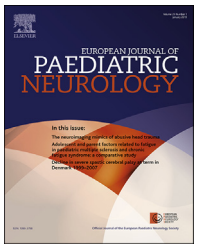
Official Journal of the European Paediatric Neurology Society



Original article

CSF neopterin, a useful biomarker in children presenting with influenza associated encephalopathy?

Emma Macdonald-Laurs [a,b,*], *Archana Koirala* [a,b], *Philip N. Britton* [a,c], *William Rawlinson* [d], *Chee Chung Hiew* [a,e], *Jocelynne Mcrae* [a], *Russell C. Dale* [a,c], *Cheryl Jones* [a,c,f,g], *Kristine Macartney* [a,c], *Brendan McMullan* [a,b], *Sekhar Pillai* [a,b]

[a] *Sydney Children's Hospital Network (SCHN), Australia*
[b] *The University of New South Wales, Australia*
[c] *The University of Sydney, Discipline of Child and Adolescent Health, Australia*
[d] *NSW Health Pathology, Australia*
[e] *Prince of Wales Hospital, Sydney, Australia*
[f] *The Royal Children's Hospital, Melbourne, Australia*
[g] *The University of Melbourne, Department of Paediatrics, Australia*

ARTICLE INFO

Article history:
Received 24 April 2018
Received in revised form
7 September 2018
Accepted 23 September 2018

Keywords:
Encephalitis
Neuroinflammation
Influenza
Neopterin
Magnetic resonance imaging
Influenza-associated encephalitis
Status epilepticus

ABSTRACT

Purpose: Neurological complications of influenza cause significant disease in children. Central nervous system inflammation, the presumed mechanism of influenza-associated encephalopathy, is difficult to detect. Characteristics of children presenting with severe neurological complications of influenza, and potential biomarkers of influenza-associated encephalopathy are described.

Methods: A multi-center, retrospective case-series of children with influenza and neurological complications during 2017 was performed. Enrolled cases met criteria for influenza-associated encephalopathy or had status epilepticus. Functional outcome at discharge was compared between groups using the Modified Rankin Scale (mRS).

Results: There were 22 children with influenza studied of whom 11/22 had encephalopathy and 11/22 had status epilepticus. Only one child had a documented influenza immunization. The biomarker CSF neopterin was tested in 10/11 children with encephalopathy and was elevated in 8/10. MRI was performed in all children with encephalopathy and was abnormal in 8 (73%). Treatment of children with encephalopathy was with corticosteroids or intravenous immunoglobulin in 9/11 (82%). In all cases oseltamivir use was low (59%) while admission to the intensive care unit was frequent (14/22, 66%). Clinical outcome at discharge was moderate to severe disability (mRS score > 2) in the majority of children with encephalopathy (7/11, 64%), including one child who died. Children with status epilepticus recovered to near-baseline function in all cases.

* *Corresponding author*. Sydney Children's Hospital Network, The University of New South Wales, Sydney Children's Hospital, High Street, Randwick 2031, New South Wales, Australia.
E-mail address: emma.macdonaldlaurs@health.nsw.gov.au (E. Macdonald-Laurs).
https://doi.org/10.1016/j.ejpn.2018.09.009

Conclusion: Raised CSF neopterin was present in most cases of encephalopathy, and along with diffusion restriction on MRI, is a useful diagnostic biomarker. Lack of seasonal influenza vaccination represents a missed opportunity to prevent illness in children, including severe neurological disease.

1. Introduction

Severe neurological complications from seasonal influenza, including influenza-associated encephalopathy/encephalitis (IAE), cause considerable morbidity and mortality in healthy children, and those with pre-existing neurological disease.[1–3] Recent estimates indicate the annual incidence of IAE in Australia is 2.8 per 1,000,000 in children under 14 years, with around 1% of hospitalized influenza cases associated with IAE.[4] Other populations show similar or higher incidence, with Japan's annual incidence of IAE recorded as 6–11/1,000,000.[2,5] Neurological complications attributed to influenza range from a mildly altered mental state, vertigo and brief febrile seizures to life threatening complications such as status epilepticus, meningitis, stroke, and demyelinating disease.[1] Antiviral agents, predominantly neuraminidase inhibitors, and immunomodulatory treatments (corticosteroids, intravenous immunoglobulin), are used to treat patients with influenza-associated neurological disease but there is limited evidence on their efficacy.[6] While it is thought that more extensive changes on MRI correlate with disease severity[7] there are no other available biomarkers that predict outcome.

