of small tight bands and spreading of polyclonal immunoglobulin. Monoclonal IgG produces a very specific ladder pattern of four to five bands equally spaced from each other on IEF, with descending intensity toward the anodic region of the blot.[11] The patient's IEF and immunoblot results demonstrated the typical polyclonal IgG spreading pattern (Fig. 1D: Lane 2,3) that could be easily distinguished from the typical ladder pattern of monoclonal IgG (Fig. 1D: Lane 4) and oligoclonal pattern (Fig. 1D: Lane 5). Subsequently, the SPE and IFE results were amended to polyclonal increase in gamma fraction, which was concordant with the clinical impression of IgG4-RD.

Although the interpretation of elevated IgG4 by SPE and IFE can be challenging in some sera, there are a few key points that should be considered. Firstly, beta-gamma bridging on SPE in this patient was strongly skewed toward the anodal region of the gamma fraction, which is consistent with the electrophoretic migration of IgG4. This can be clearly distinguished from the typical hypergammaglobulinaemia associated with infectious or inflammatory processes (Fig. 1A: Lane 3). It can also be distinguished from the beta-gamma bridging found in liver disease, which is associated with a polyclonal increase in IgA. Secondly, IEF is a useful technique to assist in identifying a focal band as polyclonal or monoclonal, when doubt exists after performance of SPE and IFE. Lastly, the sFLC kappa/lambda ratio plays an important role in distinguishing monoclonal from polyclonal hypergammaglobulinaemia. In patients with polyclonal hypergammaglobulinaemia or renal impairment, free kappa and lambda concentrations may increase due to increased immune production or decreased clearance. This results in an increase in both kappa and lambda equally, thereby maintaining a constant ratio. In contrast, only one of the FLC concentrations increases in monoclonal gammopathy, typically demonstrating a significant abnormal ratio. However, studies have shown that FLC ratio can be strongly abnormal in patients with IgG4-RD.[8,12] Thus, sFLC analysis may be less helpful than expected in differentiating polyclonal or monoclonal IgG4.

This patient's case was instructive on two levels: first the clinical misdiagnosis of sarcoidosis instead of IgG4-RD, then a laboratory misinterpretation of IgG4 biclonal gammopathy instead of a polyclonal IgG4 hypergammaglobulinaemia. We share this case to alert colleagues to the potential for misdiagnosis.

The accurate interpretation of polyclonal IgG4 is important for the diagnosis of IgG4-RD. Greater awareness of the difference in IgG subclass electrophoretic migration patterns in SPE and IFE is beneficial for the laboratory. In patients with markedly elevated polyclonal IgG4 (>5 g/L), a characteristic relatively focal, but restricted beta-gamma bridging pattern skewing to the anodal gamma fraction in SPE, is indicative for IgG4-RD. Highly sensitive and specific isoelectric focusing assay is helpful in differentiating polyclonal IgG4-RD from other gammopathies.

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Glial fibrillary acidic protein antibodies: a spectacular neuronal immunofluorescence pattern with important clinical implications

To the Editor,
Autoantibodies against intracellular antigen, glial fibrillary acidic protein (GFAP), were recently described in 16 patients presenting with meningoencephalomyelitis; a disease entity termed GFAP astrocytopathy.[1] To date, the presence of these antibodies has been described in a small cohort with heterogeneous clinical presentations.[2,3] Currently, no specific clinical or histological diagnostic criteria exist for GFAP astrocytopathy. Therefore, the presence of antibodies directed against GFAP are critical for diagnosis of this rare and emerging clinical entity.

Eight GFAP isomers have been described, with GFAP-α being the most common autoantibody target.[1,4] Laboratory methods for detection of GFAP-specific IgG include staining of neuronal tissues by either indirect immunofluorescence (IIF) or immunohistochemical methods while transfected human embryonic kidney 293 (HEK293) cell-based assay (CBA) or Western blot (WB) are used as confirmatory assays. The striking fibrillary staining pattern in the cerebellar tissue is characteristic for these autoantibodies, and when identified

should raise suspicion of GFAP antibodies. Neuronal IIF is routinely available in immunopathology laboratories and can be used to assess cerebrospinal fluid (CSF) and serum, providing a useful initial screen whilst antigen-specific confirmatory assays are not currently available in the routine diagnostic setting.

From the immunopathology laboratory perspective, there are still a few issues that need to be addressed. Firstly, the reporting of GFAP antibody pattern is not consistent among immunopathology laboratories in Australia and the neuronal IIF is currently available in 15 of the 85 centres. Secondly, the GFAP pattern is not represented in frequently used atlases of immunofluorescence patterns to date. Thirdly, a consensus on the requirement for a confirmatory laboratory method has not been reached. As such we aimed to raise awareness of this spectacular immunofluorescence pattern and its association with GFAP astrocytopathy and highlight the importance of neuronal autoantibody testing in the diagnosis of this neurological disorder.

