

# SIGNAL RECOGNITION PARTICLE IMMUNOGLOBULIN G DETECTED INCIDENTALLY ASSOCIATES WITH AUTOIMMUNE MYOPATHY

METHA APIWATTANAKUL, MD,<sup>1</sup> MARGHERITA MILONE, MD, PhD,<sup>2</sup> SEAN J. PITTOCK, MD,<sup>1,2</sup> THOMAS J. KRYZER, AS,<sup>1</sup> JAMES P. FRYER, MS,<sup>1</sup> ORNA O'TOOLE, MD,<sup>2</sup> ANDREW McKEON, MD,<sup>1,2</sup> and VANDA A. LENNON, MD, PhD<sup>1,2,3</sup>

<sup>1</sup>Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA

<sup>2</sup>Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

<sup>3</sup>Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

Accepted 9 November 2015

**ABSTRACT:** *Introduction:* Paraneoplastic autoantibody screening of 150,000 patient sera by tissue-based immunofluorescence incidentally revealed 170 with unsuspected signal recognition particle (SRP) immunoglobulin G (IgG), which is a recognized biomarker of autoimmune myopathy. Of the 77 patients with available information, 54 had myopathy. We describe the clinical/laboratory associations. *Methods:* Distinctive cytoplasm-binding IgG (mouse tissue substrate) prompted western blot, enzyme-linked immunoassay, and immunoprecipitation analyses. Available histories were reviewed. *Results:* The immunostaining pattern resembled rough endoplasmic reticulum, and mimicked Purkinje-cell cytoplasmic antibody type 1 IgG/anti-Yo. Immunoblotting revealed ribonucleoprotein reactivity. Recombinant antigens confirmed the following: SRP54 IgG specificity alone (17); SRP72 IgG specificity alone (3); both (32); or neither (2). Coexisting neural autoantibodies were identified in 28% (low titer). Electromyography revealed myopathy with fibrillation potentials; 78% of biopsies had active necrotizing myopathy with minimal inflammation, and 17% had inflammatory myopathy. Immunotherapy responsiveness was typically slow and incomplete, and relapses were frequent on withdrawal. Histologically confirmed cancers (17%) were primarily breast and hematologic, with some others. *Conclusions:* Autoimmune necrotizing SRP myopathy, both idiopathic and paraneoplastic, is underdiagnosed in neurological practice. Serological screening aids early diagnosis. Cancer surveillance and appropriate immunosuppressant therapy may improve outcome.

*Muscle Nerve* 53: 925–932, 2016

Many patients identified with autoimmune myopathy in rheumatologic practice are recognized to have “myositis-specific autoantibodies.”<sup>1</sup> The most common, Jo-1 immunoglobulin G [Jo-1 IgG, an

aminoacyl (histidyl)-tRNA synthetase autoantibody], is reported in 25%–30% of patients with dermatomyositis. IgGs specific for signal recognition particle (SRP) components (54-kDa, 72-kDa, and 7-SL RNA) have been reported with inflammatory and non-inflammatory autoimmune myopathies.<sup>2–6</sup> An ill-defined cancer association is recognized for autoimmune myopathies, particularly dermatomyositis.<sup>7</sup> Here we report fortuitous detection of unsuspected SRP-specific immunoglobulin G (IgG) in a neurological patient population evaluated serologically for paraneoplastic autoantibodies by a standardized tissue-based immunofluorescence screening assay.

## METHODS

The study was approved by the institutional review board at the Mayo Clinic. A distinctive SRP-like IgG-binding pattern was noted in 170 patients evaluated serologically for possible paraneoplastic neurological autoimmunity by routine service immunofluorescence in the Mayo Clinic Neuroimmunology Laboratory. In all cases, testing had been requested by a consulting neurologist, generally not a neuromuscular subspecialist. Of the 170 patients, clinical information was available for 77 (27 evaluated at the Mayo Clinic; limited information was obtained for 50 of 143 non-Mayo Clinic patients through consultation with the evaluating physicians). Fifty-four patients (70%; 20 of whom were seen at the Mayo Clinic) had predominant subacute myopathy (progressive proximal weakness, elevated creatine kinase, and electromyographic evidence of short-duration, low-amplitude motor unit potentials with early recruitment and fibrillation potentials). The remaining 23 patients (7 Mayo Clinic) presented with other neurological manifestations and lacked myopathy. They will be the subject of a subsequent report.

