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# Argonaute Autoantibodies as Biomarkers in Autoimmune Neurologic Diseases

Le-Duy Do, PhD,\* Christian P. Moritz, PhD,\* Sergio Muñoz-Castrillo, MD, Anne-Laurie Pinto, MSc, Yannick Tholance, PhD, Sabine Brugiére, MSc, Yohann Couté, PhD, Oda Stoevesandt, PhD, Michael J. Taussig, PhD, Véronique Rogemond, PhD, Alberto Vogrig, MD, Bastien Joubert, MD, PhD, Karine Ferraud, MSc, Jean-Philippe Camdessanché, MD, PhD, Jean-Christophe Antoine, MD, PhD,‡ and Jérôme Honnorat, MD, PhD‡

## Correspondence

Dr. Honnorat  
jerome.honnorat@chu-lyon.fr

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## Abstract

### Objective

To identify and characterize autoantibodies (Abs) as novel biomarkers for an autoimmune context in patients with central and peripheral neurologic diseases.

### Methods

Two distinct approaches (immunoprecipitation/mass spectrometry-based proteomics and protein microarrays) and patients' sera and CSF were used. The specificity of the identified target was confirmed by cell-based assay (CBA) in 856 control samples.

### Results

Using the 2 methods as well as sera and CSF of patients with central and peripheral neurologic involvement, we identified Abs against the family of Argonaute proteins (mainly AGO1 and AGO2), which were already reported in systemic autoimmunity. AGO-Abs were mostly of immunoglobulin G 1 subclass and conformation dependent. Using CBA, AGO-Abs were detected in 21 patients with a high suspicion of autoimmune neurologic diseases (71.4% were women; median age 57 years) and only in 4/856 (0.5%) controls analyzed by CBA (1 diagnosed with small-cell lung cancer and the other 3 with Sjögren syndrome). Among the 21 neurologic patients identified, the main clinical presentations were sensory neuronopathy (8/21, 38.1%) and limbic encephalitis (6/21, 28.6%). Fourteen patients (66.7%) had autoimmune comorbidities and/or co-occurring Abs, whereas AGO-Abs were the only autoimmune biomarker for the remaining 7/21 (33.3%). Thirteen (61.9%) patients were treated with immunotherapy; 8/13 (61.5%) improved, and 3/13 (23.1%) remained stable, suggesting an efficacy of these treatments.

### Conclusions

AGO-Abs might be potential biomarkers of autoimmunity in patients with central and peripheral nonparaneoplastic neurologic diseases. In 7 patients, AGO-Abs were the only biomarkers; thus, their identification may be useful to suspect the autoimmune character of the neurologic disorder.

### Classification of Evidence

This study provides Class III evidence that AGO-Abs are more frequent in patients with autoimmune neurologic diseases than controls.

\*These authors contributed equally to this work as co-first authors.

‡These authors contributed equally to this work as co-senior authors.


From French Reference Center on Paraneoplastic Neurological Syndrome (L.-D.D., S.M.-C., A.-L.P., V.R., A.V., B.J., J.-P.C., J.-C.A., J.H.), Hospices Civils de Lyon, Hôpital Neurologique, Bron, France; Institute NeuroMyoGène (L.-D.D., C.P.M., S.M.-C., A.-L.P., Y.T., V.R., A.V., B.J., K.F., J.-P.C., J.-C.A., J.H.), INSERM U1217/CNRS UMR 5310, Université de Lyon, Université Claude Bernard Lyon 1, France; University Jean Monnet (C.P.M., Y.T., J.-P.C., J.-C.A.), Saint-Etienne, France; Department of Biochemistry (Y.T.), University Hospital of Saint-Etienne, France; University Grenoble Alpes (S.B., Y.C.), CEA, INSERM, IRIG, BGE, France; Cambridge Protein Arrays Ltd. (O.S., M.J.T.), Babraham Research Campus, United Kingdom; and Department of Neurology (K.F., J.-P.C., J.-C.A.), University Hospital of Saint-Etienne, France.

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## Glossary

**Ab** = autoantibody; **AE** = autoimmune encephalitis; **CBA** = cell-based assay; **IgG** = immunoglobulin G; **LE** = limbic encephalitis; **mRS** = modified Rankin Scale; **MS** = mass spectrometry; **PNS** = paraneoplastic neurologic syndrome; **SCLC** = small-cell lung cancer; **SNN** = sensory neuronopathy.

The discovery of autoantibodies (Abs) against neuroglial antigens has revolutionized the diagnosis and understanding of autoimmune neurologic diseases and has led to the clinical description of different subtypes of autoimmune encephalitis (AE),<sup>1</sup> paraneoplastic neurologic syndromes (PNS),<sup>2</sup> and inflammatory peripheral neuropathies.<sup>3</sup> On the one hand, some neuronal Abs can play a direct role in the pathophysiology, mainly when they are directed against surface antigens such as NMDA receptor,<sup>4</sup> neurofascin 155,<sup>5</sup> or contactin 1.<sup>6</sup> On the other hand, some Abs are only indicative of an underlying cancer and can be useful to guide tumor screening in PNS,<sup>7</sup> whereas others are biomarkers of autoimmunity, such as antibodies against fibroblast growth factor receptor 3 in sensory neuronopathy (SNN).<sup>8,9</sup> Nevertheless, there are still many patients and disorders clinically indistinguishable from well-characterized autoimmune neurologic diseases, but without reliable biomarkers. In these cases, it is always difficult to establish the autoimmune nature of the disease, which is only supported by occasional inflammatory abnormalities in the CSF.<sup>10,11</sup> Hence, the discovery of new Abs is of major importance for the assertion of the autoimmune origin of these disorders and to propose an immunomodulator treatment that might lead to a better prognosis.<sup>12,13</sup>

