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Encephalitis with radial perivascular emphasis

Not necessarily associated with GFAP antibodies

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Abstract

Objective

Autoimmune steroid-responsive meningoencephalomyelitis with linear perivascular gadolinium enhancement in brain MRI is regarded as glial fibrillary acidic protein (GFAP) astrocytopathy characterized by anti-GFAP antibodies (ABs). We questioned whether anti-GFAP ABs are necessarily associated with this syndrome.

Methods

Two patients with a strikingly similar disease course suggestive of autoimmune GFAP astrocytopathy are reported. Clinical examination, MRI, laboratory, and CSF analysis were performed. Neuropathologic examination of brain tissue was obtained from one patient. Serum and CSF were additionally tested using mouse brain slices, microglia-astrocyte cocultures, and a GFAP-specific cell-based assay.

Results

Both patients presented with subacute influenza-like symptoms and developed severe neurocognitive and neurologic deficits and impaired consciousness. MRIs of both patients revealed radial perivascular gadolinium enhancement extending from the lateral ventricles to the white matter suggestive of autoimmune GFAP astrocytopathy. Both patients responded well to high doses of methylprednisolone. Only one patient had anti-GFAP ABs with a typical staining pattern of astrocytes, whereas serum and CSF of the other patient were negative and showed neither reactivity to brain tissue nor to vital or permeabilized astrocytes. Neuropathologic examination of the anti-GFAP AB-negative patient revealed infiltration of macrophages and T cells around blood vessels and activation of microglia without obvious features of clasmatodendrosis.

Conclusions

The GFAP-AB negative patient had both a striking (para)clinical similarity and an immediate response to immunotherapy. This supports the hypothesis that the clinical spectrum of steroid-responsive meningoencephalomyelitis suggestive of autoimmune GFAP astrocytopathy may be broader and may comprise also seronegative cases.

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Glossary

AB = antibody; **GFAP** = glial fibrillary acidic protein; **IgG** = Immunglobulin G.

First described in 2016, autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy has been characterized as a rare CNS disorder, typically manifesting as a steroid-responsive encephalitis, meningitis, myelitis, or meningoencephalomyelitis. Neurologic symptoms such as (sub)acute encephalopathy, blurred vision, postural tremor, and seizures often occur following an initial prodromal phase with influenza-like symptoms.[1,2] A characteristic feature in brain MRIs is a linear perivascular gadolinium enhancement in the white matter extending radially outward from the ventricles. In addition, extensive lesions of the spinal gray matter may be detected.[1] No definite diagnostic criteria have been established yet.[3] For diagnosis of autoimmune GFAP astrocytopathy, detection of GFAP antibodies (ABs) in the patient's CSF or serum is required.[1] CSF cell count and CSF protein level are usually abnormal. Disease onset has been reported after influenza-like symptoms, a preceding herpes simplex encephalitis, and varicella zoster encephalitis, respectively.[1,4] Other ABs, e.g., NMDA receptor IgG and aquaporin-4 IgG may also be detected in autoimmune GFAP astrocytopathy. Furthermore, the disease can be related to an underlying malignancy, with ovarian teratoma being the most common.[1] The majority of patients improve after treatment with immunotherapy, especially corticosteroids.[2] Yet, the pathophysiology of autoimmune GFAP astrocytopathy is unknown. Here, we compare clinical, radiologic, and serologic findings of two patients with a very similar disease course suggestive of GFAP astrocytopathy. Despite intriguing similarity, only one patient harbored anti-GFAP ABs in serum and CSF. We here discuss that the characteristic clinical syndrome of autoimmune meningoencephalomyelitis with linear perivascular gadolinium enhancement may not necessarily be associated with anti-GFAP ABs.