**Immunofluorescence Screening.** A composite substrate of cryosectioned mouse cerebellum/mid-brain/hippocampus, gut, and kidney tissues (4 μm thick) was prefixed for 10 minutes in 10% formalin. Sera were applied to the substrate after absorption at 1:240 dilution with bovine liver powder in phosphate-buffered saline (PBS) containing 1%

**Abbreviations:** ANA, antinuclear antibodies; BSA, bovine serum albumin; CK, creatine kinase; cDNA, complementary DNA; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GVHD, graft-versus-host disease; HEK, human embryonic kidney; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HRP, horseradish peroxidase; IBM, inclusion-body myositis; IgG, immunoglobulin G; IVIg, intravenous immunoglobulin G; PBS, phosphate-buffered saline; PCA-1, Purkinje cell cytoplasmic antibody type 1 (anti-“Yo”); RNP, ribonucleoprotein; SDS, sodium dodecylsulfate; Sm, Smith; SRP, signal recognition particle

**Key words:** autoantibody; necrotizing myopathy; paraneoplastic; serological marker

Current address: M. Apiwattanakul, Prasat Neurological Institute, Bangkok, Thailand

Current address: O. O'Toole, Mercy University Hospital, Cork, Ireland

**Correspondence to:** V.A. Lennon; e-mail: lennon.vanda@mayo.edu

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bovine serum albumin (BSA). After 40 minutes, we detected bound IgG in washed sections by applying fluorescein-isothiocyanate (FITC)-conjugated goat IgG reactive with all human IgG subclasses (Southern Biotechnology, Birmingham, Alabama).

**Western Blot.** A crude rough microsomal preparation was isolated from mouse pancreas by the method of Walter and Blobel.<sup>8</sup> This preparation, or recombinant SRP54 protein, was denatured by boiling 5 minutes in sample buffer containing 2% sodium dodecylsulfate (SDS) and 10% 2-mercaptoethanol. Broad range markers were molecular weight standards (Precision Plus Protein Standards Dual Color; BioRad Laboratories, Hercules, California). After electrophoretic separation in 10% polyacrylamide, the proteins were transferred electrophoretically to nitrocellulose [verified by staining with 0.1% Ponceau S (Sigma Co., St. Louis, Missouri)]. Residual nitrocellulose binding sites were blocked with 25 mM Trizma base, 192 mM glycine, and 0.02% sodium azide (pH 8.3), containing 10% fat-free milk powder. Patient sera, diluted 1:400 with blocking buffer, were applied to the transblotted proteins. After incubating 1 hour at room temperature and washing, bound IgG was detected using horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Life Technologies, Grand Island, New York). An enhanced chemiluminescence substrate was used to detect HRP (SuperSignal West Pico Chemiluminescence Substrate, Product #34080; Thermo Scientific, Waltham, Massachusetts).

**Patient Antibody Purification.** Mouse pancreatic lysate proteins were separated by electrophoresis and transblotted to nitrocellulose. Bound antigenic protein was located by Western blot staining of excised vertical edge strips. Horizontal strips bearing the antigens of interest and a control horizontal strip lacking the antigen of interest were exposed to patient sera (1:400 dilution, 2 hours). After washing the strips to remove non-specifically bound antibody [6 times in 50 mM Tris-HCl (pH 7.6), containing 300 mM NaCl], bound IgG was eluted in 0.1 M acetic acid, neutralized with 2 M Tris, dialyzed 16 hours against PBS with sodium azide 0.02%, and concentrated by Amicon Ultra tube centrifugation (final volume < 100  $\mu$ l). This IgG was applied to the composite mouse tissue substrate to evaluate its immunofluorescence staining pattern.

**Complementary DNA Cloning.** RNA from human pancreas was reverse-transcribed to provide first-strand complementary DNA (cDNA). Gene-specific primer (SRP72 R3; 5'-CCATATCTCACTAGGCAGAC-3') and Superscript III RT (Invitrogen) were used. A gene-specific primer pair (SRP72 F1; 5'-ATGGCG AGCGGCGGCAGCGG-3', SRP72 R3; 5'-CCATATCTCACTAGGCAGAC-3') was used to amplify the

cDNA. We gel purified a 2-kb product (Wizard SV Gel and PCR Clean-Up System; Promega, Madison, Wisconsin), ligated the product into pCR-Blunt II-TOPO vector (Invitrogen) and transformed TOP10 competent cells. Individual clones were selected, and the plasmid DNA was sequenced using T7 and SP6 primers. The single clone identified by restriction mapping (HindIII) and DNA sequencing was identical to human SRP72, with the exception of 1 frameshift mutation and a silent mutation at codon 7 (GGG→GGT). The frameshift mutation in the plasmid DNA was corrected using a QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, California). Plasmid DNA was used to transform XL-10 cells (Gold Ultracompetent; Stratagene). Four clones were identified by HindIII restriction mapping. DNA sequencing demonstrated correction of the frameshift mutation. One clone was selected for further propagation, and the purified plasmid DNA (Plasmid Mini Kit; Qiagen, Valencia, California) was used as template for PCR amplification to insert directional restriction sites (XhoI and SacII) using primers GFP/SRP F2 (5'-CTCGAGAGATGACGATA AGGTACAGGCCC-3') and GFP/SRP R1 (5'-CCGCG GTTCTCATCACCAGCCACC TT-3'). The PCR product was gel purified, ligated into pCR-Blunt II-TOPO vector (Invitrogen), and TOP10 competent cells were transformed. Individual clones were selected, plasmid DNA was isolated, and the sequence was verified. The SRP72 cDNA insert and pEGFP-C2 vector (XhoI-SacII digested) were gel purified before SRP72 insert ligation into the pEGFP-C2 vector. The ligated construct was used to transform TOP10-competent cells. Individual clone plasmid DNA was analyzed by restriction mapping, and 3 clones were sequence verified. One clone was selected for large-scale plasmid DNA preparation to transfect mammalian cells.

