Online supplementary tables

Table e1: Summary of imaging findings in Pattern 1

	Cerebral white matter	Cerebellar white matter	Deep gray structures	Brainstem	Cranial nerves, cord, cauda equina, meninges
Lesion incidence and types, number, white matter level	77/123 (63) Diffuse: 34/77, (44) Commonest level: deep +periventricular: 16(47) Focal: 60/77, (78) Commonest level: Subcortical + deep: 34 (57) Number: Multiple (>3): 39 (65)	71/123 (58) Diffuse: 47/71 (66) Focal: 41/71 (58) Bilateral: 46/47 (98) Asymmetrical: 38/47 Symmetrical: 9/47 Number: Multiple (>3): 17 (41)	35/123 (28) Unilateral: 11/35 (31.4) Bilateral: 24/35 (68) Basal Ganglia: 28/35 (80) Thalamus 33/35 (94)	34/123 (28) Pons: 34/34 (100) Medulla: 9/34 (7) Midbrain: 32/34	CN: 5/123 (multiple, bilateral) Cord, cauda equina: 3, 1 Meninges: 18/123
DWI	22/77 (28)	21/71 (27)	2/35 (6)	-	1 (optic nerve)
Enhancement incidence and patterns	40/77 (52) Diffuse: 9/34 (26) Commonest type: Perivascular: 6 (67) Focal: 36/60 (60) Commonest type: Nodular/Homogenous: 31 (86) Targetoid pattern: 6	43/71 (60) Diffuse: 36/47 (76) Commonest type: Perivascular: 16 (44) Focal: 33/41 (80) Commonest type: Nodular/Homogenous: 33 (100) Targetoid pattern: 4	4/35 (11)	8/34 (23) (nodular: 7, ring: 1)	CN: 5/5 (multiple, bilateral) Cord and cauda equina: 3/3 Meninges: 18/123

Table e2: Etiology and spectrum of mutations

Etiology			
Primary (40/57,70%) FHLH2 (*PRF1*): 14/40, FHLH3 (*MUNC 13-4/UNC13D*): 8/40, FHLH4 (*STX11*): 1/40, Griscelli (*RAB27A*): 1/40, XLP1 (*SH2D1A*): 4/40, XLP2 (*XIAP*): 2/40, Chediak – Higashi (*LYST*): 1/40 Presumed primary after exclusion of secondary causes: 9/40		Secondary (17/57, 30%) Infection: 10/17, Autoimmune/ Rheumatological: 6/17, Malignancy: 1/17	
Identified Mutations			
	FHLH2 (n=13)	FHLH3 (n=7)	OTHERS (n=5) (XLP1 = 3, XLP 2 = 1, Griscelli: 1)
Number of mutations	17	12	6
Missense	12	3	1
Nonsense	1	-	2
Frameshift	4	8	-
Splicing	-	1	-
Deletion	-	-	3

Table e3: Genetic variants in each mutation group

Atleast 1 Disruptive mutations (frameshift, nonsense, deletions)			
Case ID	Gene	Variant	
64	PRF1	c.50delT (p.Leu17fs)	frameshift
34	PRF1	c.50delT (p.Leu17fs)	frameshift
32	PRF1	c.50delT (p.Leu17fs)	frameshift
45	PRF1	c.563dup.p(Leu189Alafs*4); c.649T>Cp.(Ser217Pro)	frameshift, missense
43	PRF1	c.1179 C>A (p.C393X)	frameshift
50	PRF1	c.148G>A (p.Val50Met); c.1122G>A (p.Trp374Ter)	missense, nonsense
31	UNC13D	c.1800_1807delTACAACG, p.T600fs	frameshift
53	UNC13D	c.2437_2439 frameshift deletion; c.2831-13G>A intronic frameshift mutations	frameshift
52	UNC13D	c.2437_2450delins7-->fs; c.2831-13G>A-->site broken-->fs	frameshift
11	UNC13D	c.762delC, p.C255AfsX73; c.3049G>A, p. Glu1017Lys	frameshift, missense

