

Identification of adenylate kinase 5 antibodies during routine diagnostics in a tissue-based assay: Three new cases and a review of the literature

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ABSTRACT

Antibodies against adenylate kinase 5 (AK5) have been described in patients with non-paraneoplastic limbic encephalitis, mainly in men around 70 years of age. Routine testing with specific cell-based assays is not yet available. Three patients with episodic anterograde memory problems and depression had extensive limbic lesions and developed severe atrophy, mainly of the medial temporal lobes. The antibodies were identified in serum and CSF based on the typical staining pattern of AK5 antibodies on a tissue-based assay (here, unfixed mouse brain). Subsequently, they were confirmed by a research laboratory through a cell-based assay.

1. Introduction

In 2007, the Philadelphia group published in this journal on two patients in their seventies with limbic encephalitis who harbored immunoglobulin G (IgG) antibodies (abs) against the intracellular protein adenylate kinase 5 (AK5) (Tüzün et al., 2007), a protein that exerts metabolic neuron-specific functions (Ng et al., 2015). AK5 is a cytosolic protein. It is exclusively expressed in cerebral neurons and is down-regulated in patients and rodents with temporal lobe epilepsy (Lai et al., 2016; Van Rompay et al., 1999). The syndromic specificity of the AK antibodies was confirmed in 2017, when the group from Lyon/France reported on another ten patients with limbic encephalitis and AK abs (Do et al., 2017). It has become challenging for the neurologist and the laboratory doctor to keep up with the pace of detection of new specific neural antibodies. Panel tests for neural antibodies in form of immunoblots and cell-based assays have become available. However, these useful assays that permit testing for several antibody specificities in one test run cannot be updated as rapidly as desirable. Not all cases

with some suspicion of having “limbic encephalitis” but negative on the standard panels can be transferred for AK5 antibody testing to research laboratories. Here, we present another three cases of limbic encephalitis with AK5 antibodies. The recognition of the typical binding pattern of AK5 abs on a regularly performed tissue-based assay enabled the selection of samples for specific AK5 ab testing in a research laboratory. It is suggested that the report of typical binding patterns of newly described antibodies may help experts in diagnostic laboratories identifying patients at risk during a routine program.

2. Material and methods

2.1. Antibody diagnostics

Since 2011, CGB and CIB have run a diagnostic service for neural antibodies: until 2015 in the Epilepsy Center Bethel, since 2016 in the Laboratory Krone under the auspices of a fully accredited laboratory. The diagnostic approach has remained unchanged: A diagnostic

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“standard program” for patients with suspected autoimmune encephalitis, cerebellar disease, hyperexcitability syndrome or sleep disorder includes the following methods (as of 2017/2018): (1) immunoblot from detection of antibodies against the intracellular antigens Hu, Ma2, Ri, Yo, Sox1, CV2, Delta/Notch-like EGF-related receptor (DNER), Zic4, amphiphysin (Euroimmun, Lübeck, Germany, catalogue no. DL 1111-1601-7 G; protocol: as suggested by the manufacturer); (2) cell-based assays in form of Euroimmun biochips presenting the following antigens on fixed human embryonic kidney 293 cells: N-methyl-D-aspartate receptor, leucine-rich glioma inactivated protein 1, contactin-associated protein-2, glutamic acid decarboxylase 65 kD (GAD65), γ -aminobutyric acid-B receptor (GABA_BR), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) 2, dipeptidyl-peptidase-like protein-6, glycine receptor, metabotropic glutamate receptor 5, IgLON family member 5; an in-house protocol is used with a few modifications as compared to the manufacturer's suggestion, see (Bien et al., 2017); (3) tissue-based assay using unfixed 4- μ m sagittal mouse brain sections; the brains are not perfused (Euroimmun, Lübeck, Germany); serum is applied at a dilution of 1:40, CSF undiluted. The secondary antibody is goat-anti-human-IgG-Fc, Jackson ImmunoResearch, West Grove, PA, USA, 1.5 mg/ml, 1:100 (i.e., 15 μ g/ml). Both serum/CSF and the secondary antibody are incubated for 30 min at room temperature. As fluorochrome serves Alexa 594 (excitation: 590 nm, emission 619 nm). Immunofluorescence is visualized by a microscope Axio Scope.A1 (Zeiss, Jena, Germany) equipped with filter set 00 (excitation: bandpass filter 530–585 nm, emission: long pass filter 615 nm, dichroic beam splitter: 600 nm). The stainings are evaluated by experienced technical assistants; questionable or suspicious cases are reviewed by CIB or CGB. Samples suspicious for AK5 antibodies were tested through a cell based assay by JD (Tüzün et al., 2007).