In the present study, 2 different methods (immunoprecipitation coupled to mass spectrometry [MS]-based proteomics and protein microarrays) were used in parallel with the aim to identify novel Ab targets, leading to the discovery of antibodies against the Argonaute protein family (AGO-Abs), which have been already reported in systemic autoimmune disorders.

## Methods

Two distinct approaches were used to identify the Abs and their antigens. In a first approach, we used the CSF of a patient with limbic encephalitis (LE; patient XI, see below), which showed an atypical staining on indirect immunofluorescence, to perform immunoprecipitation and MS-based analyses. In a simultaneous and independent approach, protein microarrays were used for Ab characterization in sera of patients with peripheral neuropathies. Finally, different sera and CSF samples of several patient cohorts were screened via cell-based assay (CBA) and the specificity of the identified target was confirmed by CBA and immunoadsorption; an assay to determine the binding region of the antigen was also performed. Detailed description of the methods is provided in the eMaterial ([links.lww.com/NXI/A503](https://links.lww.com/NXI/A503)).

Patient sera and CSFs were obtained from the NeuroBioTec biobanks (Hospices Civils de Lyon BRC, France, AC-2013-1867, NFS96-900; and CRB42 CHU Saint-Etienne, France,

AC 2018-3372, NFS96-900). We selected for the study 250 CSF samples from patients with suspected AE/PNS and 42 sera of patients with peripheral neuropathies. As controls, we selected 312 CSF and 544 sera of various patients with or without neurologic involvement (Table 1). All samples were collected from October 2007 to December 2019.

## Standard Protocol Approvals, Registrations, and Patient Consents

The Institutional Review Board of the University Claude Bernard Lyon 1 and Hospices Civils de Lyon and the CHU of Saint-Étienne approved the study (ANR-18-RHUS-0012),

**Table 1** Samples Tested for AGO Antibodies

|   | Sample type          | Positive samples n/N |
|---|----------------------|----------------------|
| <b>Study group</b>  |                      |                      |
| Suspicion of AE/PNS   | CSF                  | 14/250               |
| Sensory neuropathies  | Serum                | 7/42                 |
| <b>Controls</b>   |                      |                      |
| Undiagnosed neurologic syndromes                                | CSF                  | 0/91                 |
| Undiagnosed neurologic syndromes                                | Serum                | 0/195                |
| Alzheimer disease   | CSF                  | 0/90                 |
| Frontotemporal dementia   | CSF                  | 0/25                 |
| Lewy body dementia  | CSF                  | 0/15                 |
| Psychosis   | CSF                  | 0/20                 |
| Normal pressure hydrocephalus                                   | CSF                  | 0/15                 |
| Patients without neurologic symptoms                            | CSF                  | 0/56                 |
| Healthy controls  | Serum                | 0/17                 |
| PNS with SCLC and Hu antibodies                                 | Serum                | 0/50                 |
| Lung cancer, neurologic asymptomatic                            | Serum                | 1/166                |
| Autoimmune diseases without neurologic involvement <sup>a</sup> | Serum                | 3/116                |
| <b>Total of controls</b>  | 312 CSF/<br>544 sera |                      |
| <b>Total of samples</b>   |                      | 25/1148              |

Abbreviations: AGO = Argonaute protein family; AE = autoimmune encephalitis; PNS = paraneoplastic neurologic syndrome; SCLC = small-cell lung cancer.

<sup>a</sup> Including systemic lupus erythematosus (n = 41), primary biliary cholangitis (n = 17), Sjögren syndrome (n = 56), and others (n = 2).

which has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written consent was obtained from all patients.

## Data Availability

Any data not published within the article are available and will be shared by request from any qualified investigator.

## Results

### Identification of Argonaute Proteins as Targets of Autoantibodies in Neurologic Diseases

Argonaute proteins were identified as targets of Abs using 2 different approaches. First, among a series of CSF from patients with suspected AE/PNS, one (from patient XI) showed an atypical immunostaining in the cytoplasm of neurons of the hippocampus (granular neurons of the dentate gyrus and CA1, CA3 pyramidal cells), cerebellum (granular cells, some cells in the molecular layer), and cerebral cortex (Figure 1A). Western blots using whole rat brain homogenates revealed with patient XI's CSF showed no signal (data not shown), suggesting that the antibodies were conformation dependent. Immunoprecipitation of whole rat brain homogenates with either patient XI or control CSF revealed the presence of patient XI-specific bands at 100 (Figure 1B, \*) and 280 kDa (Figure 1B, \*\*). Subsequent MS-based proteomic analyses identified AGO1, AGO2, AGO3, and AGO4 (theoretical MW~100 kDa), as well as trinucleotide repeat containing 6A