The Australian influenza season typically occurs between July and October. The 2017 season saw the highest levels of influenza reported since the 2009 pandemic year.[8] The authors of this report noted an apparent increase in IAE and other severe neurological complications during 2017. Here, we describe the clinical presentation, laboratory testing, neuroimaging, treatment and short-term outcome of these cases. In addition, we observed elevated cerebrospinal fluid (CSF) neopterin – a marker of central nervous system (CNS) inflammation – amongst children with IAE that has not previously described. We compared the frequency of IAE during the 2017 influenza season with previously published incidence estimates.

2. Materials and methods

2.1. Participants

We identified children aged 0–14 years, with evidence of influenza and associated severe neurological disease including status epilepticus or moderate to severe encephalopathy, admitted to two paediatric hospitals which comprise the Sydney Children's Hospital Network, the largest paediatric network in Australia. Cases were ascertained between April 1st and October 31st, 2017.

2.2. Inclusion and exclusion criteria

At the Children's Hospital at Westmead, cases were identified from those recruited under pre-existing surveillance studies: the Australian Childhood Encephalitis study (ACE), and the Influenza Complications Network (FluCAN) surveillance study.[4,9] At Sydney Children's Hospital, children were identified from neurology consultation databases. Children were included if they required hospital admission and consultation from a Paediatric Neurologist for a neurological complication or worsening of a pre-existing neurological condition due to proven influenza. All children included either presented with status epilepticus (for 30 min or longer) or reached level 2 diagnostic certainty on the Brighton encephalopathy score.[10] Children were excluded if: influenza was not confirmed, neurological symptoms were mild, hospital admission was not required, and when an alternative diagnosis could better explain the presentation. Data were retrospectively collected from electronic medical records.

2.3. Presenting characteristics, biochemical and neuroimaging

We collected demographic data, presenting clinical characteristics, intensive care unit (ICU) admission, and length of stay, laboratory results including CSF testing, and influenza testing. CSF analysis included cell count, protein, glucose, microscopy, lactate, oligoclonal bands, neopterin and influenza PCR. An elevated CSF neopterin result was defined as >30 nmol/L.[11] Electroencephalogram reports and brain magnetic resonance imaging (MRI) (T2 weighted, FLAIR and diffusion weighted imaging) were assessed by a neurologist (S.P.) and neuroradiologist (C.C.H.). The neuroradiologist was blinded to diagnosis during review of the MRI.

Influenza was most commonly acutely diagnosed through the detection of influenza RNA in respiratory samples. Both hospitals used multiplex PCR assays (Seegene, South Korea) which detected up to 16 respiratory viruses. The assay has targets for both influenza A and B and was performed daily with a turnaround time of 1–2 days. At Sydney Children's Hospital, the assay also had targets for subtypes of influenza A: H3 strains and the pandemic strain H1N1/09. When influenza serology was requested to diagnose a recent influenza illness, this was performed using a complement fixation assay (Virion, Germany).

2.4. Treatment and outcomes

Treatment given including oseltamivir, empiric aciclovir, or 3rd generation cephalosporins, IVIg, corticosteroids, and

plasmapheresis were recorded. Time from admission to commencement of oseltamivir was recorded. Each case was assigned a modified Rankin scale (mRS) based on the examination at presentation and discharge from hospital.[12] The mRS ranges from 0 (no symptoms) to 6 (death). A poor outcome was an mRS score of >2 which indicates at least moderate disability requiring assistance.

2.5. Statistics

The relation between categorical variables was investigated using the two tailed Fisher exact test. The Mann–Whitney *U* test was used to determine the relation between continuous variables.

Ethics approval was granted by the Sydney Children's Hospitals Network Ethics Committee (LNR/17/SCHN/497).

3. Results

3.1. Participants

Twenty two children were included in this case series; 59% (13/22) were female. The median age at presentation was five years (range: 1.25–14 years). Eight children (36%) had pre-existing epilepsy and/or developmental delay. One child had an immunodeficiency (hypogammaglobulinemia) and receives monthly IVIg. This child, who presented with status epilepticus, was the only child recorded as having received the seasonal influenza vaccination.