2.2. Patients' data

Patients (#1, 2) and one patient's wife by power of attorney (#3) gave written informed consent to publication of clinical data and MR images. The data were obtained retrospectively from the hospital records.

3. Results

Based on the depiction of the AK5 antibody staining pattern on the tissue based assay in the Lyon series of ten patients with high-titer AK5 antibodies, CIB and CGB identified this pattern between March 2017 and February 2019 in three patients shows the staining of the neural cell bodies together with the neuropil-like aspect in the hippocampus. (Fig. 1) All three were confirmed through a cell-based assay (Fig. 2). There was no case of an erroneous suspicion of AK5 abs (i.e., suspected on tissue-based assay but not confirmed by cell-based assay). The three AK5-positive cases were identified out of 11,472 patients whose serum, CSF or serum-CSF pairs had been sent for comprehensive testing for neural antibodies in this two-year period, i.e. 0.026%. Interestingly, all three patients had been studied before by CIB and CGB and had been diagnosed as harboring “neuropil antibodies, target unknown” because the tissue-based assay (mouse brain) was positive with antibody binding to hippocampal neuropil but there was no binding to one of the HEK cells presented on the Euroimmun biochip. The ab binding to nerve cell bodies had been identified but in the absence of a known pattern like this had not been specifically integrated into the diagnosis. Upon re-evaluation of the previous samples, these produced the same pattern as at the time when the AK5 abs were confirmed. All serum and all CSF samples showed the AK5 ab staining pattern.

The demographic features, clinical courses, laboratory results and treatments of the three patients are given in Fig. 3. This figure also gives the timepoints of the available MRI studies, which are shown in Fig. 4. The serial MR images show extended hyperintense limbic lesions in