29	UNC13D	c.2866C>T, p.Pro256Ser; c.3053_3055delCC, p.Ala1018Valfs	missense, frameshift
39	UNC13D	c.753+1G>T, c.2346_2349delGGAG (p.Arg782SerfsTer12)	splicing, frameshift
36	SH2D1A	Hemizygous deletion in exon 2	deletion
35	SH2D1A	Hemizygous deletion in exon 2	deletion
42	SH2D1A	Deletion in SH2D1A	deletion
60	RAB27A	c.550C>T (p.Arg184*); c.259G>C (p.Ala87Pro)	nonsense, missense
37	XIAP	Duplex mutation in exon 8: c.1449_1453dup (p.Tyr485CysfsTer20)	nonsense
Missense mutations with absent protein expression			
61	PRF1	c.386G>C (p.Trp129Ser)	missense
59	PRF1	c.386G>C (p.Trp129Ser)	missense
47	PRF1	c.133G>A, (p.Gly45Arg)	missense
51	PRF1	c.260G>C (p.Arg87Pro), c.470T>G (p.Phe157Cys)	missense
22	PRF1	c.1349C>T, p.T450M	missense
Missense mutations with reduced but residual protein expression			
57	PRF1	c.694C>T (p.Arg232Cys), c.731T>G (p.Leu244Pro)	missense
62	PRF1	c.116C>A(p.Pro39His)	missense
48	UNC13D	c.2896C>T, p.Arg966Trp	missense

Recurrent mutations in table e3:

Atleast 1 disruptive

- PRF1 c.50del(p.Leu17Argfs*34), n =3;

- UNC13D c2437_2439 frameshift deletion and c2831-13G>A intronic frameshift mutation, n = 2;

Missense with absent protein expression

- PRF1 c.386G>C (p.Trp129Ser), n = 2

Table e4: Etiology, imaging characteristics and outcomes in sub-pattern 1.2

Pat. ID	Gene/ etiology	NK Cell Perforin expression/ CD107a	At what point in illness,	Clinical	Symmetry	Nodule size (T1-C+)	T2 signal extending beyond T1	Lesion evolution on follow - up	Treatment, Outcome

		Degranulation expression (abnormal value, control value)	systemic HLH status				enhancing nodule		
38	Heterozygous variant of unknown significance in STX11. No mutations in other HLH genes	Normal/ absent (0%,3.8%)	7months after systemic HLH onset.	S, E, diplopia, exaggerated reflexes	As	4mm	Yes	Multifocal, diffuse	HLH-04, IT MTX, HSCT – stabilisation followed by worsening, death
57	*PRF1* Compound heterozygous missense c.694C>T p.(Arg232Cys); c.731T>G p.(Leu244Pro)	Decreased (24%, 51%)/ normal	At onset, preceding systemic HLH.	G, S, L, D	S	3mm	Yes	Multifocal, diffuse	Steroids, IVIG, alive
60	*RAB27A*(Griscelli syndrome) Compound heterozygous c.550C>T p.(Arg184*) nonsense c.259G>C p.(Ala87Pro) missense	NA	At onset, preceding systemic HLH.	G, L diplopia	As	5mm	Yes	Restricted to brainstem	HLH-04, IT MTX – clinical and radiological response, alive
31	*UNC13D* Homozygous deletion *c.1800_1807delTACAACG, p.T600fs*	Normal/Absent (0%,15%)	3months after systemic HLH onset.	S, irritability	S	4mm	Yes	No follow-up	HLH-04, death
30	Presumed primary	NA	12months after systemic HLH	G, S, M, D	As	10mm	Yes	No follow-up	Non responsive to steroids, HLH-04 could not be initiated, death
47	*PRF1* Homozygous missense c.133G>A, p.Gly45Arg	Absent (0%)/normal	Systemic and CNS HLH already present, BS lesions developed with progression	S, L	As	5mm	Yes	Multifocal, diffuse	HLH-94, IT MTX, death

(Abbreviations: G: gait difficulty, D: dysarthria, M: meningismus, S: seizures, E: encephalopathy, L: limb weakness, As: asymmetric, S: symmetric, NA: not available)