Eleven children (50%) met level 2 Brighton criteria for encephalitis and were designated as influenza associated encephalopathy/encephalitis (IAE).[10] Two children with IAE had previous episodes of acute disseminated encephalomyelitis (ADEM) with complete clinical and radiological recovery (Table 3 cases 1&2). One child had mosaic tetrasomy X, while another had epilepsy and developmental delay. The remaining 11 children in our series had status epilepticus. In contrast to the children with IAE, over half of this group ($n = 6$) had pre-existing neurological disease including two children with refractory genetic epilepsies (Dravet syndrome and CDKL5).

3.2. Presenting characteristics

The majority of children had a fever (82%) and two-thirds had respiratory symptoms. Half presented with neurological symptoms within two days of onset of their influenza illness. Sixteen children (73%) presented with an altered level of consciousness. Seizures occurred in 19 children (86%) at any stage of illness and status epilepticus was frequent ($n = 17$, 77%). Other neurological findings at presentation were weakness ($n = 9$, 41%), pyramidal signs ($n = 8$, 36%), movement disorder ($n = 7$, 32%) and ataxia ($n = 6$, 27%). Hallucinations, meningism, cranial neuropathy and pupillary changes were infrequent (<20%).

3.3. Biochemical and neuroimaging

The majority of children ($n = 18$, 82%) had influenza A. Of those sub-typed ($n = 8$) half were H1 (2009) and half were H3. Four children had other respiratory pathogens co-identified on NPA (rhinovirus, coronavirus, *Mycoplasma pneumoniae*). Enterovirus was detected in the NPA of one child but was absent in CSF. One blood culture was positive for *Staphylococcus epidermidis*, and this was assessed to be a contaminant.

Lumbar puncture was performed in 14 children where it was considered clinically indicated (Table 2). Of those who did not have a lumbar puncture performed most were children who presented with status epilepticus alone, usually with known pre-existing epilepsy. One case, with acute necrotising encephalopathy (ANE), who was deemed to be too unwell to undergo a second lumbar puncture for measurement of neopterin. Where sampled, CSF showed pleocytosis and elevated protein in only a third (each $n = 5$). Influenza PCR on CSF was positive in 1 of 5 children tested, in an immunocompetent previously well 10 year old. The CSF neopterin was elevated in 9 of 11 children tested; in seven children it was considerably elevated ranging from 74 to 669 nmol/L (normal < 30 nmol/L), one had a borderline result (34 nmol/L). CSF neopterin was measured in one child presenting with status epilepticus and was 31.75 nmol/L (borderline result). While most children with IAE had a raised CSF neopterin (8/10, 80%), only three had CSF pleocytosis (10, 18, 19 cells/mm^3) and two had an elevated CSF protein (0.7 g/L and 7.0 g/L). Oligoclonal bands were measured on serum and/or CSF in 10/11 children with IAE and were not present in any. Some children with IAE had anti-neuronal antibodies testing performed on serum and/or CSF, usually NMDA and VGKC (Table 3). These were negative apart from two cases with mildly elevated anti-thyroid antibodies. Other routine laboratory data were normal or only mildly abnormal in most children (Table 1).

MRI brain was performed in 15 children and showed new abnormalities in eight (53%), all with IAE. The common acute MRI abnormalities were the presence of T2-FLAIR hyperintensities, diffusion restriction (each, $n = 8$), and gadolinium enhancement ($n = 4$). The spectrum of radiological features are shown in Fig. 1A–I. Diverse clinico-radiological syndromes were diagnosed including: ANE ($n = 1$), acute encephalopathy with biphasic seizures (AESD) ($n = 2$), posterior reversible encephalopathy (PRES) ($n = 1$), hemiconvulsion hemiplegia syndrome (HHS) ($n = 1$), and cerebellitis.

Genetic testing of Ran-binding protein 2 (RANBP2) was performed in the child with ANE (Table 3 case 2) and the child who died from an ANE-like illness (Table 3, case 1). Both were negative. One child who met criteria for IAE was subsequently found to have a mutation in the polymerase gamma (POLG) gene (Table 3, case 11).