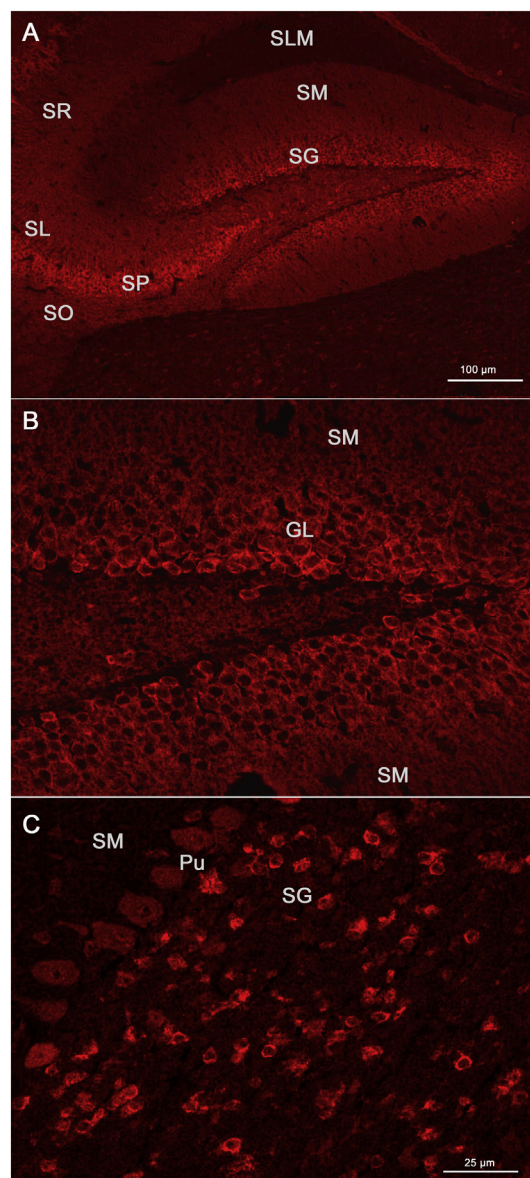


Fig. 1. Antibodies against adenylate kinase (AK5 abs): undiluted cerebrospinal fluid on a sagittal section of unfixed mouse brain (tissue-based assay), secondary antibody; anti-human immunoglobulin G heavy and light chain. (A) Hippocampus. Note the staining of the neural cell bodies in the stratum pyramidale (SP) and in the stratum granulosum (SG) of the dentate gyrus on the one hand and the neuropil aspect in the stratum molecular (SM) of the dentate gyrus, in the stratum radiatum et lacunosum (SR, SL) and in the stratum oriens (SO) but not in the stratum lacunosum-moleculare (SLM). Bar: 100 μ m. (B) Hippocampus, higher magnification of the tip of the endfolium and the surrounding dentate gyrus. This image shows the characteristic antibody binding to the intracellular aspect of the neural cell bodies. (C) Detail from the cerebellar cortex with stratum molecular (SM), the Purkinje cell layer (Pu), and the stratum granulosum (SG). Here, the intracellular binding to the neural cell bodies is particularly well visible. Bar (valid for B and C): 25 μ m.

early scans and severely atrophic brains (mainly temporomedially) in the long run.

There was no refined neuropsychological testing available in two of the three patients. They scored 12/18 (patient #2) and 10/18 (patient #3) in the DemTect, a broad dementia screening test. These results are interpreted as “mild cognitive impairment”. The patient with in-depth testing 6 months after onset had the following problems: temporally insufficient orientation; chronotaxis; memory difficulties; especially in encoding of new verbal information; reduced drive; blunted affect.

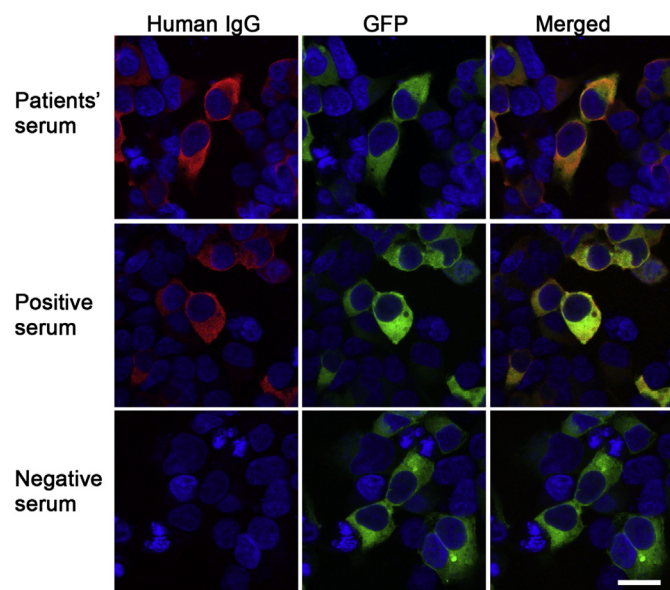


Fig. 2. Human embryonic kidney 293 cells expressing adenylate kinase 5 (AK5) carrying green fluorescence protein (GFP) were incubated with patient's serum (upper row), a positive control serum (middle row) and a negative control serum (lower row). The reactivity of human IgG was developed with a secondary fluorescence-labeled anti-human immunoglobulin G (IgG, column "human IgG"). The column "GFP" shows the fluorescence of AK5 carrying GFP. The column "Merge" shows the co-localization of patient's IgG with AK5 expression. Bar: 20 μ m.

No abnormalities in the following domains: language and speech; reading; cognitive speed; short-term memory (working memory); visuospatial performance; executive performance; cognitive flexibility.

The 3 patients did not experience epileptic seizures apart from patient #3 who had one generalized tonic-clonic seizure 4 years after disease onset in the context of a pneumonia.

The demographic, clinical, paraclinical, treatment and outcome data of these three patients together with those of the previous publications (Do et al., 2017; Ng et al., 2015; Tüzün et al., 2007) are summarized in Table 1.

4. Discussion

4.1. Novel findings

This study shows that AK5 abs can be detected on a tissue-based assay as long as no routine testing by cell-based assay is available. Research laboratories hosting HEK cells that can be transfected with the antigen will eventually confirm the suspicion. It is therefore suggested that such an assay is always included in routine diagnostics and is evaluated by experienced examiners who are aware of the AK5 staining pattern.