**Recombinant Proteins.** Recombinant human SRP54 (hexahistidine tagged) was purchased from Diarect AG (Freiburg, Germany). Recombinant human SRP72 [green fluorescent protein (GFP)-tagged] was made in-house by transfecting human embryonic kidney (HEK)293 cells (FuGENE 6 Transfection Reagent; Roche, Indianapolis, Indiana) with plasmid DNA encoding SRP72 (obtained using the Qiafilter Maxi Kit, Promega).

**SRP-Subunit-Specific Antibody Assays.** SRP54-IgG reactivity was tested by enzyme-linked immunoassay (ELISA). Sera, diluted in doubling steps from 1:60 in PBS containing 10% normal goat serum, were applied to Immulon II plates coated with recombinant SRP54 protein (0.5  $\mu$ g/ml). After holding for 2 hours at 37°C, the wells were washed 3 times with PBS containing sodium azide (0.02%) and goat serum (2%), then alkaline phosphatase-

conjugated goat IgG specific for human IgG (Rockland Immunochemicals, Inc., Gilbertsville, Pennsylvania) was added. After 1 hour at 37°C, wells were washed, substrate was added (*p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine buffer, pH 9.8), and the plates were held for 1 hour at 37°C. The reaction product was measured photometrically (wavelength 405 nm; ELx800; Bio-Tek Instruments, Inc.). Values exceeding 150% of the mean optical density yielded by corresponding dilutions of 4 healthy control human sera were considered positive. Positive sera were retested and titrated further as necessary to determine the endpoint dilution. Results were expressed as titer (i.e., reciprocal of the final positive dilution).

SRP72 IgG reactivity was tested by immunoprecipitation assay. Patient sera (30 µl) were added to 100 µl of sonicated transfected cell lysate containing 15,000 GFP counts (in 2% Triton X-100, 10 mM Tris, 1 mM ethylene-diamine tetraacetic acid, 700 mM NaCl, 0.02% sodium azide; clarified by ultracentrifugation). After shaking at 4°C overnight, immune complexes were captured by adding 30 µl of recombinant Protein G–Sepharose 4B (Novex/Invitrogen, Grand Island, New York), pelleted centrifugally after 2 hours (2,000 × *g*; 2 minutes, 4°C), washed 3 times with 1 ml of lysis buffer, and resuspended in 200 µl of lysis buffer. Precipitated GFP SRP72 in 100-µl aliquot was measured spectrophotometrically in a black flat-bottom plate (Tecan GENios Pro). Results were expressed in nanomoles per liter by reference to a GFP standard (Promega).

**Other Autoantibody Tests.** All patient sera were tested for neural-specific IgG autoantibodies according to comprehensive evaluation standardized in the Mayo Clinic Neuroimmunology Laboratory: mouse tissue–based immunofluorescence to screen for neuronal and glial nuclear, cytoplasmic, and synaptic antibodies; radioimmunoprecipitation for cation channel antibodies and glutamic acid decarboxylase 65 (GAD65) antibody; and ELISA for skeletal muscle striational antibodies.<sup>9</sup> Service testing for thyroid and antinuclear antibodies was performed on all Mayo Clinic patient sera in the Mayo Clinic Immunology Laboratory.

**Statistical Analysis.** Either Student *t*-test, Fisher exact test, Wilcoxon rank sum test, or Mann–Whitney *U*-test was employed as appropriate.

## RESULTS

**Immunohistochemical Characteristics.** Between July 1, 2007 and June 30, 2012, 150,000 patient sera were tested for paraneoplastic IgG autoantibodies on a service basis using a standardized composite tissue-based immunofluorescence screening assay.