3.4. Treatment

Fourteen (66%) children were admitted to the ICU and nine (41%) required mechanical ventilation. Thirteen (59%) children received oseltamivir. Median time to commencement of oseltamivir from presentation was 1 day (mean 5.6 days, IQR 0–9 days), but >3 days in five cases. Two ICU ventilated children were commenced on oseltamivir nine days after admission. In contrast, nineteen (86%) children were treated with a 3rd generation cephalosporin, while 14 (64%) received aciclovir. Nineteen (86%) received anticonvulsants and 16 (73%) continued these on discharge. First-line

Table 1 – Demographics and clinical features of children with Influenza and severe neurological complications admitted to the admitted to the Sydney Children's Hospitals network during the 2017 influenza season.

Characteristic	IAE,[a] $n = 11$ (%)	SE,[b] $n = 11$ (%)	p-Value
Female sex	6 (55)	7 (64)	1.0
Age (median, range)	6 (15 months–14 years)	3 (20 months–12 years)	0.36
Pre-existing epilepsy	2 (18)	6 (55)	0.18
Received seasonal influenza vaccine	0 (0)	1 (9)	–
Days between respiratory symptoms and neurological presentation in days (median, range)	4 (1–21)	3 (1–14)	0.663
Seizures	8 (73)	11 (100)	0.21
Status epilepticus	6 (55)	11 (100)	0.04
Influenza A positive (NPA[c] PCR/serology)	8 (73)	10 (91)	0.59
Influenza B positive (NPA[c] PCR)	3 (27)	1 (9)	0.59
CSF influenza PCR positive	1/5 (40)	0/0 (0)	–
CSF pleocytosis (n, %, range) [WCC > 5.0×10^6/L]	5 (45) [0–242]	0/3, [0–1]	0.26
Elevated CSF protein (n, %, range) [0.15–0.45 g/L]	5 (45), [0.19–7.0]	0/3 [0.10–1.17]	0.26
CSF neopterin elevated (n, %) >30	8/10 (80)[d]	1/1 (100)[e]	0.45

Note: tests used – Fisher exact for categorical and Mann–Whitney for continuous data points.
[a] IAE: influenza related encephalopathy.
[b] SE: Status epilepticus.
[c] NPA: Nasopharyngeal aspirate.
[d] Including one borderline result (33.86 nmol/L).
[e] Borderline result (31.75 nmol/L).

Table 2 – Treatment and outcome of children with Influenza and neurological complications admitted to the Sydney Children's Hospitals network during the 2017 Influenza season.

Outcomes	IAE[a] ($n = 11$)	SE[b] ($n = 11$)	p-Value
Admitted to intensive care	9 (82)	5 (45)	0.18
Required mechanical ventilation	7 (64)	2 (18)	0.08
Treatment[c]			
Oseltamivir	7 (64)	6 (55)	1.0
3rd generation cephalosporin	11 (100)	8 (73)	0.21
Aciclovir	8 (73)	6 (55)	0.66
Intravenous immunoglobulin	7 (64)	0 (0)	0.004
High dose corticosteroids	9 (82)	0 (0)	<0.001
Plasmapheresis	1 (9)	0 (0)	1.0
Anticonvulsant	8 (73)	11 (100)	0.21
Days in ICU (mean, range)	7.7 (1–37)	2 (1–3)	0.03
Days in hospital (mean, range)	33.5 (5–108)	4.8 (2–14)	0.001
Outcome at discharge			
Death	1 (9)	0 (0)	1.0
Abnormal/Poor[d]	7 (64)	0 (0)	0.004
Mean mRS at admission	0.2	1.7	0.086
Mean mRS at discharge	3.3	2.1	0.126
Mean change in mRS	3.1	0.4	0.001

Tests used – Fisher exact for categorical and Mann–Whitney for continuous data points.
[a] IAE: influenza related encephalopathy.
[b] SE: status epilepticus.
[c] Treatment received during hospital admission.
[d] Defined as an increase of more than 2 points on mRS (new disability or worsening of preexisting disability by >2 points).

immunomodulatory treatment (corticosteroids and/or IVIg, plasmapheresis) was given to nine children with IAE (corticosteroids ($n = 9$), IVIg ($n = 7$) and plasmapheresis ($n = 1$)) but none of those with status epilepticus alone.