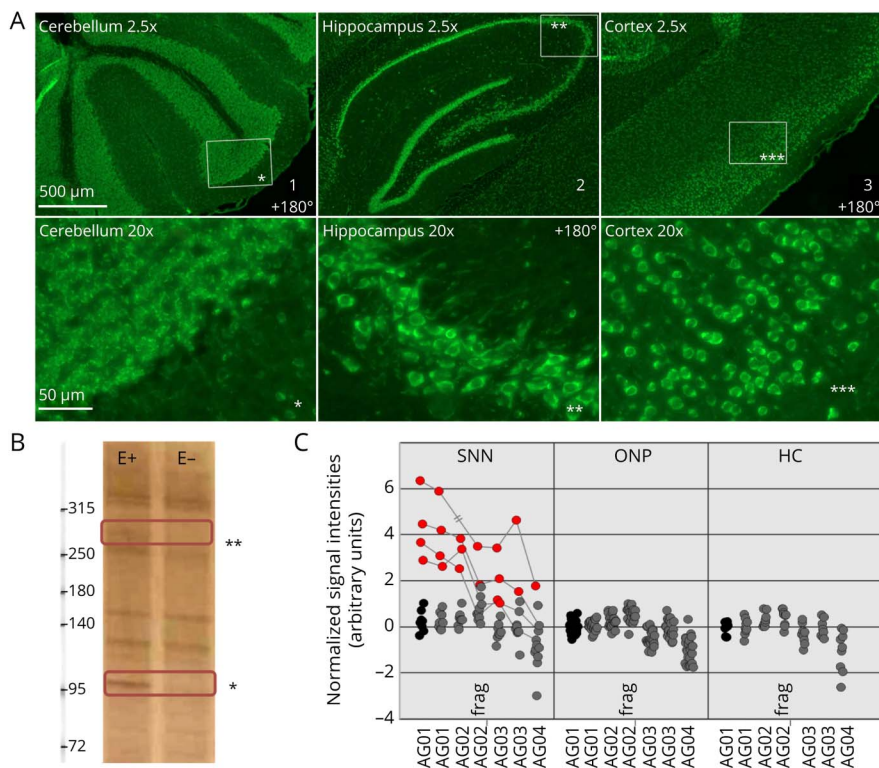
(TNRC6A), TNRC6B, and TNRC6C (theoretical MW~200 kDa), as the most enriched proteins in patient XI's compared with control's immunoprecipitate.

In parallel, the sera of 12 patients with SNN and of 34 controls were screened by protein microarrays, which revealed that AGO1 and AGO2 proteins were significantly targeted by 3 SNN patient sera; none of the control sera targeted any of the AGO proteins. A fourth SNN serum targeted AGO1, but not AGO2. Four and one of these patients with SNN significantly targeted AGO3 and AGO4 proteins, respectively, but with lower reactivities (Figure 1C).

### Specificity of AGO-Abs

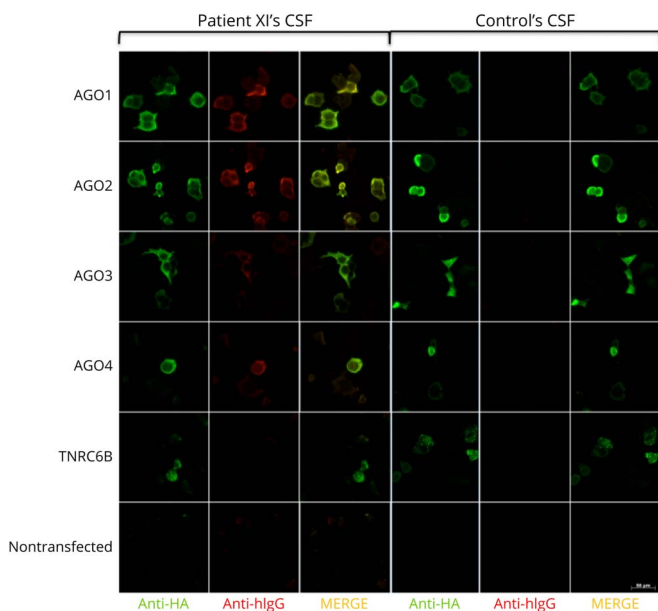
To validate the antigenic specificity of AGO-Abs, different CBAs were constructed, each with one of the AGO plasmids. Patient XI's CSF reacted with AGO1–4–transfected cells, whereas a control CSF showed no reactivity (Figure 2, lines 1–4), and no signal was observed with TNRC6 transfection (Figure 2, line 5) and nontransfected cells (line 6). Furthermore, after simultaneous AGO1- and AGO2-Ab depletion, the reactivity of patient XI's CSF in indirect immunofluorescence on rat brain sections was lost (eFigure 1, [links.lww.com/NXI/AS02](https://links.lww.com/NXI/AS02)), confirming that the atypical staining pattern previously observed was solely caused by antibodies targeting AGO proteins. In addition, 3 of 4 sera from patients with SNN that were positive by protein microarray specifically reacted with AGO1 and AGO2-HEK293–transfected cells (patients I, II, and III; data not shown).