We present three cases of GFAP astrocytopathy, in whom a diagnosis was based on the presence of suggestive staining using IIF on commercial neuronal slides according to the manufacturer's instructions (Inova Diagnostics, USA) and clinical correlation. Subsequently, antigen-specific testing by CBA (Euroimmun, Germany) confirmed this antibody specificity (Fig. 1). The clinical and laboratory findings are summarised in Table 1.

At present, IIF on neuronal tissue is a simple, cost effective and widely available means of screening for neuronal antibodies of multiple specificities. Patterns of staining can be identified by experienced readers, which in some instances are sufficiently specific to be reported (for example Hu, Ri, Yo), and in others require confirmatory testing. GFAP antibodies display a characteristic Bergmann radial pattern seen in pial, subpial, subventricular and perivascular locations of the cerebellum tissue substrate (Fig. 1).[1,5] Whilst there is some overlap with other neuronal antibodies, such as aquaporin 4 antibodies seen in neuromyelitis optica, the presence of staining in the cell body and end process throughout all layers of the cerebellum is specific for GFAP.[3] Ultimately, the specificity of the pattern needs to be confirmed by an antigen-specific confirmatory test which as yet is not available in the routine diagnostic setting.

We propose that the diagnostic laboratory algorithm should include this step, especially when less typical patterns of staining are seen on IIF. Until fast, reliable and cost effective confirmatory assays are available in diagnostic laboratories, this pattern cannot be reported with certainty, ultimately limiting the number of laboratories routinely reporting this pattern.

Controversy still exists regarding the clinical relevance of staining seen in serum and CSF samples, for establishing a diagnosis of GFAP astrocytopathy. Recent reports suggest that GFAP antibody positivity in CSF may represent a more important clinical finding[6,7] owing to the association with meningoencephalitis.[2,3] On the contrary, Dubey *et al.* demonstrated that the majority of patients in their cohort had detectable GFAP antibodies in both the serum and CSF.[7]