Case report 1

During winter season, a 53-year-old Caucasian man was admitted to hospital due to influenza A infection. In the following weeks, the patient developed cognitive impairment, ataxia, tremor, and a left gaze–evoked nystagmus (for details, see table 1). Cranial MRI revealed areas with diffuse periventricular T2 hyperintensities and linear perivascular gadolinium enhancement in the supratentorial white matter extending radially outward from the ventricles (figure, A). CSF diagnostics revealed 86 cells/μL and 1,075 mg/L protein. High titers of anti-GFAP IgG ABs (titer 1:320) were found in CSF and serum. A typical staining pattern restricted to astrocytes could be detected after incubation of mouse brain slices and astrocyte and microglia cocultures with the patient's CSF. A confirmatory cell-based assay with the GFAPα isoform (Euroimmun, Lübeck, Germany) was positive when incubated with the patient's CSF (figure, B). After other differential diagnoses were ruled out (table 2), autoimmune GFAP astrocytopathy was diagnosed. The patient was treated with methylprednisolone 1000 mg/d for 5 consecutive days. A rapid clinical improvement and a reduction of anti-GFAP IgG AB titers (CSF 1:100, serum 1:100) could be observed. MRI follow-up revealed regressive gadolinium enhancement and decreased periventricular T2 hyperintensities. In addition, EEG follow-up showed improvement with basal alpha activity. When the daily oral prednisolone dose was reduced to less than 20 mg during the following months, the patient's condition deteriorated again as he developed tremor. We therefore initiated 6 cycles of immunoadsorption and subsequently started rituximab treatment. This led to a clinical improvement with almost complete remission of clinical symptoms within 2 weeks.

Case report 2

In the same month, a 63-year-old Caucasian man presented with influenza-like symptoms. He later developed cognitive impairment, aggressive behavior, ataxia, and apraxia (for details, see table 1). MRI revealed pronounced T2 hyperintensities and gadolinium enhancement extending radially along the vessels within the supratentorial white matter (figure, C). CSF analysis revealed 53 cells/μL and 2,130 mg/L protein. Repetitive testing for antineuronal, anti-myelin oligodendrocyte glycoprotein, and anti-GFAP ABs was negative including incubation of both the patient's serum and CSF in mouse and monkey brain slices, vital respectively fixed glia cocultures after permeabilization, and a GFAP cell-based assay (figure, D). As most differential diagnoses were ruled out (table 2), the patient underwent frontal brain biopsy. Biopsy featured parenchymal blood vessel associated infiltration of macrophages and T cells. Brain tissue itself showed activation of microglia, infiltration of macrophages, and astrogliosis (figure, E). Although not specific, histopathologic findings are compatible with the few available histology reports of proven cases of autoimmune GFAP astrocytopathy in the literature.[5] We did not observe direct signs of clasmatodendrosis or loss of aquaporin-4 (not shown), which is typically seen in anti–aquaporin-4 AB-positive neuromyelitis optica spectrum disorder.

Following treatment with IV immunoglobulins, the patient's condition deteriorated as he developed brainstem symptoms including dysarthria, dysphagia, and impaired consciousness. Artificial ventilation was required. Following methylprednisolone pulse therapy of 1000 mg/d for 5 consecutive days, a rapid clinical improvement was observed, and the patient could be extubated and was able to eat and walk again. Brain MRI confirmed a significant regression of T2 lesions and

Table 1 Clinical characteristics of case reports 1 and 2

Case report 1 (GFAP AB positive)	Case report 2 (GFAP AB negative)
53-year-old man	63-year-old man
Prodromal symptoms	
Upper respiratory tract infection with dry cough for several weeks (laboratory confirmed influenza A)	Upper respiratory tract infection with dry cough for 3 weeks during influenza season
Weight loss (10 kg in 4 weeks)	Weight loss (6 kg in 3 weeks)
Fever and night sweats	Fever
Fatigue	Fatigue
Neuropsychiatric symptoms	
Progressive personality change for several months with apathy	Progressive personality change for several months with apathy, confabulations, and aggressive behavior
Neurologic symptoms	
Left gaze–evoked nystagmus, ataxia in arms and legs, gait ataxia, intention and resting tremor of the upper limbs, and repetitive aphasia	Ataxia of arms and legs, gait ataxia, dysarthria, dysphagia, decreased consciousness, and apraxia
Neurocognitive symptoms	
Deficits in attention, word fluency, and memory	Deficits in attention, word fluency, abstraction ability, learning, and memory
Treatment	
Response to methylprednisolone	Deterioration after IVIG
Relapse after methylprednisolone tapering; stabilization additional treatment with immunoadsorption and rituximab	Response to methylprednisolone
	Relapse after methylprednisolone tapering
	Stabilization with immunoadsorption; additional treatment with azathioprine

Abbreviations: GFAP = glial fibrillary acidic protein; IVIG = intravenous immunoglobulin.