Table e5: Etiology, imaging characteristics and outcomes in sub-pattern 1.3

Pat. ID	Gene/etiology	NK Cell Perforin expression/ CD107a Degranulation expression (abnormal value, control value)	At what point in illness	Clinical	Status of rest of the white matter	Status of systemic HLH	Evolution of lesions
58	*UNC13D* (Genetic variants unavailable)	Normal/ Decreased (8%/15%)	At onset, limited to CNS	G,H,E,D	Uninvolved at presentation, TD	Not involved at this presentation, subsequently present	Multifocal white matter lesions
2	Recurrent histoplasmosis	NA	3 years into systemic HLH	S, E, diplopia, increased tone	Lesions present	Involved	Lesion Resolution with atrophy
8	Presumed primary	NA	At onset	G,H,E, F	Uninvolved, TD, Hy	Involved within 2 weeks	Multifocal cerebral and cerebellar lesions
11	*UNC13D* Compound heterozygous *c.762delC, p.C255AfsX73* (frameshift) *c.3049G>A, p. Glu1017Lys* (missense)	NA	At onset	G,H,E, F	Uninvolved, TD, Hy	Found to have Systemic involvement	Multifocal cerebral and cerebellar lesions
22	*PRF1* Homozygous missense c.1349C>T, p.T450M	Absent (0%)/normal	3 years into systemic HLH	G,H	Cerebellar, BS lesion, TD, Hy	Involved	Recurrent cerebellitis Multifocal cerebral, BS, cerebellar lesions
32	*PRF1* Homozygous frameshift *c.50del(p.Leu17ArgfsX34)*	Absent (0%)/ normal	At onset	G, H, S	Uninvolved, TD	Found to have Systemic involvement	Recurrent cerebellitis, Diffuse cerebellar lesions

(Abbreviations: G: gait difficulty, H: hypotonia, F: fever, S: seizures, E: encephalopathy, N: nystagmus, Hy: hydrocephalus, TD: tonsillar descent, NA: not available)

Table e6: Mutation and imaging pattern age based sub analysis

Age of onset < 12 months		
	Pooled mutation group (n = 16)	Missense mutations with residual protein expression
Pattern 1.1	4	0
Pattern 1.2, 1.3	2	0
Pattern 2	10	0
Age of onset > 12 months		
	Pooled mutation group (n = 6)	Missense mutations with residual protein expression (n = 3)
Pattern 1.1	0	3
Pattern 1.2, 1.3	3	0
Pattern 2	3	0

Online supplementary figures

Online Figure legends

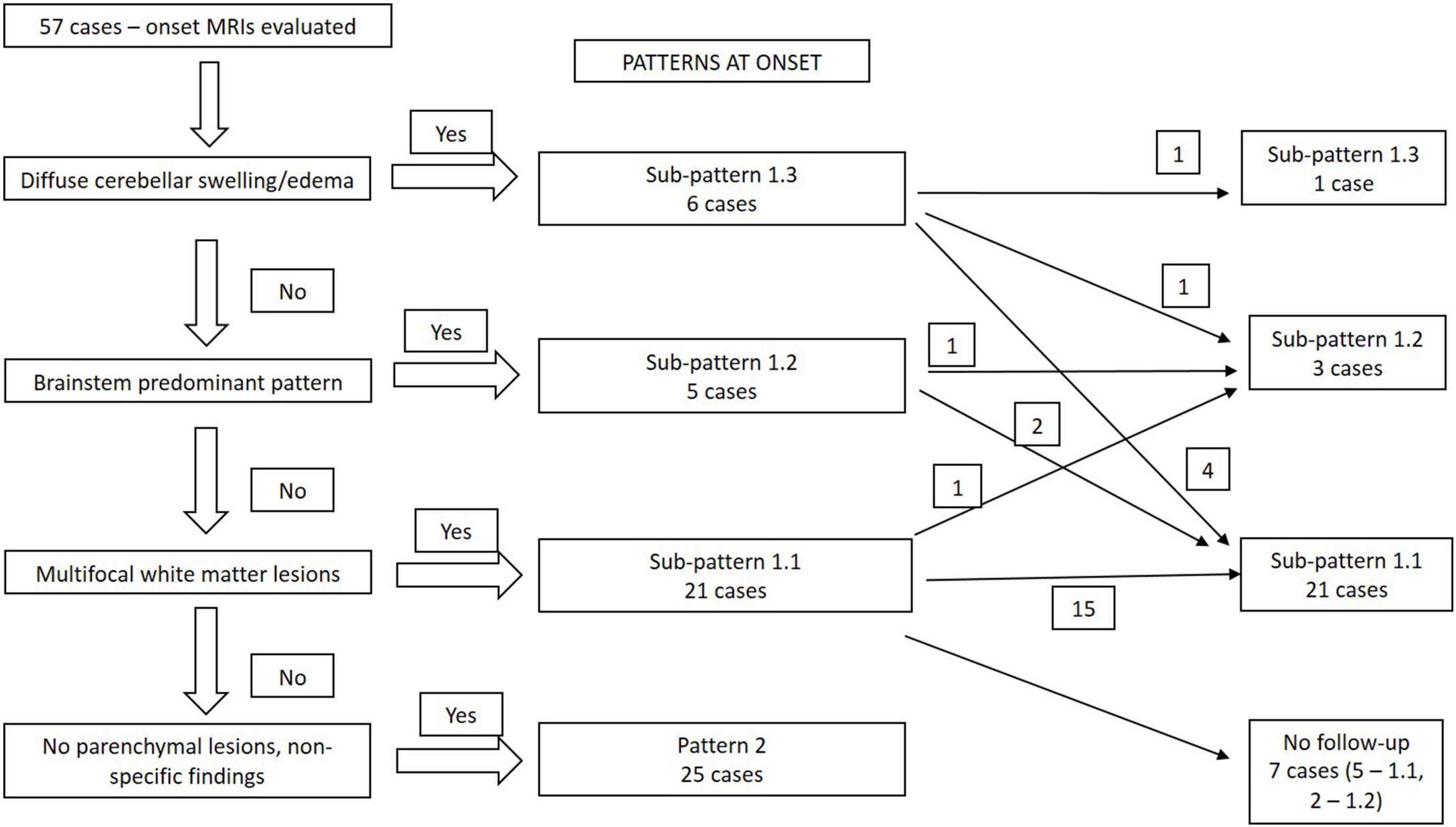
Fig e1: Flow chart of the case allocation to onset MRI pattern groups and subsequent follow-up