**Figure 1** Identification of AGO-Abs



(A) Immunostaining of the adult rat brain with CSF (1:10) of patient XI resulted in a strong reactivity with the stratum pyramidale of the hippocampus, granule cells of the cerebellum, and cerebral cortex. The scale bar is indicated for each magnification. (B) Immunoprecipitation of the antigens with patient XI's CSF. Note the specifically enriched band around 100 kDa (\*) and the less intense band around 280 kDa (\*\*) obtained after immunoprecipitation with patient XI's CSF (E+) compared with control CSF (E-). Protein bands were visualized via silver staining. (C) Quantified reactivities of 12 patients with SNN, 22 patients with ONPs, and 9 HCs with 7 AGO variants on protein microarrays. Red dots represent significantly positive samples ( $z$ -score > 4). AGO-Abs = antibodies against the Argonaute protein family; HC = healthy control; ONP = other peripheral neuropathy; SNN = sensory neuropathy.

**Figure 2** Confirmation of AGO-Abs Using Cell-Based Assay



HEK293 cells were transfected with VP5-AGO1-4 or VP5-TNRC6B plasmid, for a transient overexpression. Fixed and permeabilized cells were then immunostained with anti-HA antibody (in green) and patient XI's CSF (1:10; in red). Only AGO-transfected cells (in green) reacted with patient XI's CSF. No reactivity was observed on TNRC6B-transfected cells or non-transfected cells. Scale bar = 50  $\mu$ m. AGO-Abs = antibodies against the Argonaute protein family.

To validate the clinical specificity of AGO-Abs, a total of 856 control samples (312 CSF and 544 sera; Table 1) from patients with and without neurologic diseases (including 116 with systemic autoimmunity and no neurologic involvement and 50 from PNS with Hu-Abs and small-cell lung cancer [SCLC]) were screened by CBA. Among these controls, only 4 serum samples (4/544, 0.7%) and no CSF (0/312, 0.0%) were found positive for AGO-Abs. The 4 positive sera came from 1 patient with SCLC without neurologic symptoms and 3 patients with Sjögren syndrome.

To identify additional patients with AGO-Abs besides the index LE case and the 3 patients with SNN positive by CBA, the CSF of 250 patients with high suspicion of AE/PNS and the sera of 42 patients with peripheral neuropathies were screened by CBA. Among them, 12 (4.1%) new patients with AGO-Abs were identified; 8 of them had available CSF showing exactly the same aforementioned pattern by indirect immunofluorescence. In addition, 3,254 CSF sent to the reference center for suspicion of AE/PNS were prospectively screened for an AGO-Abs immunostaining pattern (between August 28, 2019 and February 25, 2020), and 5 (0.1%) new AGO-Abs cases were identified.

### Clinical and Paraclinical Characteristics of AGO-Ab Neurologic Patients

A total of 21 patients with AGO-Abs and neurologic symptoms were identified, their median age was 57 years (range 25–85 years), and 15/21 (71.4%) were women (Table 2). The most common clinical presentation was SNN (8/21, 38.1%), followed by LE (6/21, 28.6%), cerebellar syndrome (2/21, 9.5%), other peripheral neuropathies (2/21, 9.5%), rhombencephalitis (1/21, 4.8%), and opsoclonus-myoclonus

(1/21, 4.8%). One patient (1/21, 4.8%) presented with a transitory low level of consciousness that was not described in detail in the charts. The CSF was inflammatory (pleocytosis  $\geq 5$  cells) in 8/16 (50.0%) patients; oligoclonal bands were positive in 4/9 (44.4%) patients. All but one of the patients with CNS involvement had an abnormal MRI (Figure 3). Cancer was diagnosed in only 5/21 (23.8%) patients. Auto-immune comorbidities (Sjögren syndrome in 7/21 patients, 33.3%) and/or co-occurring Abs (mainly anti-Sjögren syndrome-related antigen A, detected in 8/21 patients, 38.1%) were present in 14/21 (66.7%) patients, whereas in the remaining 7 patients (7/21, 33.3%), AGO-Abs were the sole biomarker of autoimmunity. A total of 14/21 (66.7%) patients received immunotherapy, including steroids (n = 11), IV immunoglobulin (n = 9), cyclophosphamide (n = 3), rituximab (n = 3), plasma exchanges (n = 2), azathioprine (n = 2), and/or methotrexate (n = 2). The median modified Rankin Scale (mRS) score at onset was 3 (range 2–5) for the 19 patients with available information, the median mRS score at last follow-up for 16 patients was 2.5 (range 0–6), and follow-up was not available for 5 patients. Among the patients treated with immunotherapy for whom enough follow-up data were available (n = 13), 8/13 (61.5%) improved (lower mRS score at last follow-up compared with the one at clinical onset), 3/13 (23.1%) remained stable (unchanged mRS score), and for 2/13 (15.4%), their condition worsened. In total, 2 (2/21, 9.5%) patients died: patient XIV, due to progression of the SCLC, and patient XVI, because of several complications of hepatic cirrhosis of alcohol origin.