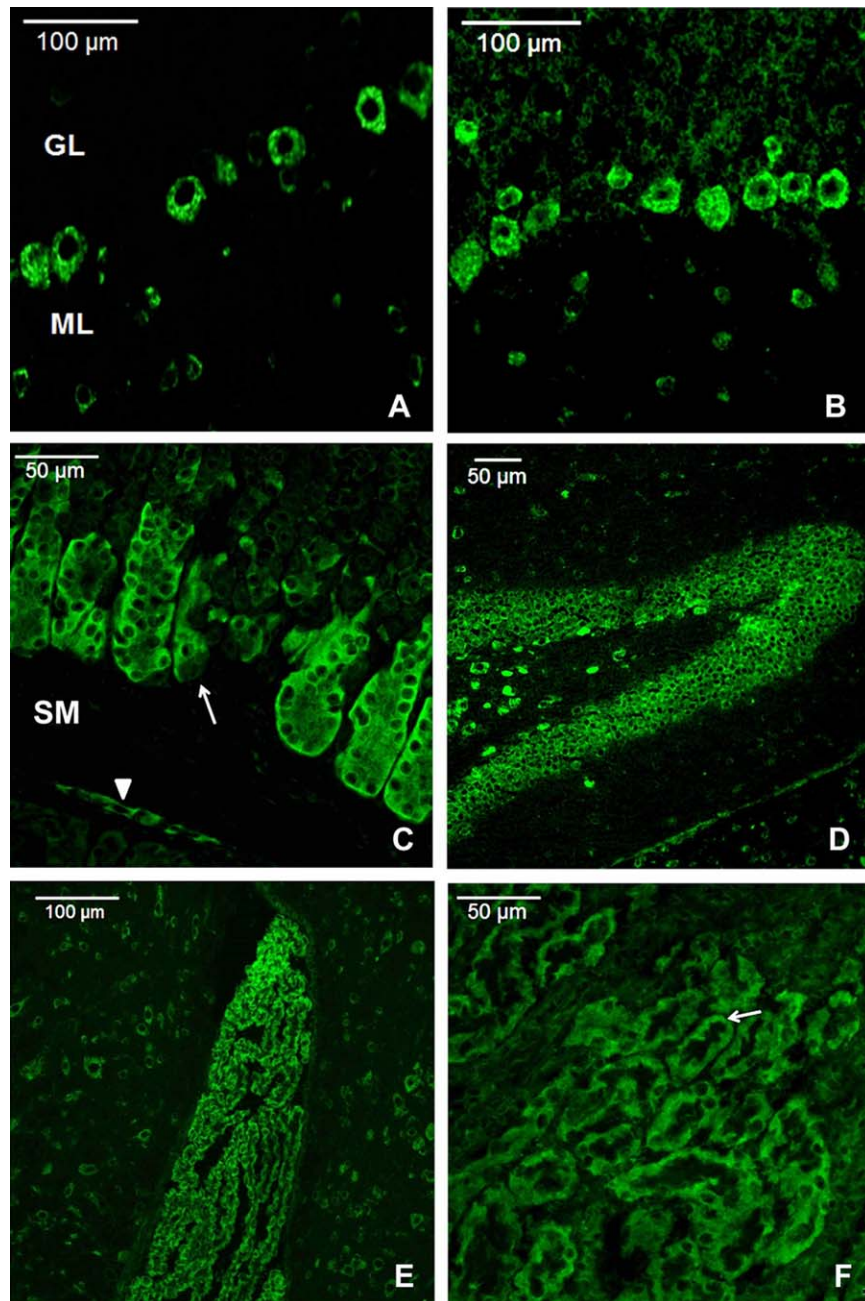
In 170 sera we noted an autoantibody pattern not previously described and not yielded by thousands of healthy and disease control sera. Although not organ-specific, this IgG bound prominently to cytoplasm of cerebellar cortical neurons (Purkinje, molecular, and Golgi) and resembled PCA-1 IgG (anti-Yo; Fig. 1A),<sup>10</sup> except for also binding to cytoplasm of cerebellar granular neurons (“chicken-wire pattern”; Fig. 1B). Like PCA-1 IgG, the novel IgG bound to all large neurons, including myenteric ganglionic neurons (Fig. 1C, arrowhead); it also bound distinctively to hippocampal neurons (Fig. 1D). This IgG bound prominently to certain non-neural elements, including cytoplasm of proximal gastric mucosal epithelial cells (Fig. 1C, arrow), choroid epithelium (Fig. 1E), and renal tubules (cortical more than medullary; Fig. 1F). The resemblance of the cytoplasmic staining in large neurons of the cerebellar cortex to PCA-1 immunoreactivity suggested the autoantigen may reside in endoplasmic reticulum, as this laboratory demonstrated with immunoelectron microscopy for the PCA-1 antigen.<sup>10</sup> Sera from the 54 patients presenting with myopathy were further analyzed.