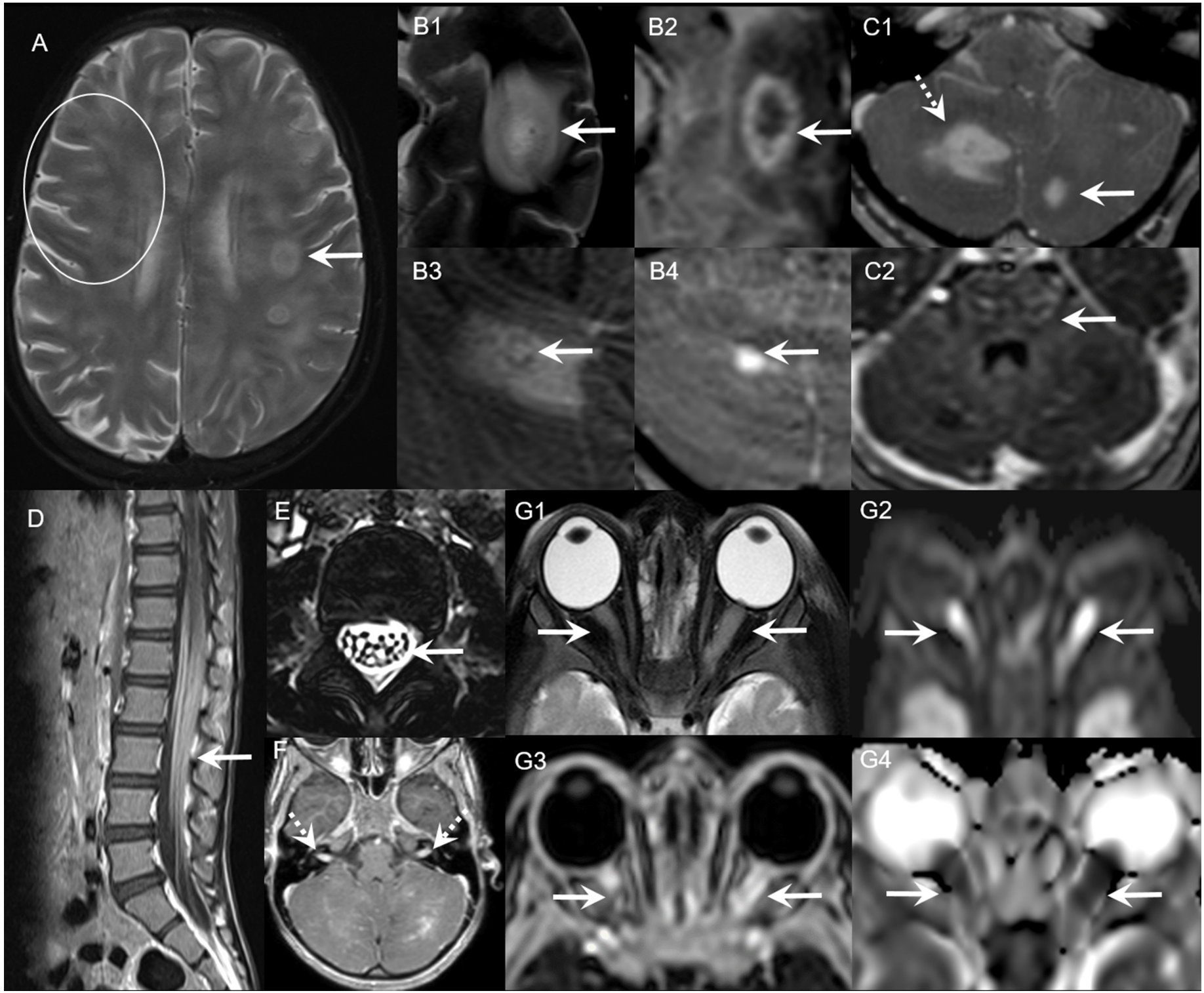
Fig e2: Axial T2-WI (A, B1, B3, E, G1), T1 post-contrast (B2, B4, C1-2, D, F, G3) and DWI (G2) and ADC (G4) images. Image A shows examples of diffuse (white circle) and focal lesions (arrow). Image B1-4 shows target lesions with trilaminar appearance and ring (B1,2) and core enhancement (B3,4, white arrow). Images C1-2 show nodular (solid arrow, C1), homogenous (dashed arrow) and perivascular patterns of enhancement (solid arrow, C2). Diffuse cauda equina thickening and enhancement are noted in images D, E. Bilateral 7^{th}/8^{th} and 2^{nd} cranial nerve enhancement is seen in Images F, G3 respectively. Bilateral optic nerve swelling with diffusion restriction and protrusion of the optic nerve head are noted in images G1-2, G4.