### Characteristics of AGO-Abs

Among all the samples tested herein so far, the ones that were positive for AGO-Abs reacted on CBA more strongly for

**Table 2** Clinical Characteristics of Patients With AGO Antibodies

| Patient | Sex, age, y | Neurologic presentation                                     | Ancillary tests   | CSF  | Other Abs           | Autoimmune comorbidities                  | Cancer <sup>a</sup>                     | Treatment                                    | mRS score at onset → mRS score at last follow-up (delay in months) |
|---------|-------------|---|---|--|---------------------|---|---|--|--|
| I       | M, 54       | SNN   | EMG + SNN   | NA   | No                  | Sarcoidosis                               | No                                      | IVIg + steroids + PEX + CP + RTX + AZA + MTX | NA   |
| II      | F, 40       | SNN   | EMG + SNN   | NA   | ANA, SSA            | Sjögren syndrome                          | No                                      | Steroids + IVIg + CP                         | 3 → 3 (144)  |
| III     | F, 53       | SNN   | EMG + SNN   | NA   | SSA, SSB            | Sjögren syndrome                          | No                                      | NA   | NA   |
| IV      | M, 74       | SNN   | EMG + SNN   | 0.7 g/L proteins, no cells, OCB-                   | No                  | No  | No                                      | No   | 2 → 3 (5)  |
| V       | F, 57       | SNN   | EMG + SNN   | 0.42 g/L proteins, no cells, OCB-                  | No                  | No  | Colon adenocarcinoma (at SNN diagnosis) | IVIg   | 2 → 2 (2)  |
| VI      | F, 56       | Bilateral asynchronous trigeminal neuralgia; 2 y later, SNN | EMG + SNN; MRI contrast enhancement of both trigeminal nerves | 1.22 g/L proteins, 12 cells/mm <sup>3</sup>        | ANA, SSA            | Sjögren syndrome, vitiligo                | Breast cancer (2 y before)              | PEX + IVIg + steroids                        | 2 → 1 (22)   |
| VII     | F, 55       | SNN   | EMG + SNN   | NA   | SSA, SSB            | Sjögren syndrome                          | No                                      | CP + RTX + steroids                          | 3 → 3 (3)  |
| VIII    | F, 54       | SNN   | EMG + SNN   | 0.43 g/L, 1 cell/mm <sup>3</sup>                   | ANA                 | Sjögren syndrome                          | No                                      | Steroids + AZA                               | 4 → 3 (156)  |
| IX      | M, 79       | Sensory demyelinating polyneuropathy                        | EMG demyelinating abnormalities in 4 limbs                    | NA   | ANA, MAG            | No  | No                                      | RTX  | 2 → 1 (168)  |
| X       | M, 71       | Sensory-motor axonal polyneuropathy                         | EMG axonal abnormalities in 4 limbs                           | 0.35 g/L, 1 cell/mm <sup>3</sup>                   | No                  | No  | No                                      | No   | 2 → NA   |
| XI      | F, 78       | LE: amnesia and psychiatric symptoms; movement disorders    | MRI bilateral hippocampal atrophy; EEG-                       | 0.23 g/L proteins, 5 cells/mm <sup>3</sup> , OCB+  | No                  | Sjögren syndrome                          | No                                      | IVIg   | 2 → 0 (29)   |
| XII     | F, 25       | LE: seizures, amnesia, and psychiatric symptoms             | MRI bitemporal FLAIR abnormalities; EEG+                      | 0.5 g/L proteins, 84 cells/mm <sup>3</sup> , OCB+  | SSA                 | No (minor salivary gland biopsy negative) | No                                      | Steroids + IVIg                              | 5 → 2 (9)  |
| XIII    | F, 41       | LE: seizures, amnesia, and psychiatric symptoms             | MRI bitemporal FLAIR abnormalities; EEG+                      | 0.38 g/L proteins, 2 cells/mm <sup>3</sup> , OCB-  | No                  | Cutaneous lupus with arthritis            | No                                      | Steroids + IVIg                              | 3 → 2 (2)  |
| XIV     | F, 74       | LE: seizures (status), amnesia, and psychiatric symptoms    | MRI right temporal FLAIR abnormality; EEG+                    | 0.91 g/L proteins, 15 cells/mm <sup>3</sup> , OCB+ | GABA <sub>B</sub> R | No  | SCLC (4 mo before)                      | Steroids + IVIg                              | 5 → 6 (8)  |
| XV      | F, 85       | LE: seizures and amnesia                                    | MRI left temporal FLAIR abnormality; EEG+                     | Normal proteins, 8 cells/mm <sup>3</sup>           | NA                  | No  | No                                      | Steroids                                     | 3 → 1 (0.5)  |