**Western Blot Characteristics.** Detailed testing of the first 25 sera revealed that IgG in 12 bound to native microsomal proteins of 54-kDa (6) or 72-kDa (10) molecular size, consistent with SRP54 and SRP72 subunits; 4 sera were dual-reactive (Fig. 2A shows 2 representative sera). IgG eluted from native Western blot bands corresponding to 54- and 72-kDa proteins (but not IgG eluted from a control protein bands) yielded the same immunofluorescence pattern as the original serum antibody when applied to mouse tissue sections (data not shown).

**Recombinant SRP54 and SRP72 Binding Data.** Reactivity with recombinant SRP54 protein was confirmed with both Western blot (Fig. 2B) and ELISA. All 54 sera were tested by ELISA, and 49 were positive for SRP54 IgG (91%). Only 44 sera were available for SRP72 specificity testing by immunoprecipitation, and 35 were positive (79%). Only 3.7% lacked evidence of either SRP54 IgG or SRP72 IgG.

**Clinical and Demographic Information.** Table 1 summarizes demographic, serological, clinical, and laboratory data for the 54 patients. Median age at onset of weakness was 50 (range 16–83) years; 57% were women. One-third were non-Caucasian. All patients presented with a subacute, progressive proximal-predominant weakness; 23 had bulbar weakness, and 21 had respiratory weakness (intensive care admission was required for 8). Seven patients had coexisting neurological disorders,



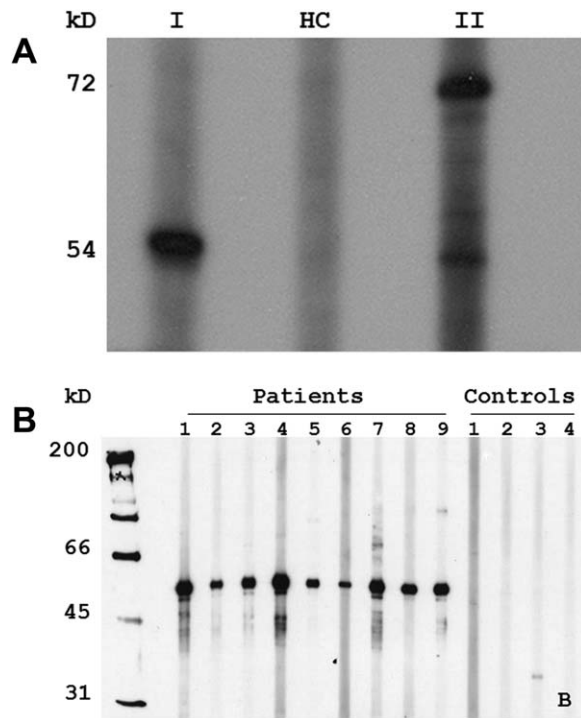


**FIGURE 1.** Indirect immunofluorescence patterns of cytoplasmic staining yielded on mouse tissue sections by patient serum IgGs. **(A)** In cerebellar cortex, PCA-1 IgG (anti-Yo, marker of cerebellar degeneration related to Mullerian and breast adenocarcinomas) stains perikarya of Purkinje neurons (chunky pattern) and molecular layer (ML) neurons. Granular layer (GL) is non-stained except for occasional Golgi neurons. **(B)** SRP IgG similarly stains Purkinje and molecular layer neurons and also yields a “chicken-wire” appearance in granular layer neurons. **(C)** Proximal epithelial cells in gastric mucosa (arrow) and ganglionic neurons of myenteric plexus (arrow-head). Subjacent renal tubular sections are less reactive. SM indicates gastric smooth muscle. **(D)** SRP-immunoreactive neurons in the dentate granular layer and hilus of the hippocampus. **(E)** SRP immunoreactivity in choroid plexus epithelium, midbrain neurons, and **(F)** renal cortical tubule cells (arrow).

including: encephalopathy (2); sensorimotor peripheral neuropathy (4); and dysautonomia (1). Cardiomyopathy was reported in 5 patients.

**Laboratory Findings.** All available serum creatine kinase values were elevated (median 8,750 IU/L), except for a patient with graft-versus-host disease (GVHD) following autologous hemopoietic stem

cell transplantation for acute myeloid leukemia (diagnosed in the course of radiation therapy for mammary ductal carcinoma *in situ*). EMG results (available for 39 patients) all showed evidence of myopathy and, except for the GVHD patient, fibrillation potentials. Muscle histopathology reports (available for 44 patients) confirmed myopathy. Of 18 patients whose biopsied tissue was available for



**FIGURE 2.** Western blot immunoreactivities of IgGs in representative patient sera yielding SRP-like immunostaining patterns. **(A)** Mouse pancreatic microsomal proteins. Patient I: IgG binds to a 54-kDa band; patient II: IgG binds more prominently to a 72-kDa band than to the 54-kDa band. Healthy control (HC) IgGs bind to neither band. **(B)** Recombinant SRP54 protein. Serum of 9 patients, but no control, binds to a prominent 54-kDa band.