Another finding refines previous observations, too: The encephalitic brain lesions in AK5 ab-associated limbic encephalitis are extended and leave severely atrophic brains behind with accentuation on the medial temporal lobes. The extent of destruction is only paralleled by herpes simplex encephalitis and not by any known autoimmune encephalitis. AK5 abs - like onconeural antibodies - are directed against an intracellular antigen. An autopsy immunopathology study suggested that not the antibodies (which are directed to an inaccessible intracellular target) but rather cytotoxic T cells underly and sustain the encephalitic process (Ng et al., 2015). This immunopathology has also been described in encephalitides with antibodies against other intracellular targets (Bien et al., 2012; Popkirov et al., 2017).

Thirdly, all our cases had elevated CSF cells counts at first

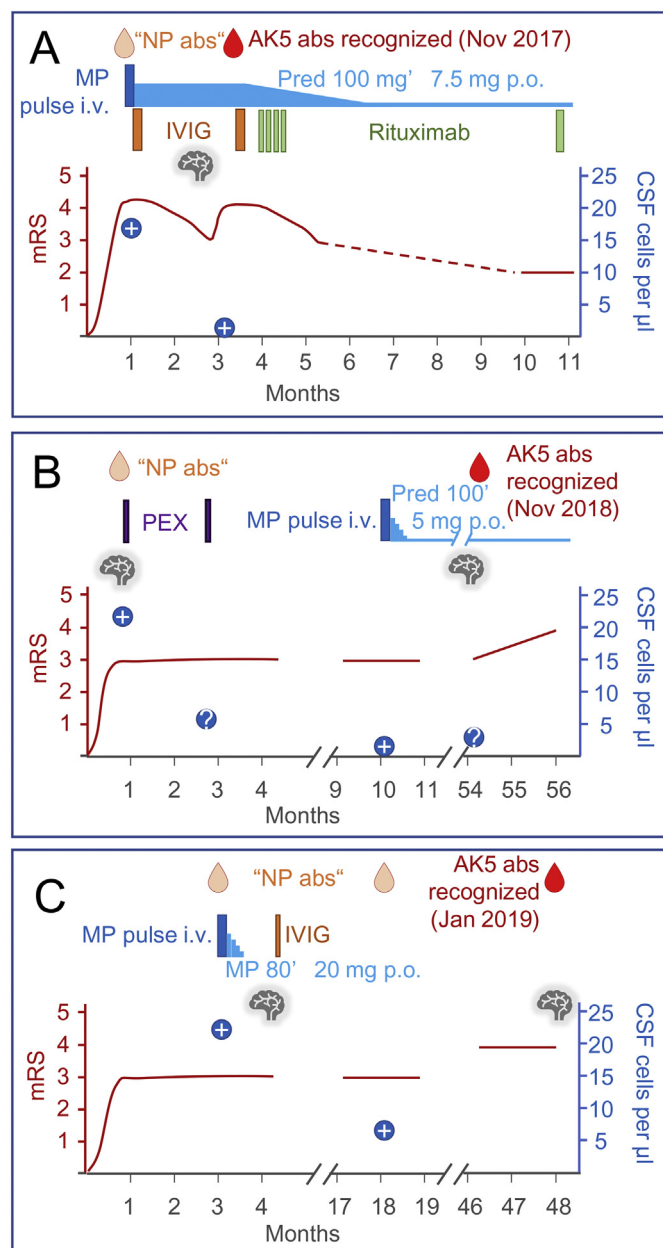


Fig. 3. Course of the three patients: modified Rankin Scale, immunological treatments, standard CSF findings (cells, intrathecal immunoglobulin G synthesis), neural antibody results. (A) patient 1; (B) patient 2; (C) patient 3.

Abbreviations: Abs = antibodies; AK5 = adenylate kinase 5; CSF = cerebrospinal fluid; i.v. = intravenous; IVIG = intravenous immunoglobulins; MP = methylprednisolone; mRS = modified Rankin scale; NP = neuropil; p.o. = per os; PEX = plasma exchange; Pred = prednisolone. The brain pictograms indicate the timepoints of the MRI studies shown in Fig. 4. The blue dots indicate the time points (on the x-axis) and the cell count (on the right y-axis) of the CSF studies. "+" means: intrathecal synthesis (autochthonous oligoclonal bands); "?" means: no information on intrathecal synthesis available. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presentation (around 20 cells/ μ l). Over the disease course, the cell counts went down, apparently unrelated to the extent of immunological therapy. The intrathecal synthesis of IgG, however, did not disappear (Fig. 3).