3.5. *Outcomes*

The median length of ICU and hospital stay was four days (range 1–37) and 8 days (range 2–108 days) respectively. Children with IAE were more likely to have both longer hospital (mean 33.5 days vs 4.8 days; $p = 0.001$) and PICU admissions (mean 7.7 days vs 2 days; $p = 0.03$) compared to children with status epilepticus. One child with IAE died following an ANE-like illness, although MRI findings were atypical (Table 3, case 1). Her post-mortem was inconclusive: showing generalised cerebral oedema and some features of acute hemorrhagic leucoencephalitis. Ten out of 11 children with IAE had an mRS score of 0 (normal) at baseline. At discharge from hospital 7/11 (64%) of children with IAE had a higher in mRS score (mRS > 2; moderate disability) compared to those with status epilepticus. The change in mean mRS was significant between the two groups: children with IAE had a change in mRS of 3.1 points while those in the status epilepticus group had a mean increase of mRS of 0.4 points (p-value: 0.001). Nearly all children (7/8, 88%) with MRI diffusion restriction had a poor outcome. The child with MRI diffusion restriction and a good outcome had posterior reversible encephalopathy syndrome (PRES). Among the 7 children with IAE and a considerably elevated neopterin, four had an mRS > 2 while three had mild deficits (mRS 1, 2 and 2). There was a mean increase in the mRS of 2.8 points the seven children with highly elevated CSF neopterin (74–669 nmol/L).

4. Discussion

In this case series we observed two groups of children who presented with severe influenza related neurological disease. One group of children fulfilled criteria for IAE, while the other group, most often with pre-existing neurological disease, presented with status epilepticus but otherwise did not fulfill

Table 3 – Case summaries of children with Influenza-associated encephalitis (IAE) in Sydney, 2017.

Case	Age (years) sex (M/F)	Clinical syndrome	Flu type	MRI findings	CSF white cell count ($\times 10^6$/L)	Influenza positive on CSF.	Neuronal/ inflammatory antibody testing	ICU	Treatment given	Neopterin (nmol/L)	mRS (Baseline; discharge)
1	14y, F	Encephalitis, Previous cerebellitis aged 7 years, full recovery.	B	T2 hyperintensity involving the margins of the lateral ventricles, clastrum, mammillary bodies, and caudate nucleus [Fig. 1G and H]	1	Not performed.	NMDAR, VGKC, NMO, TPO antibodies – negative.	Yes	3rd generation cephalosporin Oseltamivir Aciclovir IVIG Corticosteroid Plasmapheresis	174	0; 6 (Death)
2	13y, F	ANE Previous ADEM aged 4y, full recovery.	A	Multiple symmetrical foci of diffusion restriction & T2 hyperintensity in the cerebellum pons, cerebral white matter and basal ganglia. Left thalamic haemorrhage. [Fig. 1E]	0	Not performed.	Not performed.	Yes	3rd generation cephalosporin Oseltamivir Aciclovir IVIG Corticosteroid	none	0; 3
3	10y, M	Hyperkinetic movement disorder. Mild cerebral palsy. GMFCS 1.	B	Normal.	3.3	Negative.	NMDAR, VGKC antibodies – negative. TPO & thyroglobulin antibodies positive.	No	3rd generation cephalosporin IVIG Corticosteroid	77.3	0; 2
4	9y, M	Encephalitis with focal status epilepticus. Previously well.	A	Normal.	3.9	Positive. Influenza A	NMDAR, VGKC antibodies – negative. TPO and thyroglobulin antibodies positive.	No	3rd generation cephalosporin Aciclovir IVIG Corticosteroid	203	0; 1
5	7y M	Encephalitis with possible PRES Previously well.	B	Initial scan normal. Repeat 5 days later: Cortical and subcortical vasogenic oedema in posterior and anterior watershed areas with a few non-specific foci of restricted diffusion.[Fig. 1D]	10	Not performed.	Not performed.	Yes	3rd generation cephalosporin Oseltamivir Aciclovir	74	0; 2
6	6y F	Cerebellitis Previously well.	A	T2 and FLAIR hyperintensities in both cerebellar hemispheres. Enhancement of the cerebellar folia with gadolinium. [Fig. 1F]	242	Negative	NMDAR, VGKC, GAD, PCA1, PCA2, Hu, Ri, Ma 1&2, amphiphysin antibodies, MOG - negative	Yes	3rd generation cephalosporin Oseltamivir Aciclovir IVIG Corticosteroid	16	0; 4