Continued

**Table 2** Clinical Characteristics of Patients With AGO Antibodies (continued)

| Patient | Sex, age, y | Neurologic presentation   | Ancillary tests  | CSF  | Other Abs                          | Autoimmune comorbidities                  | Cancer <sup>a</sup>                    | Treatment       | mRS score at onset → mRS score at last follow-up (delay in months) |
|---------|-------------|---|--|--|------------------------------------|---|--|-----------------|--|
| XVI     | F, 67       | LE: seizures (status), amnesia, and psychiatric symptoms                  | MRI right temporal FLAIR abnormality; EEG+             | 0.47 g/L proteins, 2 cells/mm <sup>3</sup>         | NA                                 | No  | No                                     | No              | 5 → 6 (1.5)  |
| XVII    | F, 30       | Subacute cerebellar syndrome  | MRI left hemispheric hypersignal → cerebellar atrophy  | 0.7 g/L proteins, 465 cells/mm <sup>3</sup> , OCB+ | SSA                                | No (minor salivary gland biopsy negative) | No                                     | Steroids + IVIG | 2 → 4 (37)   |
| XVIII   | M, 67       | Chronic cerebellar syndrome   | MRI cerebellar atrophy                                 | Normal, OCB-                                       | No                                 | No  | Neck epidermoid carcinoma (3 y before) | No              | 4 → NA   |
| XIX     | F, 35       | Rhombencephalitis: headache, vertigo, vomiting, nystagmus, and gaze palsy | MRI brainstem FLAIR abnormality and LETM imaging; EEG- | 0.9 g/L proteins, 424 cells/mm <sup>3</sup>        | ANA, SSA, GAD, and anticardiolipin | Autoimmune hepatitis and Sjögren syndrome | No                                     | Steroids        | 2 → 0 (2)  |
| XX      | M, 58       | Opsoclonus-myoclonus  | CT normal; EEG-  | Normal proteins, no cells, OCB-                    | No                                 | No  | Lingual carcinoma (4 mo before)        | No              | 3 → NA   |
| XXI     | F, 76       | Transitory low level of consciousness                                     | MRI normal; EEG-                                       | 1 g/L protein, 14 cells                            | SSA and SSB                        | No  | No                                     | No              | 5 → 4 <sup>b</sup> (1)   |

EEG-, normal; EEG+, temporal epileptic abnormalities. EMG + SNN, at least 1 sensory action potential (SAP) absent or SAP < 30% of the lower limit of normal in the upper limbs, and no more than 2 nerves with abnormal motor nerve conduction studies in the lower limbs, according to previously published criteria.<sup>31</sup> Abbreviations: Ab = autoantibody; AGO = Argonaute protein family; ANA = antinuclear antibody; AZA = azathioprine; CP = cyclophosphamide; F = female; FLAIR = fluid-attenuated inversion recovery; GABA<sub>B</sub>R = gamma-aminobutyric acid receptor B; GAD = glutamic acid decarboxylase; IVIG = IV immunoglobulin; LE = limbic encephalitis; LETM, longitudinally extensive transverse myelitis; M = male; MAG = myelin-associated glycoprotein; mRS = modified Rankin Scale; MTX = methotrexate; NA = not available; OCB = oligoclonal band; PEX = plasma exchange; RTX = rituximab; SCLC = small-cell lung cancer; SNN = sensory neuronopathy; SSA = Sjögren syndrome-related antigen A; SSB = Sjögren syndrome-related antigen B.

<sup>a</sup> Cancer diagnosis in relation to the neurologic diagnosis is provided in brackets.

<sup>b</sup> Previous paraplegia due to compressive myelopathy.

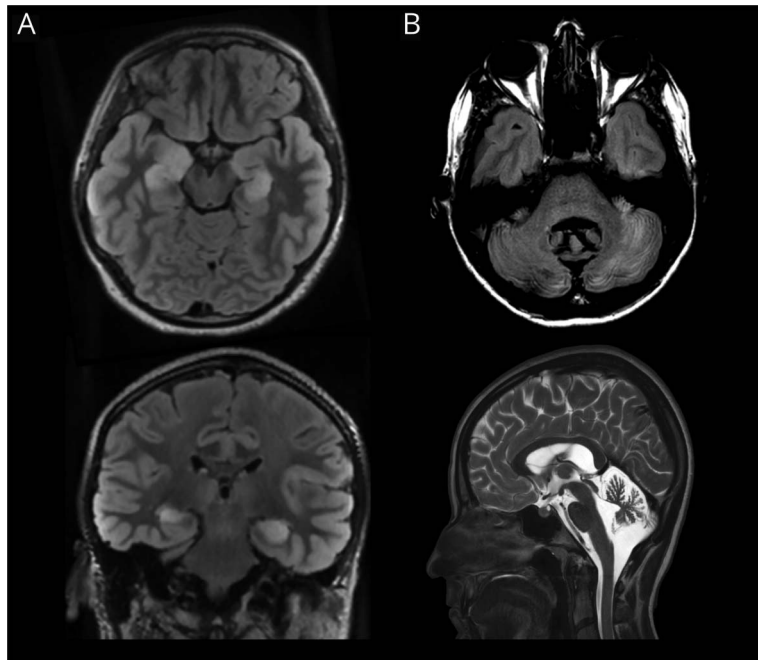
AGO1 and AGO2 compared with AGO3 and AGO4. The median serum titer of AGO-Abs was 40,000 (range [200–2,560,000]) and was higher than the median CSF titers (400; range [10–512,000]). The median AGO1- and AGO2-Ab CSF and serum titers were higher than the median AGO3- and AGO4-Ab titers (data not shown). These results, together with those from the protein microarrays and the AGO1/AGO2 immunoadsorption, indicate that the antibodies in the studied patients targeted mainly AGO1 and AGO2 rather than AGO3 and AGO4. AGO1/2-Abs in the CSF were predominantly of immunoglobulin G (IgG)1 subclass in the 8 patients analyzed, although other subclasses were also present in 4 patients; in the sera, IgG1 was also the main subclass in 11/12 patients (the remaining one being exclusively IgG3), but other subclasses co-occurred in 6 patients.