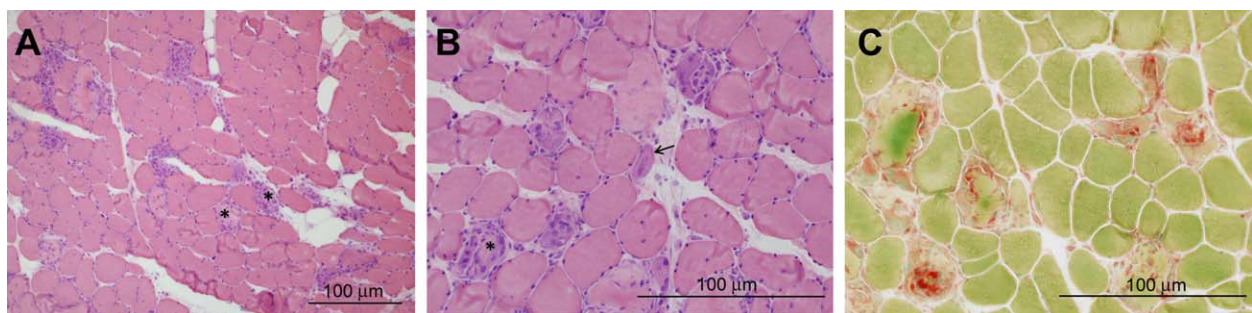
review, 14 showed predominantly necrotizing myopathy (scattered necrotic fibers and regenerating fibers) with minimal or no lymphocytic infiltration, and 3 showed an inflammatory myopathy with endomysial and perimysial lymphocytic inflammatory reaction. The GVHD patient's biopsy showed predominantly perifascicular myofiber atrophy, multifocal capillary depletion, and membrane attack complex deposition in intramuscular capillaries, but no necrosis, regeneration, or inflammation, suggestive of GVHD-associated myopathy.<sup>11</sup> No biopsied tissue had autoaggressive inflammatory

mononuclear cells attacking healthy muscle fibers. Although scant muscle fiber vacuoles were present in 2 biopsies, the pattern of weakness (proximal, symmetrical), favorable immunotherapy responses, and lack of autoaggressive inflammation and congophilic inclusions argued against the diagnosis of inclusion-body myositis. Except for the GVHD patient, no patient had pathological findings of dermatomyositis, necrotizing vasculitis, or granulomatous myopathy.

**Evidence of Coexisting Autoimmunity.** Coexisting autoimmune conditions were reported for 17 of 46 (37%) patients with a documented autoimmune history (Raynaud phenomenon 8, hypothyroidism 3, connective tissue disease 3, pernicious anemia 2, Sjögren syndrome 2, alopecia areata 2, rheumatoid arthritis 1, and diabetes mellitus 1). Remote sarcoidosis with liver involvement was reported in 1 patient.

Coexisting neural autoantibodies, identified in 15 of the 54 patients (28%), were specific for voltage-gated potassium channel-complex, N-type voltage-gated calcium channel, ganglionic acetylcholine receptor, GAD65, and sarcomere (striational; see Table 1). PCA-1 IgG was negative in all patients. Only Mayo Clinic patients (20) had available data for non-neural autoantibodies. Fifty percent of patients had non-organ-specific autoantibodies [antinuclear antibody (ANA) >> ribonucleoprotein, Smith (Sm), double-stranded DNA, anti-phospholipid antibody, or rheumatoid factor]; 40% had thyroid autoantibodies (Table 1). No patient was positive for Jo-1 antibodies.

**Oncological Findings or History.** Malignant neoplasms were documented in 9 patients, either contemporaneously or in past history (breast adenocarcinoma 3, lymphoma 2, acute myeloid leukemia 1, colon adenocarcinoma 1, renal cell carcinoma 1, thyroid papillary carcinoma 1, prostate carcinoma 1). The patient with acute myeloid leukemia was diagnosed in the course of therapy for initially diagnosed breast carcinoma. In 3



**FIGURE 3.** Muscle biopsy of a patient with necrotizing SRP autoimmune myopathy. Hematoxylin–eosin-stained sections **(A, B)** show several scattered necrotic muscle fibers (asterisk) and a few regenerating fibers (arrow). Macrophages invading necrotic fibers stain bright red in the acid phosphatase–reacted section **(C)**.