7	4y F	AESD, Reye like syndrome 2 previous episode of status epilepticus, known epilepsy.	A (H3)	Multiple foci of T2/FLAIR hyperintensity and diffusion restriction in the subcortical white matter of the left frontal and deep white matter of parietal lobes. Reduction in volume of right temporal lobe with hyper intense signal in right hippocampus.[Fig. 1A]	0	Negative	NMDAR, VGKC antibodies – negative.	Yes	3rd generation cephalosporin Corticosteroid	201	2; 5
8	4y F	Encephalitis Mosaic tetrasomy X, cerebral palsy, developmental delay.	A (H109)	Stable appearance from previous scans. Moderate atrophy of the superior vermis of the cerebellum. Non-specific T2 hyperintensiites in the subcortical white matter.	2.7	Not performed.	Not performed.	Yes	3rd generation cephalosporin Oseltamivir	34	4; 4
9	2y M	AESD Previously well. History of simple febrile seizures.	A	Bilateral restricted diffusion in the peri-rolandic and posterior white matter with sparing of the basal ganglia [Fig. 1B]	0	Not performed.	Not performed.	Yes	3rd generation cephalosporin Oseltamivir Aciclovir Corticosteroid	317	0; 3
10	1.3y F	HHS Previously well.	A (H109)	T2/FLAIR hyper- intensity and mild diffusion restriction of the left temporal and left occipital lobes. Lacate peak on MRS on the left side. [Fig. 1C]	4.1	Negative	NMDAR, VGKC, TPO antibodies - negative	Yes	3rd generation cephalosporin Oseltamivir Aciclovir IVIG Corticosteroid	669	0; 3
11	1.2y M	Subcortical myoclonus Previously well. This child was found to have a mutation in the POLG gene.	A	Initial scan: non-specific left thalamic T2 hyperintensity. Repeat 3 weeks later: Diffusion restriction lateral part of left thalamus. [Fig. 1I]	6	Not performed.	Not performed	Yes	3rd generation cephalosporin Aciclovir IVIG Corticosteroid	28	0; 4

ANE, acute necrotizing encephalopathy; AESD, acute encephalopathy with biphasic seizure disorder and late diffusion restriction; ADEM, acute disseminated encephalomyelitis; HHS, hemconvulsion hemiplegic syndrome; ICU, intensive care unit, POLG, polymerase gamma, PRES, posterior reversible encephalopathy syndrome. MOG, myelin oligodendrocyte glycoprotein; NMDAR, N-methyl-D-aspartate antibody; VGKC, voltage gated potassium channel antibody; GAD, glutamic acid decarboxylase antibody; TPO, thyroperoxidase antibody; NMO, neuromyelitis optica antibody; PCA1, purkinje cell cytoplasmic antibody 1; PCA2, purkinje cell cytoplasmic antibody 2.