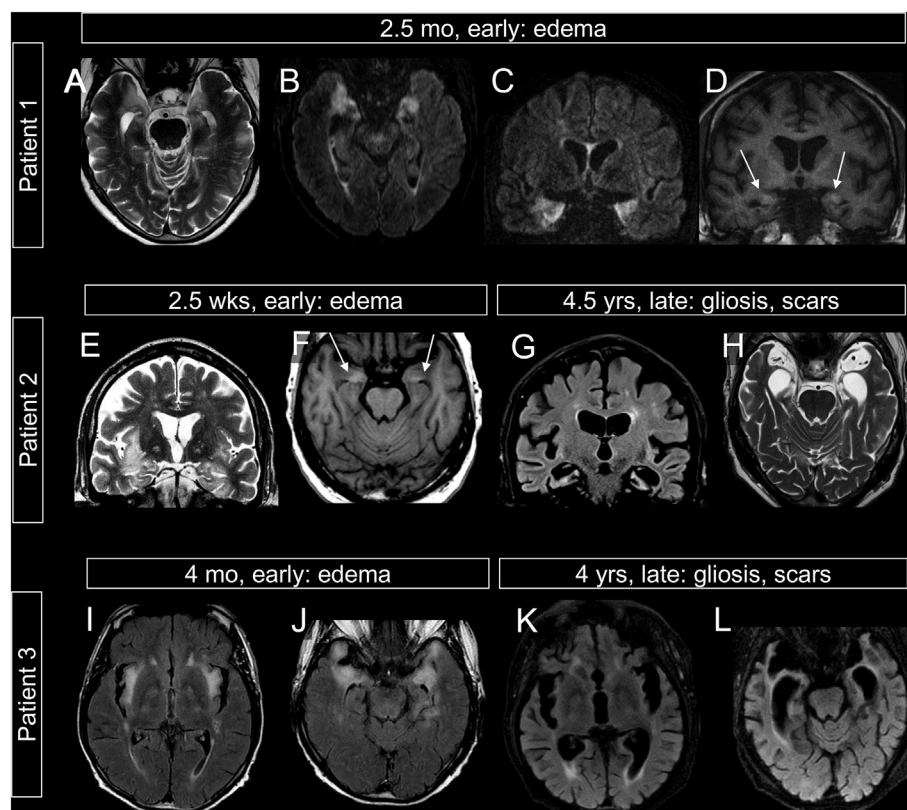


Fig. 4. Early and late MRI changes in patients with adenylate kinase 5 antibodies.

(A–D) Patient 1. (E–H) Patient 2. (I–L) Patient 3. (D, F): arrows: bilateral temporomedial hyperintensity on native T1 (probably corresponding to laminar necrosis). Sequences: T2 (A, E, H.); fluid-attenuated inversion recovery (B, C, G, I–L); T1 without contrast medium (D, F).

4.2. Confirmatory findings

AK5 abs are rare. The Lyon group found them as frequently as AMPAR or GABA_BR abs among 891 CSF samples from patients with “possible autoimmune encephalitis” (Do et al., 2017). Their exact frequency depends on the degree of patient preselection: Do and colleagues (Do et al., 2017) identified two of their ten cases within a cohort of 50 patients (4%) with (a) limbic encephalitis and (b) uncharacterized antibody staining patterns on tissue-based assays. Over the subsequent six months, they identified another two among 891 cases (0.22%) who were said to have “possible autoimmune encephalitis” according to internationally accepted diagnostic criteria (Graus et al., 2016). In our very recent unselected cohort from a routine diagnostic service, the frequency was only one tenth of this (0.026%). This probably reflects the increasingly liberal tendency to test patients for neural antibodies even “without the full supporting clinical features, in the often vain hope of identifying an atypical but immunotherapy-responsive disorder” (Dalmau and Vincent, 2017).