**Table 1.** Clinical and serological characteristics of 54 patients with autoimmune SRP myopathy

Demographic characteristics	
Gender, women:men [n]	31:23
Ethnicity, number with information	35
Caucasian	22 (63)
African American [n (%)]	8 (23)
Hispanic	3 (9)
Asian	1
Native American	1
Onset age, years [median (range)]	50 (16–83)
Follow-up duration, months [median (range)]	NA
SRP serology	
SRP IFA titer [median (range)]*	7,680 (240–122,880)
SRP54 ELISA positive [n (%)] Titer [median (range)]	49 (91) 12,800 (120–819,200)
SRP72 IPA positive [n (%)] Median (range) (nmol/L)	35 (79% of 44 tested) 95 (12–102,400)
Muscle biopsy results	
Available [n (%)]	44 (81)
Necrotizing [n (%)]	36 (82)
Inflammatory [n (%)]	7 (16)
Other	1 (2) (GVHD)
Coexisting neoplasm	
Histologically confirmed malignancy [n (%)]	9 (17)
Coexisting autoimmunity	
Autoimmune disease [n (%)] Neural autoantibody [n (%)]	17/46 (37) 15 (28)
VGCC N-type [n; median (range)] in nmol/L*	3; 0.05 (0.05–0.07)
VGKC-complex [n; median (range)], in nmol/L	5; 0.09 (0.03–0.20)
Ganglionic AChR [n (range)], in nmol/L	2 (0.04–0.15)
GAD65 [n; median (range)], in nmol/L	5; 0.09 (0.07–0.14)
CRMP5, Western blot positive	1
Striational [n; median titer (range)]	4; 240 (120–480)
Other autoantibody [n (%)]	
ANA	8/20 (40)
ENA	4/20 (20)
Thyroperoxidase/thyroglobulin	8/20 (40)

IFA, tissue-based immunofluorescence assay; IPA, immunoprecipitation assay; n, number; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel; NA, data not available; ENA, extractable nuclear antibodies. See text for other abbreviations.

\*Autoantibody normal range values: SRP IFA < 1:240; SRP54 ELISA < 1:60; SRP72 IPA 0–10 nmol/L; VGCC N-type 0.00–0.03 nmol/L; VGKC-complex 0.00–0.02 nmol/L; ganglionic (nicotinic  $\alpha$ 3) acetylcholine receptor 0.00–0.02 nmol/L; GAD65 0.00–0.02 nmol/L; striational < 1:120.

patients, the diagnosis of myopathy preceded cancer discovery (mean interval 5 months), and in 4 patients myopathy diagnosis followed cancer discovery (mean interval 50 months); the timing of cancer discovery was unknown for 2 patients.

**Treatment Responses.** Clinical information 6 months or more posttreatment was available for 31 patients (57%). All received an initial trial of high-dose corticosteroids. Overall, 26 patients (84%) improved; 81% of responders required additional immunotherapy (usually intravenous immune globulin and methotrexate or mycophenolate mofetil). The median time elapsed from symptom onset to treatment was 6 (range 1–36) months. Therapy in 3 responders commenced at 24, 30, and 36 months after symptom onset. Recovery was typically slow. Most patients had improved after 18 months of therapy, but not to baseline. More than 70% of responders relapsed on withdrawal of immunosuppression, necessitating extended therapy. One patient who deteriorated significantly during

relapse failed to regain lost function despite rapid reintroduction of therapy.

**Prospective Analysis of SRP IgG Detection Frequency.** Between January 1 and December 31, 2012, we detected SRP IgG in 26 patient sera (0.06%) among the 40,400 evaluated by service immunofluorescence assay for neural autoantibodies. In the same period, we detected (and confirmed by native Western blot assay) PCA-1 (anti-Yo) in 27 patient sera (0.07%).

## DISCUSSION

The method of patient ascertainment distinguishes our study of SRP autoantibody associations from any previous report. Earlier studies have selectively tested for SRP autoantibody in patients with rheumatologic or muscle disease (inflammatory or otherwise). SRP IgG was not suspected in the patients in our study, but it was identified incidentally through service screening for neural-restricted paraneoplastic IgG autoantibodies by tissue-based immunofluorescence assay. The patients presented with diverse subacute neurological problems,



usually dominated by profound weakness. Because the ordering neurologist's early consideration was a paraneoplastic etiology, serological testing for "myositis-pertinent" autoantibodies had not been considered in most cases. In some patients, previous SRP antibody testing by a commercial laboratory had been reported to be negative.

The unanticipated serological finding correlated in 70% of patients with a final diagnosis of myopathy (particularly necrotizing autoimmune myopathy). Subsequent testing with recombinant antigens revealed IgGs specific for the 54- or 72-kDa subunits of SRP in 96% of patients. The final clinical diagnosis of necrotizing autoimmune myopathy in the 9% of patients negative for SRP54 IgG (4% lacked evidence for either SRP54 IgG or SRP72 IgG) suggests another SRP component as antigen in some, or greater sensitivity of the tissue-based immunofluorescence assay than ELISA or immunoprecipitation for detection of SRP antibody. The latter possibility awaits prospective investigation. Typical of necrotizing autoimmune myopathy, creatine kinase was elevated, and electromyography supported the diagnosis of an active proximal-predominant myopathy in all patients.<sup>4</sup> In 75% of patients, muscle biopsies were found to be consistent with necrotizing myopathy, with minimal or no inflammation.<sup>12</sup> Evidence of autoaggressive inflammation was lacking in the 17% of patients who had an inflammatory myopathy. It is noteworthy that 13% of our cohort had coexisting neurological problems (peripheral neuropathy, encephalopathy, or dysautonomia). This suggests that neurological involvement associated with SRP IgG may be more widespread than previously recognized.