It was not possible to identify the binding region of the AGO-Abs by CBA with the 4 different subdomains of AGO, as transfection with any of the truncated mutants abrogated reactivity, while the full-length AGO-positive control was reactive (data not shown).

## Discussion

Using 2 complementary methods, we identified AGO proteins as the target of Abs in patients having autoimmune neurologic diseases of the peripheral and CNS, therefore suggesting that AGO-Abs might be potential biomarkers of autoimmunity.

AGO proteins constitute a highly conserved subfamily of 4 RNA-binding proteins (AGO 1–4) that plays a major role in RNA silencing pathways, repressing translation through the interaction with microRNAs and short interfering RNAs.<sup>14</sup> AGO proteins are present in cytoplasmic structures known as glycine/tryptophan (GW-) or processing (P-) bodies, which are involved in mRNA degradation and also include other components such as the TNRC6 proteins.<sup>14,15</sup> Of interest, several proteins implicated in RNA metabolism have been described as antigens of Abs detected in autoimmune systemic diseases, such as Sm or Ro proteins,<sup>16</sup> and even in patients with PNS because Hu and Ri antigens are RNA-binding proteins as well.<sup>17,18</sup> AGO-Abs, initially labeled as Su-



(A) Axial (top) and coronal (bottom) fluid-attenuated inversion recovery (FLAIR) brain MRI of patient XII with limbic encephalitis, showing bilateral swelling and hyperintensity of medial temporal lobes, extending also to a lesser degree to the lateral temporal cortex. (B) Axial FLAIR (top) brain MRI of patient XVII presenting with cerebellar ataxia, showing vermis atrophy and left cerebellar hemisphere hyperintensity. Pancerebellar atrophy is better demonstrated on the sagittal T2 brain MRI (bottom) of the same patient.

Abs, were reported in the serum of patients with systemic lupus erythematosus, scleroderma, Sjögren syndrome, and other rheumatologic autoimmune diseases.<sup>19-21</sup> The Su antigen was later identified by immunoprecipitation as the 100-kDa RNA-binding protein AGO2 and localized in the cytoplasmic GW/P-bodies.<sup>22,23</sup> More recent studies expanded the clinical associations of AGO-Abs to primary antiphospholipid syndrome and inflammatory myopathies,<sup>24,25</sup> reinforcing the link between AGO-Abs and systemic autoimmunity.

We report herein a series describing in detail the presence of AGO-Abs in patients with neurologic diseases. In a study investigating the clinical correlation of antibodies against several antigens of the GW/P-bodies, 2 of 6 patients with serum AGO-Abs had peripheral neuropathies and 1 patient with Sjögren syndrome had a nonspecified ataxia.<sup>26</sup> Nevertheless, only serum was analyzed, and no precise clinical descriptions were provided. In the present series, SNN and LE were the 2 most common clinical phenotypes, representing two-thirds of the cases, and of interest, Sjögren syndrome co-occurred more frequently in patients with SNN than in LE cases. AGO-Abs were also present in patients with other neurologic presentations (cerebellar syndrome, opsoclonus-myoclonus, and length-dependent polyneuropathies), resembling the varied clinical associations observed in patients with PNS and antibodies against intracellular neural antigens such as Hu.<sup>2</sup> The presence of inflammatory CSF abnormalities and the frequent co-occurrence of other Abs or autoimmune diseases also support the autoimmune nature of the neurologic conditions associated herein with AGO-Abs. Besides, the fact that 3 of 4 positive controls were

patients with Sjögren syndrome also supports the value of AGO-Abs as biomarkers of autoimmunity. Conversely, AGO-Abs seem not to be a paraneoplastic biomarker because only 5 of 21 patients had a cancer; moreover, there was a clear temporal relationship between the tumor and the neurologic disorder in only 3 of them, and in 1 case, AGO-Abs co-occurred with GABA<sub>B</sub>R-Abs already described to be strongly associated with SCLC. Moreover, no AGO-Abs were identified in a cohort of well-defined PNS with SCLC and Hu-Abs.

The initial identification of the target of AGO-Abs led to the observation of 2 immunoprecipitated bands, a 100-kDa band attributed to AGO and a 250-kDa band.<sup>20,23</sup> Likewise, we observed herein the same result and identified the 280-kDa band as TNRC6 proteins, whereas it was previously suggested to be the double-stranded RNA-specific endonuclease Dicer,<sup>23</sup> which is necessary to form the small RNAs bound to AGO proteins.<sup>14</sup> Furthermore, by developing a specific CBA, we showed that the Abs from the patients of the current series targeted only AGO proteins and not TNRC6. Moreover, AGO-Abs seem to be conformation specific<sup>21</sup> because Western blots did not show any signal and CBA was only reactive when the full-length AGO was transfected. Although, as previously reported,<sup>23</sup> AGO-Abs reacted against the 4 AGO proteins; AGO1 and AGO2 were the main targets based on the antibody titration, protein microarrays, and immunoadsorption results. This shared reactivity has been linked to the high homology among the 4 AGO proteins (reaching up to 80%), although an epitope spreading phenomenon might also explain this finding.<sup>23</sup>