The clinical, CSF and MRI presentation of patients with AK5 antibodies is very consistent, also in this third small series coming from a laboratory independent from the previous two that reported on AK5 abs. All known cases have had non-paraneoplastic limbic encephalitis according to the Graus criteria (Graus et al., 2016), were mostly (73%) males around 70 years (range 57–80 years), had temporomedial FLAIR-signal increase on early MRIs (93%) and increase CSF cell count (80% of cases, mean/median of 17 cells/ μ l) and intrathecal IgG synthesis (92%). The AK5 abs were always present in CSF and serum; the Do study gave titers, which suggested that AK5 abs were in most cases intrathecally synthesized. Only 2/15 improved, the others remained stably impaired ($N = 7$) or even deteriorated ($N = 6$). This syndromic consistency surpasses that of other well-defined autoimmune encephalitides. Key symptoms are episodic anterograde memory problems and depression. The one case tested in depth clearly had this type of memory problems, similarly to the other patient with detailed neuropsychology reported (Ng et al., 2015). The cognitive problems can to

be so circumscribed mnemonic in nature that patients appear only “mildly impaired” on broad screening tests for dementia like the DemTect or the Mini Mental Status Test. Seizures are exceptional. Bilateral tonic-clonic seizures (but not focal seizures) occur occasionally in late stages.

4.3. Still unresolved issues

It remains open if one would detect more AK5 ab positive cases if a cell-based assay was accessible for routine diagnostics. Cell-based assays expressing intracellular antigens are feasible, e.g. with antigens Yo (Probst et al., 2015), GAD65 (Schou et al., 2016) or Rho-associated protein kinase 2 (Popkirov et al., 2017). Such assays require fixation of HEK cells with membrane-permeabilizing acetone. It cannot be excluded that one would detect low-titer AK5 antibodies in patients with more divergent phenotypes or even different (non-encephalitic) diseases, i.e., non-specific AK5 abs.

It is not quite clear how an antibody directed against an intracellular antigen can produce such a neuropil staining pattern in addition to the intracellular signal. Of note, CV2 and amphiphysin abs, also directed against intracellular targets, also give neuropil patterns in the hippocampus in addition to the well-known features visible in the cerebellum (own observations, unpublished).

Regarding therapy, the novel three cases have not clarified if early aggressive therapy [as advocated by some (Ng et al., 2015)] is related to improved outcomes and vice versa. No support for this comes from patients #2 and 3 who did not deteriorate in periods of 7 and 15 months without any immunotherapy. However, patient #1 with the most intense therapy improved and can now live largely independently despite ongoing memory problems.

4.4. Conclusion

It is of value if new antibodies are published including photos of the reactivities they produce on rodent brain, i.e., on tissue-based assays.

Table 1
Demographic, clinical and paraclinical data of the patients in the literature and in this series.