Fig e3: Images A1-4, B1-4 summarise findings of sub-pattern 1.1 (multifocal white matter lesions). Axial FLAIR images at onset MRI (A1-4) show multifocal cerebral (white arrow, A1,2) and cerebellar (black arrow, A3,4) white matter lesions. Post treatment follow-up images show reduction in size of the lesions (dashed white and black arrows, B1-4) with parenchymal atrophy.

Fig e4: Histological features in Case ID 57 -There were foci of chronic inflammation (arrows) in cortex and leptomeninges (A) and in the white matter (B) as seen in these H&E sections. These foci within the parenchyma are predominantly perivascular in location and showed features reminiscent of an ill-formed non-necrotising granuloma (C) without fibrinoid necrosis of vessel walls. There were rare cells suspicious but not definitive haemophagocytosis could be established (D, arrows). The inflammatory infiltrates comprised predominantly of CD3+ T-lymphocytes (E) along with moderate numbers of CD20+ B-lymphocytes (F). Macrophages and activated microglia were highlighted on CD68 stain (G). The background brain parenchyma showed significant gliosis as highlighted by GFAP (H). There was no obvious evidence of demyelination, although pockets of loss of myelin in association with inflammatory foci was noted on SMI94 stain (I). The markers for micro-organisms including commonly tested viral markers were negative (not shown).