Autoantibodies reactive with the 54-kDa component of the hexameric SRP,<sup>2</sup> and subsequently antibodies reactive with the 7-SL RNA component,<sup>5</sup> were described initially as a marker of polymyositis. Because early diagnostic criteria for inflammatory muscle disorders did not require a histological diagnosis nor did they recognize autoimmune necrotizing myopathy as a distinct entity,<sup>13</sup> early serological reports may have misclassified patients with necrotizing myopathy as severe polymyositis cases. The SRP54 IgG association with autoimmune necrotizing myopathy has been recognized increasingly in the past 9 years. SRP72-reactive IgG has also been reported.<sup>1</sup> We emphasize the value of serological testing as a diagnostic aid for necrotizing autoimmune myopathy, because it is not uncommon for lack of evident inflammation in biopsied muscle to dissuade a non-neuromuscular specialist neurologist from entertaining the diagnosis of immune-mediated myopathy. A recently recognized unrelated IgG autoantibody,

specific for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR, the rate-controlling enzyme of the cholesterol-biosynthesizing mevalonate pathway), is a second biomarker of a histopathologically indistinguishable and similarly immunotherapy-responsive autoimmune necrotizing myopathy.<sup>14</sup> Although initially recognized as statin therapy related, a majority of HMGCR IgG-positive cases are idiopathic.<sup>14</sup> Additional examples of the particular diagnostic value of autoimmune serology include patients who have slow progression or are untreated and have longstanding weakness. As in 1 of our patients, SRP myopathy may be misdiagnosed as muscular dystrophy.<sup>15</sup> The myopathy cohort we have described is similar overall in demographic and clinical characteristics to previously studied cohorts with SRP IgG-associated myopathies.<sup>1,5</sup> Patient ascertainment bias through physician-ordered paraneoplastic serological screening plausibly accounts for the higher cancer frequency documented here than previously reported for patients with SRP IgG (17% vs. 10%).<sup>4,16</sup> This suggestion is supported by the reported 18% frequency of cancer history in a control group of adult Mayo Clinic neurology patients for whom service paraneoplastic serological evaluation yielded no positive findings.<sup>17</sup> Breast adenocarcinoma and hematologic malignancy were most common, but we also encountered carcinomas of colon, thyroid, kidney, and prostate. Limited follow-up information for 3 patients (smokers with pulmonary radiological abnormalities noted) suggested a cancer frequency exceeding 17%.

The beneficial responses observed in recipients of aggressive and sustained immunotherapy emphasize the importance for neurologists to be aware of the entity of autoimmune necrotizing myopathy. No standardized treatment has been established, but, based on our continuing experience and the work of others,<sup>14,18</sup> we currently recommend at least 3 months of combined therapy with intravenous immune globulin (IVIg), corticosteroids, and a steroid-sparing immunosuppressant, followed by long-term treatment with the steroid-sparing agent. However, treatment must be individualized. Tapering of immunosuppressant drugs should be done cautiously to avoid relapse. IVIg is beneficial early in the disease course to manage relapses or when corticosteroids are contraindicated. The majority of patients will require long-term immunosuppression. Acknowledging our patient ascertainment bias, we nevertheless recommend that patients with autoimmune necrotizing SRP myopathy be screened for occult malignancy, guided by risk factors and past and family history (mammogram, prostate evaluation, whole-body positron emission tomography-computed

tomography, and upper and lower gastrointestinal endoscopy).

In conclusion, autoimmune SRP myopathy is a severe but treatable entity that appears to be underdiagnosed in neurological practice. At least 1 in every 6 of these patients has an associated malignancy. The novel SRP IgG immunostaining pattern we have described enables serological detection of at least 2 SRP subunit-specific autoantibodies (SRP54 and SRP72). The diagnosis of necrotizing autoimmune myopathy may be challenging because the finding of minimal or no evidence of inflammation on muscle biopsy may point non-subspecialist neurologists away from an immune-mediated etiology and dissuade them from implementation of immune-modulating therapy. Improvement is usually slow and requires long-term immunotherapy with more than a single agent. Relapses are frequent when immunotherapy is reduced. Early treatment is ideal.

The authors thank Nancy Peters for technical assistance.

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