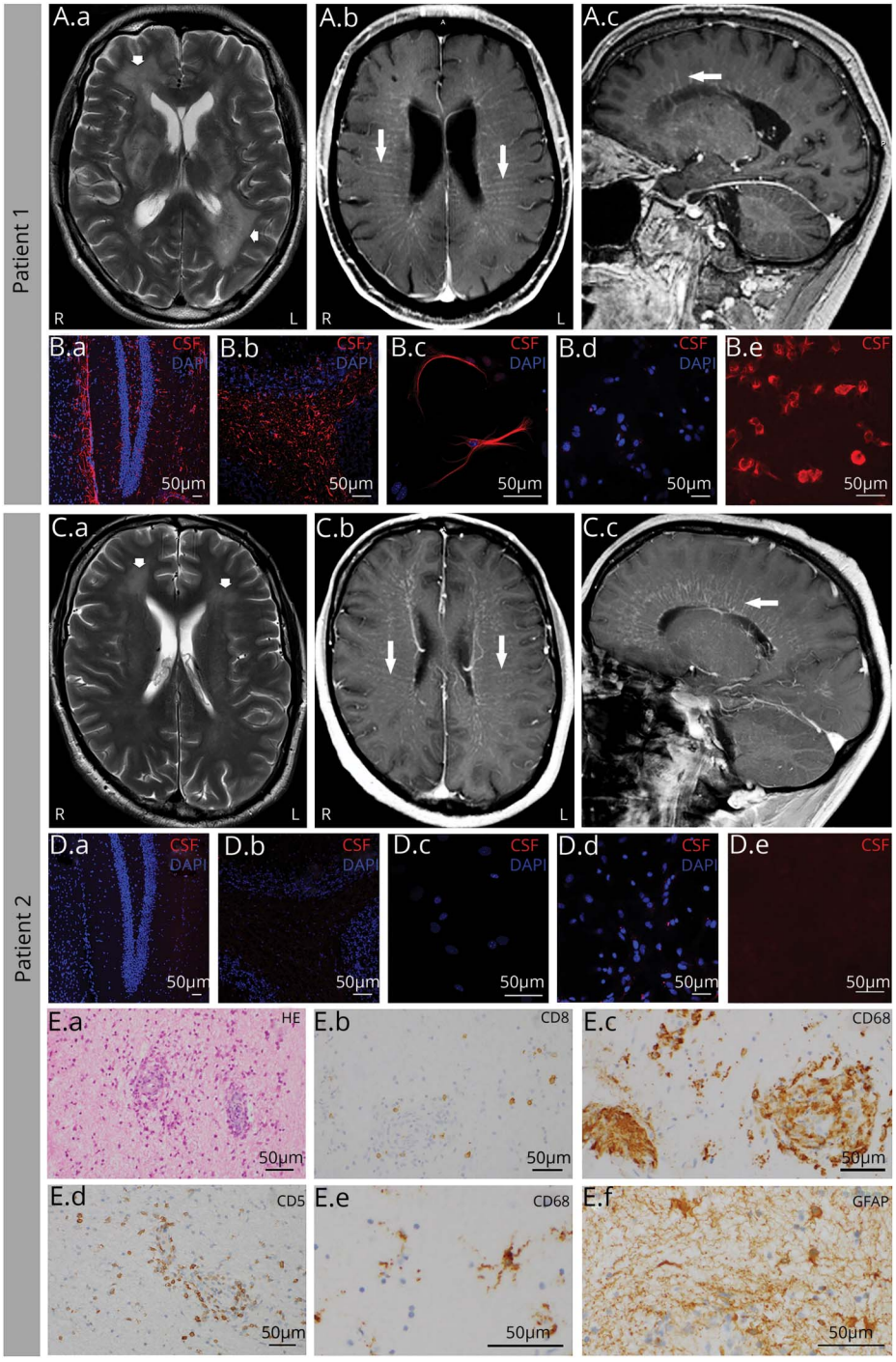
decreased radial perivascular gadolinium enhancement. Thus, oral prednisolone treatment was continued and tapered over the next months. EEG follow-up showed a significant improvement with basic alpha but still intermittent bifrontal delta activity. CSF measurement 3 months after first hospital admission revealed lower but still increased cell count (33 cells/μL) and protein levels (1,100 mg/L protein). Thereafter, 5 cycles of immunoadsorption were initiated with subsequent clinical improvement. However, after tapering prednisolone to less than 10 mg daily symptoms reoccurred, MRI demonstrated radial perivascular gadolinium enhancement, and CSF showed a persistent pleocytosis of 31 cells/μL. Additional treatment with azathioprine was initiated, and prednisolone was increased to 80 mg again and tapered over the following months.