	Sex	Age at onset	Time to first presentation; symptoms	MRI	CSF: cells per μ l, signs of intrathecal IgG synthesis	Therapy	Most recent follow-up: time since onset; presentation
This series, pat. 1	F	68 y	3 w; anterograde amnesia, temporal disorientation, depression, severe lack of hallucinations	See Fig. 4	17, OCB +	Steroids, IVIG, rituximab (see Fig. 3)	11 m; back home, still memory difficulties, but can look after herself (improved)
This series, pat. 2	M	72 y	2 w; anterograde amnesia, temporal disorientation, depression	See Fig. 4	21, OCB +	PEX, steroids (see Fig. 3)	4½ y; lives with his wife, increasing lack of drive, severe memory problems
This series, pat. 3	M	74 y	3 m; anterograde amnesia, concentration difficulties, depression	See Fig. 4	23, OCB +	Steroids, IVIG (see Fig. 3)	4 y; lives with his wife, appears demented, had 1 GTCS
Pat. 1 (Tüzün et al., 2007), and (Ng et al., 2015)	M	71 y	?; rapidly progressing amnesia, delusional thoughts, confusion, agitation, aggressive behavior	Right temp-med T2/FLAIR signal†, mild contrast +	0, OCB +	Steroids, IVIG, PEX	2 y; severely demented, had one GTCS, died
Pat. 2 (Tüzün et al., 2007)	M	72 y	?; rapidly progressing confusion, personality change, difficulty recognizing familiar faces.	Bitemp-med signal† with contrast +	18, IgG index 0.92 (normal: < 0.7)	Steroids, IVIG	1 m; death due to neurological symptoms after having had several GTCS.
Pat. 1 (Do et al., 2017)	M	79 y	3 w; massive anterograde amnesia	Bilhippocampal, frontal and insular FLAIR signal†	8, OCB +	IVIG, Cyclophosphamide	18 m, dementia with anterograde amnesia, aphasia, anosognosia, amimia, apraxia
Pat. 2 (Do et al., 2017)	M	63 y	6 w; depression, subacute pure anterograde amnesia	Normal	2, OCB +	Steroids, IVIG, cyclophosphamide, rituximab	2 y; stable amnesia, prosopagnosia, spatial difficulties
Pat. 3 (Do et al., 2017)	F	69 y	6 w; depression, subacute anterograde amnesia	Bitemp-med and right insular FLAIR signal†	53, no data	Steroids, IVIG	1 y; dementia, aphasia, aggressiveness
Pat. 4 (Do et al., 2017)	M	71 y	3 w; anterograde amnesia, temporo-spatial disorientation, visual agnosia, apraxia, apathy	Bitemp-med FLAIR signal†	13, OCB +	IVIG, steroids, cyclophosphamide, rituximab, MMF	5 y; stable, pure anterograde amnesia
Pat. 5 (Do et al., 2017)	M	62 y	6 w; depression, subacute anterograde amnesia	Bitemp-med FLAIR signal†	4, OCB +	IVIG, steroids	5 y; stable, pure anterograde amnesia
Pat. 6 (Do et al., 2017)	M	57 y	3 w; subacute mild anterograde amnesia, depressive mood, anxiety attacks	Bitemp-med FLAIR signal†	29, OCB +	IVIG	8 mo; improvement; slight memory disturbances
Pat. 7 (Do et al., 2017)	M	71 y	3 w; subacute anterograde amnesia	Bitemp-med FLAIR signal†	6, OCB +	None	1 y; stable
Pat. 8 (Do et al., 2017)	F	64 y	?; anxiety, depression, confusion	Bitemp-med FLAIR signal†	9, no data	IVIG, steroids	1 y; stable
Pat. 9 (Do et al., 2017)	F	61 y	?; depression, anxiety, memory loss	Bitemp-med FLAIR signal†	23, no data	IVIG	6 m; stable
Pat. 10 (Do et al., 2017)	M	80 y	3 w; subacute anterograde amnesia	Bitemp-med FLAIR signal†, slight contrast +	26, OCB +	IVIG, steroids	1 m; stable
Summary N = 15	11 M (73%)	Mean 69, med 71, min 57, max 80 y	Mean 3, med 5, min 2, max 13	Temp-med signal + : 14/15 (93%)	Mean 17, med 17, min 0, max 53. > 5 cells/ μ l: N = 12 (80%). Intrathecal IgG synthesis: 12/13 (92%)		Follow-up: mean 24, med 12, min 1, max 60 mo. Deteriorated: N = 6 (40%), stable: N = 7 (47%), improved: N = 2 (13%)

Abbreviations: ? = unknown; bitemp-med = bitemporomedial; contrast + = contrast enhancement; F = female; FLAIR = fluid-attenuated inversion recovery; GTCS = generalized tonic-clonic seizure; IgG = immunoglobulin G; IVIG = intravenous immunoglobulins; M = male; m = month(s); max = maximum; med = median; min = minimum; MMF = mycophenolate mofetil; OCB = autochthonous oligoclonal bands in CSF; Pat. = patient; PEX = plasma exchange; signal† = signal increase; hyperintensity; temp-med = temporomedial; w = week(s); y = year(s).

This can help other labs to identify tentatively such cases and to send them for confirmation to research lab running a specific cell-based assay. AK5 abs are quite specific for limbic encephalitis in men around 70 years with dominant memory and mood problems in the absence of seizures and, especially, with lesions that extend beyond the hippocampi and leave behind severely destructed limbic areas comparable to herpes simplex encephalitis. Especially in such cases, a close look for the AK5 staining pattern is worthwhile.

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Declaration of interests (disclosures)

Dr. Steinke gave scientific advice to Boehringer Ingelheim (Ingelheim, Germany) and obtained honoraria for speaking engagement from Biogen (Cambridge, MA, USA), Boehringer Ingelheim, Merck (Darmstadt, Germany), Novartis (Basel, Switzerland) and Roche (Basel, Switzerland).

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The other authors have no disclosures.

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