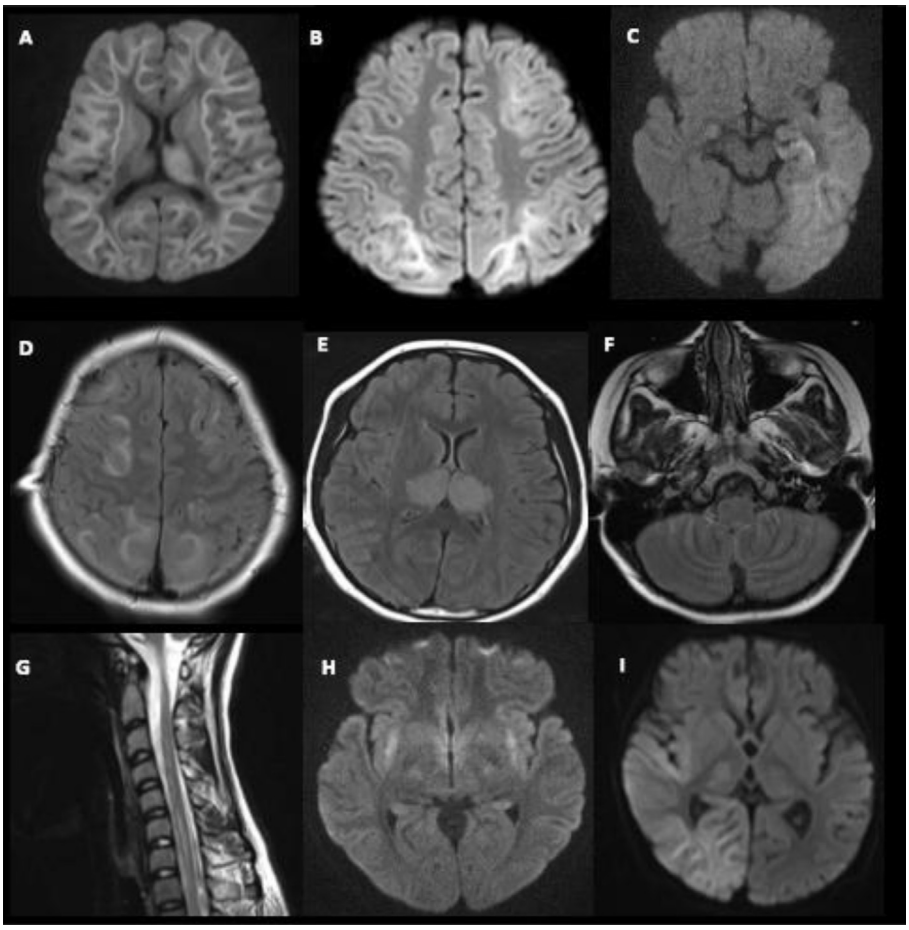


Fig. 1 – Selected MRI images showing the spectrum of imaging abnormalities in Influenza-associated encephalitis/encephalopathy. A–I. A: Symmetrical diffusion restriction in the subcortical white matter and left thalamus (MRI: axial, DWI) in a 4 year old girl with acute encephalopathy with biphasic seizure disorder and late diffusion restriction (AESD) and Reye-like syndrome (Case 7). B: Symmetrical diffusion restriction posteriorly and left frontal lobe (MRI: axial, DWI) in a 2 year old girl with AESD (Case 9). C: Diffusion restriction in the left temporal lobe especially subcortical white matter (MRI: axial, DWI) in a 1.3 year old girl (Case 10). D: T2 hyperintensities in the posterior white matter (symmetrical) and patchy subcortical frontal white matter (MRI: axial T2 FLAIR) in a 7 year old boy with posterior reversible encephalopathy syndrome (Case 5). E: T2 hyperintensities in bilateral thalami which are swollen (MRI brain: axial, T2) in a 13 year old girl with acute necrotising encephalopathy. F: Prominent gadolinium enhancement of the cerebellar folia (MRI brain: axial, T1 with contrast) in a 6 year old girl (Case 6). G: Longitudinal T2 hyperintensity involving C3–7 (MRI cervical spine: sagittal, T2) in a 14 year old girl with encephalitis (Case 1). H: Diffusion restriction involving bilateral external capsule/claustrum (MRI: axial, DWI) in a 14 year old girl with encephalitis (Case 1). I: Diffusion restriction involving predominantly the right temporal lobe white matter (MRI brain: axial DWI) in a 1 year old boy with subcortical myoclonus (Case 11).

criteria for IAE. Amongst cases of IAE, elevated CSF neopterin appeared to correlate with the presence of diffusion restriction on MRI brain and adverse outcome. Use of oseltamivir was infrequent among all cases, although use of antibiotics occurred in the majority. Only one child had documented influenza vaccine, even amongst those with pre-existing neurological co-morbidity, despite the fact that this is recommended in Australia.[13]