Abbreviations: H&E – haematoxylin and eosin; CD – cluster of differentiation; GFAP – glial fibrillary acidic protein; SMI94 – a type of myelin basic protein


57 cases – onset MRIs evaluated
Diffuse cerebellar swelling/edema
Yes
No
Brainstem predominant pattern
Yes
No
Multifocal white matter lesions
Yes
No
No parenchymal lesions, non-specific findings
Yes
PATTERNS AT ONSET
Sub-pattern 1.3
6 cases
Sub-pattern 1.2
5 cases
Sub-pattern 1.1
21 cases
Pattern 2
25 cases
1
1
1
2
1
4
15
Sub-pattern 1.3
1 case
Sub-pattern 1.2
3 cases
Sub-pattern 1.1
21 cases
No follow-up
7 cases (5 – 1.1,
2 – 1.2)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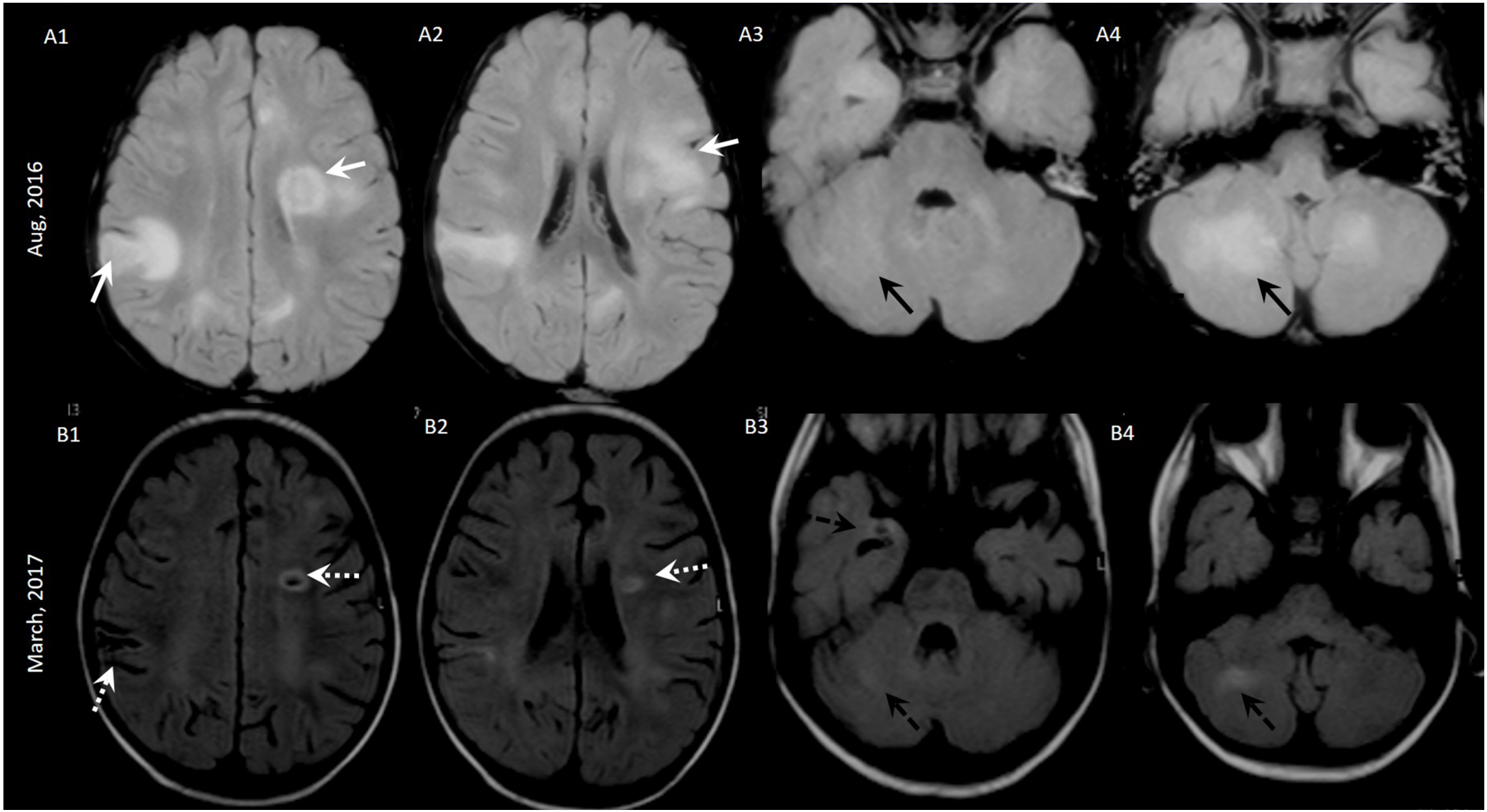
B1
B2
C1
B3
B4
C2
D
E
G1
G2
F
G3
G4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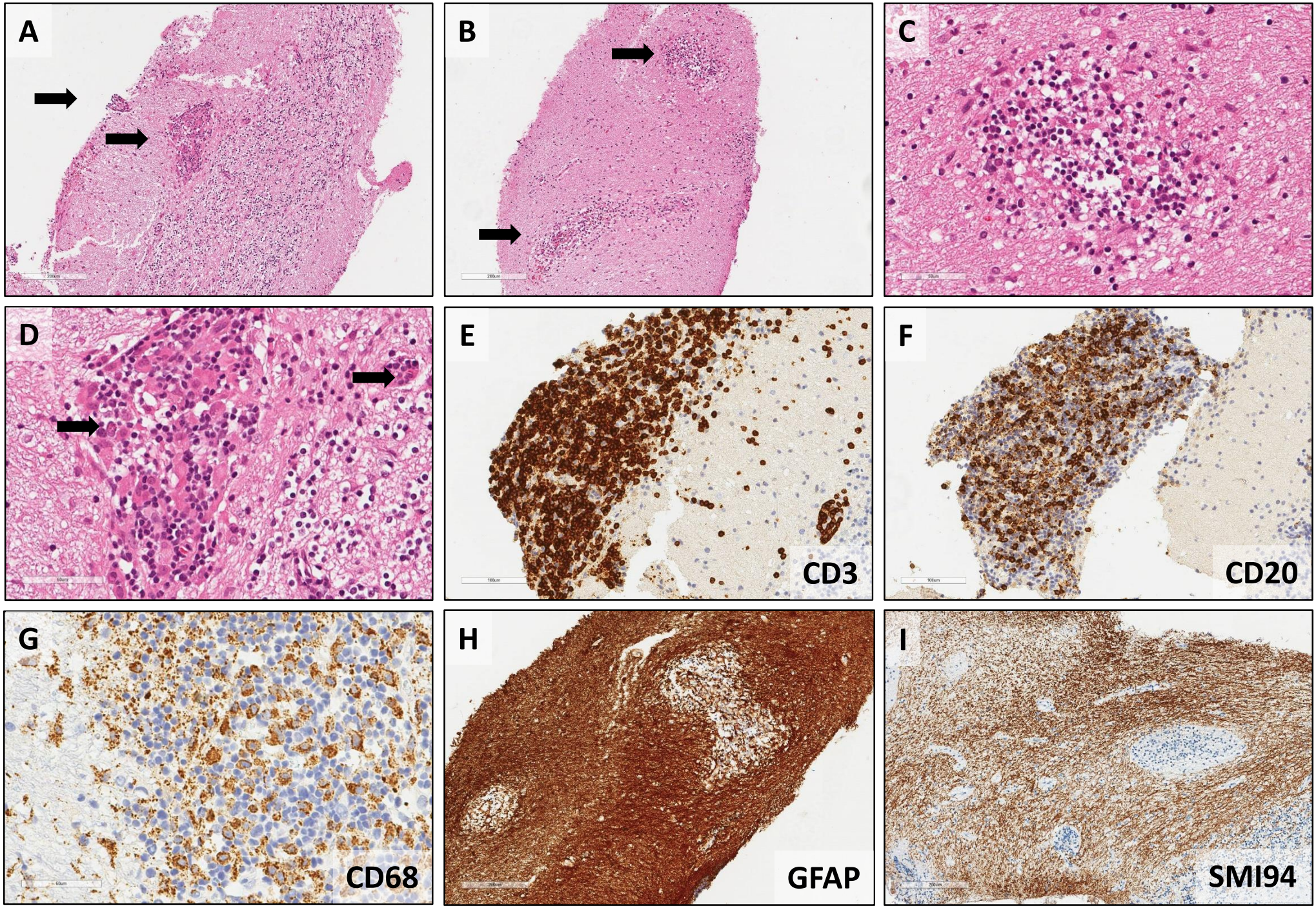
A2
A3
A4
Aug, 2016
B1
B2
B3
B4
March, 2017


A
B
C
D
E
CD3
F
CD20
G
CD68
H
GFAP
I
SMI94