Discussion

Detection of anti-GFAP ABs and typical MRI findings are regarded as essential features in the diagnosis of autoimmune GFAP astrocytopathy. We here compare two patients with a similar disease course suggestive of autoimmune GFAP astrocytopathy. Yet, only in one patient, anti-GFAP ABs were detected. Recent reports questioned the relevance of anti-GFAP ABs in this clinical syndrome.[3,6] In line with these reports, we here support the hypothesis that autoimmune meningoencephalomyelitis with characteristic MRI findings and steroid responsiveness may present with diverse immunologic findings, and the presence of anti-GFAP ABs is not obligatory. So far, it is not clear whether the presence of anti-GFAP-ABs in some patients with this disorder is just an immunologic accompaniment or whether these patients with anti-GFAP-ABs represent a particular subgroup with a specific pathophysiology targeting the astrocyte.

Because anti-GFAP ABs bind to astrocytic cytosolic intermediate filaments and ABs are not internalized, a direct pathophysiologic relevance of these ABs is unlikely. As known from autoimmune encephalitis and neuromyelitis optica spectrum disorders, pathophysiologically relevant ABs usually target membrane surface proteins.[7] In contrast, in paraneoplastic disorders, specific onconeuronal ABs to intracellular antigens are not pathogenic but excellent biomarkers.[7] In autoimmune GFAP astrocytopathy, a role of autoreactive T cells triggering a GFAP-specific autoimmune response has been suggested.[3] In

Figure Patients' MRIs, immunofluorescence, and histologic findings



Patient 1: (A.a) T2-weighted images demonstrate diffuse periventricular hyperintense lesions (thick arrows, A.a). Axial (A.b) and sagittal (A.c) T1-weighted images with gadolinium show linear perivascular enhancement extending radially through the periventricular white matter (thin arrows). (B) Characteristic staining pattern of GFAP-positive astrocytes in mouse brain slices incubated with CSF of patient 1 (B.a: hippocampus, coronal; magnification ×100; (B.b): Cerebellum, sagittal; magnification ×200). Incubation of astrocyte-microglial cocultures with CSF of patient 1 showed characteristic staining of astrocytes in fixed cells after permeabilization (B.c; magnification ×400), but not in vital cells (B.d; magnification ×200). Incubation of a cell-based assay transfected with the GFAPα isoform with the patient's CSF revealed a positive staining pattern (B.e; magnification ×200). Scale bars: 50 µm each. Patient 2: (C) brain MRI shows similar features as shown in A with periventricular T2 lesions (thick arrows, C.a) and linear perivascular gadolinium enhancement (thin arrows, C.b, C.c). (D) Immunofluorescence stains with CSF of patient 2 revealed no specific staining in brain tissue (D.a: Hippocampus, coronal; magnification ×100; D.b: cerebellum, sagittal; magnification ×200) nor in permeabilized (D.c; magnification ×400) and vital cells (D.d; magnification ×200) in astrocyte-microglial coculture. Incubation of a cell-based assay transfected with the GFAPα isoform with the patient's CSF did not show binding (D.e; magnification ×200). Scale bars: 50 µm each. (E) Histological analysis of patient 2 brain biopsy showed blood vessel–associated infiltration by hematopoietic cells (E.a; hematoxylin and eosin stain; magnification ×200; E.d; CD5-positive T lymphocytes; magnification ×200). Only scattered infiltration by single cytotoxic T cells was observed (E.b; CD8; magnification ×200). The majority of infiltrating cells were identified as macrophages (E.c; CD68; magnification ×200). Brain tissue showed microglial activation (E.e; CD68; magnification ×200) and astrogliosis (E.f; GFAP; magnification ×200), but no obvious signs of clasmatodendrosis. Scale bars: 50 µm each. GFAP = glial fibrillary acidic protein.

