [14]H&E+dark NADH+dark alkaline phosphatase)	42.6±12.2§	3.8±1.3	5.2±2.1	ND	0
‰ of LC3b$^+$ non necrotic SMFs (light EH+light NADH+dark acid phosphatase) surrounded by CD68$^+$ macrophages	10.27±1.21§	0.98±0.86	1.09±0.65	ND	0



Legend: [1]S.D.:standard deviation; [2]MMT: manual muscle testing; [3]HAQ: Healt Assessment Questionnaire; [4]CPK: Creatin Phospho Kinase; [5]ANA: Anti-Nuclear Antibody; [6]ESR: Erythrocyte Sedimentation Rate, [7]CRP: C-Reactive Protein; [8]MSAs: Myositis-specific autoantibodies (one patient positive for anti-small ubiquitin-like modifier activating enzyme (SAE) and two for anti-nuclear helicase/ ATPase (Mi-2); [9]MAAs: Myositis-associated autoantibodies (one patient positive for anti-small nuclear ribonucleoprotein and two for anti-3-ubiquitine ligase (Ro52/SSA); [10]HMGCR: anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase; [11]SRP: anti-signal recognition particle (SRP); [12]SMFs: skeletal muscle fibers; [13]MHC: Major histocompatibillity complex; 14H&E: hematoxylin and eosin stain, NADH: nicotinamide adenine dinucleotide *tetrazolium reductase* stain; *: p value=<0.05; #: p value=<0.01; §: p value=<0.001.

RESULTS. We confirm the typical different features of IIMs expanding the phenotype of NAM that exhibits a specific histological pattern, characterized by significant necrosis, simultaneous SMF regeneration, but little inflammation driven by macrophages. Prominent activation of ubiquitin system together with enhanced expression of LC3b were noted (Table, Fig. A, B, G,H, L). Number of LC3b-positive non-necrotic myofibers correlated with motor performance at MMT-8 and plasmatic creatin kinase levels of NAM patients at diagnosis stage (Pearson's r=0.98; p<0.05).

DISCUSSION. Impaired autophagosome maturation with consequent accumulation of multiprotein aggregates is considered a key factor of myofiber degeneration and muscle weakness characteristic of sIBM [1]. The autophagic pathway seems significantly increased also in NAM as it could be predictive of greater risk of severe disease. In sIBM specimens, autophagy-activated SMFs are associated with lymphocyte immune activation [1], whereas in NAM biopsies the autophagy-activated SMFs are preferentially surrounded by CD68+ macrophages (Table; Fig.A,G) suggesting a role of autophagy in maintaining an inflamed microenvironment possibly due to autophagy-dependent antigen presentation, also suggested by the enhanced expression of MHC-II on sarcolemma (Table, Fig. L). In NAM, we additionally found that LC3 abundantly localized in sarcoplasma of the numerous CD56+ regenerating SMFs (Table, Fig.B, H) suggesting two possible alternative explanations of the enhanced autophagy flux: (a) protein recycling during SMF differentiation for preservation of muscle mass and SMF integrity or (b) perpetuating SMF immune activation that continue to drive muscle damage.

CONCLUSION. Autophagy is involved in the pathogenesis of all IIMs [2]. Better understanding of the differences of autophagic activation and interaction with the immune system in each type of IIMs may be expected to lead to new and distinct therapies for the different IIM types.

REFERENCES. [1] Girolamo F, Lia A, Amati A, Strippoli M, Coppola C, Virgintino D, Roncali L, Toscano A, Serlenga L, Trojano M. Overexpression of autophagic proteins in the skeletal muscle of sporadic inclusion body myositis. (2013) Neuropath Appl Neurobiol. 39:736-749. [2] Cappelletti C, Galbardi B, Kapetis D, Vattemi G, Guglielmi V, Tonin P, Salerno F, Morandi L, Tomelleri G, Mantegazza R, Bernasconi P. Autophagy, inflammation and innate immunity in inflammatory myopathies. (2014) PLoS One. 9(11):e111490

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