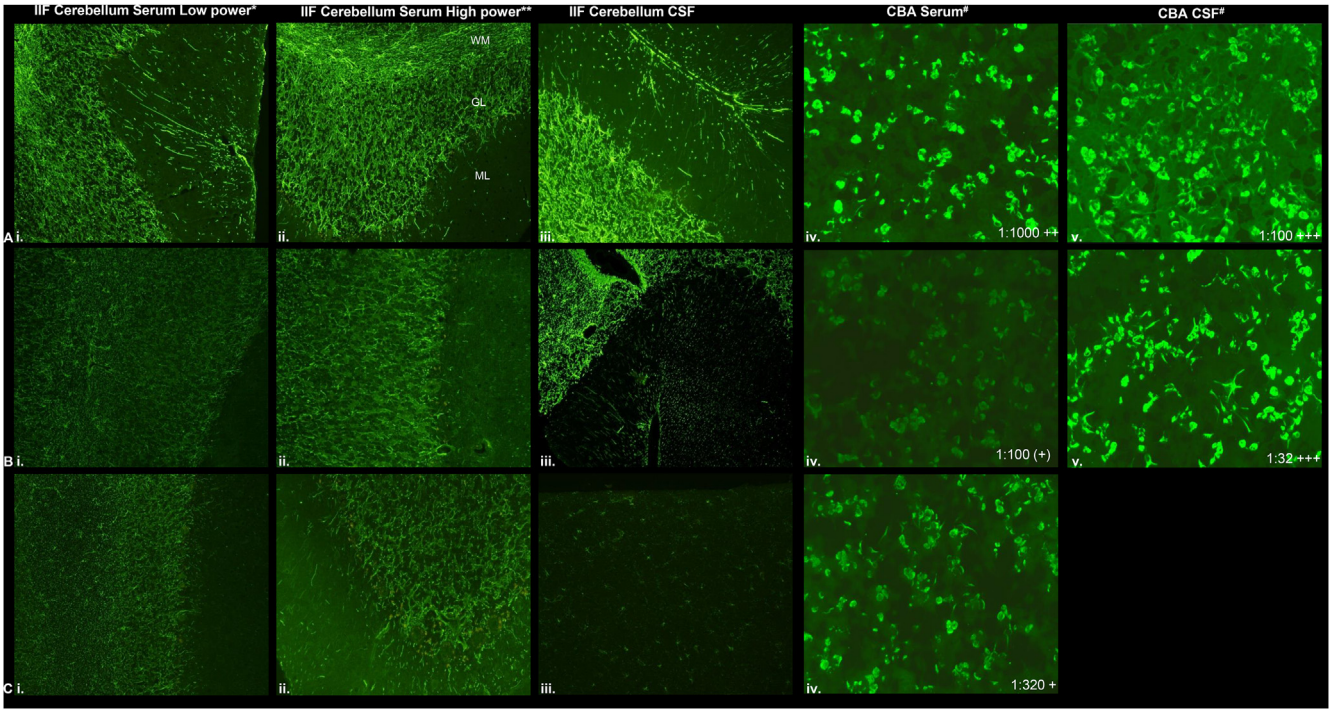

Fig. 1 Indirect immunofluorescence (IIF) on rat cerebellum and cell based assay (CBA) findings for the three cases. (A) Case 1. (i) Strong linear fibrillary staining in molecular and granular layers. (ii) Staining in granular layer and white matter. (iii) Staining in granular layer and along the vessel sparing Virchow−Robin space. (iv) Moderate positive ++ 1:1. (v) Strong positive +++ 1:100. (B) Case 2. (i) Moderate fibrillary staining in molecular and granular layer. (ii) Moderate staining in granular layer. (iii) Strong staining in granular and molecular layer along vessels sparing Virchow−Robin space. (iv) Trace positive (+) 1:100. (v) Strongly positive +++ 1:32. (C) Case 3. (i) Moderate fibrillary staining in granular and molecular layers. (ii) Moderate staining in granular and molecular layer. (iii) Negative. (iv) Weak positive + 1:320. (v) Weak positive + 1:1 (image not available). *Low power 10× magnification **High power 20× magnification. (iv/v) CBA dilutions noted in lower right corner. GL, granular layer; ML, molecular layer; WM, white matter.

Table 1 Clinical findings of three cases of GFAP astrocytopathy

Case	Age/sex	Clinical phenotype	MRI	CSF	Treatment and progress
1	26 F	Meningoencephalitis	Brain: linear perivascular radial enhancement of white matter perpendicular to ventricle Spine: cord myelitis	Marked pleocytosis with elevated protein	Initial response to systemic glucocorticoids, IVIG and azathioprine Levetiracetam for focal seizure Rituximab for relapse at 8 months
2	63 M	Encephalopathy	Brain: unremarkable	Moderate pleocytosis with elevated protein	Clinical improvement with prednisolone
3	73 F	Peripheral neuropathy	Brain and spine: unremarkable	No pleocytosis with increased protein	Conservative management

CSF, cerebrospinal fluid; F, female; IVIG, intravenous immunoglobulin; M, male; MRI, magnetic resonance imaging.

Whilst the lack of autoantibodies in the CSF usually goes against their pathogenic role in CNS disease, it is proposed by Castillo-Gomez *et al.* that the CNS can act as a substrate binding pathological antibodies and preventing their breach of the extracellular matrix-CSF barrier.[8] This may set the scene for central effects of an autoantibody with CSF negativity.[8] Whilst our small sample size limits conclusions in this area, considering the typical diagnostic laboratory algorithm for neurological autoantibody testing,[9] we propose the importance of testing on both serum and CSF samples to improve diagnostic yield.

In conclusion, GFAP astrocytopathy is a newly described neuroinflammatory disorder, the diagnosis of which can be challenging and is strongly supported by neuronal autoantibody testing. IIF on both CSF and serum is an ideal screening modality but requires experienced readers familiar with the specific staining pattern, whilst confirmatory tests such as CBA are likely to play an important and emerging role in this condition.

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CPSF6-RARG-positive acute myeloid leukaemia resembles acute promyelocytic leukaemia but is insensitive to retinoic acid and arsenic trioxide

To the Editor,
Acute promyelocytic leukaemia (APL) is a special subtype of acute myeloid leukaemia (AML) which features promyelocytic leukaemia cells, prominent coagulopathy, *PML-RARA* fusion generated by t(15;17)(q24;q21), and high cure rate in response to all-trans retinoic acid (ATRA) plus arsenic trioxide (ATO) and/or chemotherapy.[1] In <5% of morphologically defined APL, *PML-RARA* is absent. In addition to *GTF2I-RARA* reported by our group,[2] 16 variant *RARA* fusion genes have been described in patients with different sensitivities to ATRA.[1,3] Moreover, rearrangement involving *RARB* and *RARG*, the other two members of the retinoic acid receptor (RARs) family, has been reported in a group of APL-like leukaemia (APLL) patients, such as *TBLR1-RARB,*