Table 2 Diagnostics of case reports 1 and 2 before treatment

Case report 1 (GFAP AB positive)	Case report 2 (GFAP AB negative)
Blood tests	
Negative, e.g., for vasculitis, viral and bacterial infections	Negative, e.g., for vasculitis, viral and bacterial infections (including Borna virus, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, and dengue virus), and toxoplasmosis
CSF	
Lymphocytic pleocytosis	Lymphocytic pleocytosis (predominantly T lymphocytes)
Increased albumin quotient (20.2)	Increased albumin quotient (19.8)
Increased lactate (2.5 mmol/L)	Increased lactate (3.4 mmol/L)
Initially, synthesis of IgG, IgM, und IgA; oligoclonal bands positive in CSF only	Initial intrathecal IgM and IgA synthesis; oligoclonal bands negative
Normal soluble IL2 receptor; negative viral DNA (CMV, HSV, VZV, EBV, and HHV6)	Normal soluble IL2 receptor; normal protein 14–33, JC virus PCR negative; negative viral DNA (CMV, HSV, VZV, EBV, and HHV6)
Autoimmune and onconeuronal ABs	
Negative (anti-mGluR1, anti-mGluR2, NMDA-R, GAD65, GABA-bR, Glycin-R, LGI1, CASPR2, amphiphysin, anti-Yo, Hu, Ri, anti-PNMA2, anti-SOX1, anti-recoverin, anti-CV2, anti-titin, and anti-DPPX)	Negative (anti-Hu, Ri, ANNA-3, Yo, Tr/DNER, Ma/Ta, GAD65, amphiphysin, aquaporin-4, MOG, NMDA-R, GABA-B receptor, LGI1, CASPR2, IGLON5, ZIC4, DPPX, CARP VIII, Glycin-R, mGluR1, mGluR5, GABA-aR, Rho GTPase activating protein 26, recoverin, GluRD2, flotillin, ITPR1, Homer 3, and neurochondrin)
PET/CT	
No tumor	No tumor
Electroencephalography	
Generalized, frontally emphasized theta activity with intermittent rhythmic delta activity	Bifrontal rhythmic delta activity

Abbreviations: AB = antibody; CMV = Cytomegalievirus; EBV = Epstein-Barr virus; GFAP = glial fibrillary acidic protein; HSV = Herpes simplex virus; IgA = Immunoglobulin A; IgM = Immunoglobulin M; VZV = Varicella-zoster virus.

a rat model, $CD3^+$ T cells have been shown in close proximity to GFAP-positive astrocytes, thereby inducing an immune response with production of IgG ABs to GFAP.[8] This might be a first direct link of how the humoral response is initiated. Of note, in our patients and also in others, an observational link exists between viral infections, e.g., influenza A, herpes simplex, and varicella zoster and subsequent GFAP autoimmunity.[1] The association of viral CNS infections and other forms of autoimmune encephalitis is well established. Here, antigen presentation due to neuronal damage caused by viral inflammation or virus-induced costimulation of immune cells is favored to be a pathophysiologic mechanism involved in promoting encephalitis.[9] Although influenza A infection does not necessarily lead to neuronal damage in the CNS, it may be a predisposing factor to develop GFAP autoimmunity.

The specificity of anti-GFAP ABs for meningoencephalomyelitis needs to be clarified in further studies, as serum anti-GFAP ABs can also be found in other diseases, e.g., traumatic brain injury, vascular dementia, and astrocytoma, or even in healthy controls.[10]

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Ha-Yeun Chung, MD	Jena University Hospital, Jena	Author	Drafted the manuscript and involved in treatment of the patients
Klaus Kirchhof, MD	Jena University Hospital, Jena	Author	Interpreted MRI data
David Boeckler, MD	Sana Hospital Borna, Borna	Author	Involved in treatment of the patients
Stefan Merkelbach, MD	Heinrich-Braun Hospital, Zwickau	Author	Involved in treatment of the patients
Peter Kuzman, MD	Leipzig University Hospital, Leipzig	Author	Provided neuropathology findings
Wolf C. Mueller, MD	Leipzig University Hospital, Leipzig	Author	Provided neuropathology findings
Christian Geis, MD	Jena University Hospital, Jena	Author	Supervised the project, involved in treatment of the patients, and writing of the manuscript
Albrecht Günther, MD	Jena University Hospital, Jena	Author	Supervised the project, involved in treatment of the patients, and writing of the manuscript

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