To date, there are no available data on the potential effect of AGO-Abs on the pathogenesis of rheumatologic diseases.<sup>21</sup> Of interest, AGO-Abs have also been described in association with hepatitis C virus,<sup>27</sup> which has been shown to interact with AGO2 and inhibit RNA silencing processes.<sup>28</sup> It has been proposed that AGO2 could be modified through its binding to viral proteins leading to a loss of self-tolerance and AGO-Ab production.<sup>21</sup> In terms of pathogenic relevance in autoimmune neurologic diseases, it is unlikely that AGO-Abs play a major role, as is the case for other Abs against intracellular neural antigens.<sup>29</sup>

Overall, AGO-Ab prevalence among systemic autoimmune diseases is 10%–20% compared with less than 1% in an unselected population.<sup>30</sup> Thus, though not tightly associated with a single disorder, AGO-Abs appear as reliable biomarkers of autoimmunity. Likewise, our results revealed only 1 sample of the 856 screened controls being positive without evidence of neurologic or systemic autoimmune disease. Nevertheless, AGO-Abs were not frequently found in the retrospective cohort of high suspicion of AE/PNS and inflammatory neuropathies or in the prospective sample. In addition, a considerable subset of neurologic patients with AGO-Abs described herein also presented well-characterized Abs or co-occurring autoimmune diseases; thus, the diagnostic value of AGO-Abs seems restricted to selected cases.

In conclusion, we report herein AGO-Abs as potential biomarkers of autoimmunity in neurologic diseases, especially in patients with associated systemic diseases. In few patients, AGO-Abs might be the only biomarkers of the autoimmune origin of the neurologic syndrome.

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## Disclosure

The authors report no disclosures relevant to the manuscript. Go to [Neurology.org/NN](http://Neurology.org/NN) for full disclosures.

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## Appendix Authors

| Name                              | Location  | Contribution  |
|-----------------------------------|---|---|
| <b>Le-Duy Do, PhD</b>             | Hospices Civils de Lyon, and Universit  Claude Bernard Lyon 1, France                       | Study concept and design, acquisition of data, analysis and interpretation of the data, and drafted and revised the manuscript for intellectual content |
| <b>Christian P. Moritz, PhD</b>   | Universit  Claude Bernard Lyon 1, France; and Universit  Jean Monnet, Saint- tienne, France | Study concept and design, acquisition of data, analysis and interpretation of the data, and drafted and revised the manuscript for intellectual content |
| <b>Sergio Mu iz-Castrillo, MD</b> | Hospices Civils de Lyon, and Universit  Claude Bernard Lyon 1, France                       | Acquisition of data, analysis and interpretation of the data, and drafted and revised the manuscript for intellectual content                           |
| <b>Anne-Laurie Pinto, MSc</b>     | Hospices Civils de Lyon, and Universit  Claude Bernard Lyon 1, France                       | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content                                       |
| <b>Yannick Tholance, PhD</b>      | Universit  Claude Bernard Lyon 1, France; and University hospital of Saint- tienne, France  | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content                                       |
| <b>Sabine Brugiere, MSc</b>       | Universit  Grenoble Alpes, France   | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content                                       |

## Appendix (continued)

| Name                                       | Location  | Contribution   |
|--|---|--|
| <b>Yohann Couté, PhD</b>                   | Université Grenoble Alpes, France   | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content  |
| <b>Oda Stoevesandt, PhD</b>                | Babraham Campus Cambridge, United Kingdom   | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content  |
| <b>Michael J. Taussig, PhD</b>             | Babraham Campus Cambridge, United Kingdom   | Analysis and interpretation of the data and revised the manuscript for intellectual content  |
| <b>Véronique Rogemond, PhD</b>             | Hospices Civils de Lyon, and Université Claude Bernard Lyon 1, France   | Analysis and interpretation of the data and revised the manuscript for intellectual content  |
| <b>Alberto Vogrig, MD</b>                  | Hospices Civils de Lyon, and Université Claude Bernard Lyon 1, France   | Analysis and interpretation of the data and revised the manuscript for intellectual content  |
| <b>Bastien Jobert, MD, PhD</b>             | Hospices Civils de Lyon, and Université Claude Bernard Lyon 1, France   | Analysis and interpretation of the data and revised the manuscript for intellectual content  |
| <b>Karine Ferraud, MSc</b>                 | Université Claude Bernard Lyon 1, France; University hospital of Saint-Etienne, France  | Acquisition of data and revised the manuscript for intellectual content  |
| <b>Jean-Philippe Camdessanché, MD, PhD</b> | Université Claude Bernard Lyon 1, France; University Jean Monnet, Saint-Etienne, France; and University hospital of Saint-Etienne, France | Acquisition of data, analysis and interpretation of the data, and revised the manuscript for intellectual content  |
| <b>Jean-Christophe Antoine, MD, PhD</b>    | Université Claude Bernard Lyon 1, France; University Jean Monnet, Saint-Etienne, France; and University hospital of Saint-Etienne, France | Study concept and design, acquisition of data, analysis and interpretation of the data, drafted and revised the manuscript for intellectual content, and study supervision |
| <b>Jérôme Honnorat, MD, PhD</b>            | Hospices Civils de Lyon, and Université Claude Bernard Lyon 1, France   | Study concept and design, acquisition of data, analysis and interpretation of the data, drafted and revised the manuscript for intellectual content, and study supervision |

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