Influenza associated neurological complications are thought to occur due to an inflammatory or immune-mediated response to influenza infection rather than direct viral invasion.[14,15] Among those with IAE we observed, similar to previous authors[2,6,16] that CSF pleocytosis and detection of influenza in the CSF occurred in a minority ($n = 5$; 35%, and $n = 1/4$; 25%).[3,7,17,18] However CSF neopterin, a biomarker of inflammation, was elevated in most children with IAE ($n = 9$, 81%). Neopterin, a catabolic product of guanosine triphosphate (GTP), is synthesized by human macrophages upon stimulation from interferon gamma and can be measured in urine, serum and CSF.[19] While serum neopterin levels are useful in the diagnosis and monitoring of systemic infectious or inflammatory diseases, such as HIV,[20] CSF neopterin, reflecting intrathecal production by microglial cells, more accurately detects CNS inflammatory diseases (infectious or immune mediated).[11,19] A recent review assessing biomarkers of CSF inflammation found, among clinically available tests,

that CSF neopterin performed better than the presence of oligoclonal bands or CSF pleocytosis in detection of CNS inflammation.[21]

There is limited data regarding prognostically significant biomarkers in IAE. Pro-inflammatory cytokines may impair the blood brain barrier and induce apoptosis of neurons.[14] Elevated cytokines such as interleukin-6 (IL-6) and tumour necrosis factor alpha have been demonstrated in children with IAE and correlates with poorer outcome.[14,22,23] Testing CSF IL-6 outside the research setting is currently unavailable. Clasmatodendrosis, abnormal morphological changes in astrocytes, occurring presumably due to the effect of pro-inflammatory cytokines, has recently been suggested to be a pathological feature of IAE on autopsy.[24] Clasmatodendrosis was found in the cerebral white matter, thalamus, corpus callosum, cerebellum, thalamus and hippocampus of children with IAE and may correlate MRI changes commonly seen.[24] Previous authors have associated abnormalities on MRI brain with poorer outcome.[7] In our cohort MRI brain abnormalities were diverse and common, particularly diffusion restriction in the subcortical white matter. Diffusion restriction correlated with a poor outcome, apart from in the child who had PRES, and was associated with an elevated CSF neopterin in most cases. Further studies of IAE are required to evaluate whether significant elevations of CSF neopterin, particularly in combination with diffusion restriction and other MRI changes, could predict short and long-term outcome.

Oseltamivir, a neuramidase inhibitor which prevents release of influenza virus from infected cells[25] has been shown to reduce influenza symptoms in otherwise healthy children by 29 h (95% CI 12–47 h,$p = 0.001$).[26] Only 59% (13 children) were treated with oseltamivir and there was a significant delay in commencement in 5 cases (>3 days in hospital). In contrast, empirical 3rd generation cephalosporin (86%) and aciclovir use (64%) was more frequent. This may be related to the perception among practitioners that anti-influenza therapy has little benefit. We suggest in accordance with local guidelines,[8] that children with encephalitis should be empirically treated with oseltamivir during the influenza season (May to October). The evidence for use of immunotherapy (IVIg, corticosteroids) in IAE, is limited[27,28] however, in our case series, most children with IAE were treated with first-line immunotherapy with uncertain benefit. No serious side effects were reported.

In 2017, the burden of influenza in Australia (particularly the Eastern states) was the highest seen since the 2009 pandemic.[8] Based on IAE incidence estimates published by Britton et al.[2] from the 2013–15 influenza seasons in Australia and the population coverage of our hospitals, we calculated that we would expect 5.2 (1.3–14.5) cases of IAE in children (<14 years) per year. The IAE case frequency observed in our cohort was twice the expected point estimate based on these previous incidence estimates but within the 95% confidence interval,[2] and so contribute to validating the estimates from Britton et al.[2]

The short-term outcome of our cohort, particularly those with IAE, was alarming with 64% having a poor outcome. While there was a significant rate of ICU admission among the group of children with status epilepticus (45%) this was not as high as children with IAE (82%) and, most often, non-IAE children did not experience a significant change in their mRS. This supports previous observations that survivors of IAE during the 2009 H1N1 pandemic, and in more recent non-pandemic influenza seasons in Australia, experienced significant ongoing disability.[29,30] We have previously shown in a large retrospective encephalitis cohort study that ICU admission, MRI diffusion restriction and status epilepticus and were risk factors for a long-term abnormal outcome.[31] These risk factors were common (82%, 73%, 45%) in children with IAE from our cohort. The medium and long-term outcome in our cohort should be assessed including formal neuropsychological testing. Further research is required to understand and modulate the CNS inflammatory cascade present in IAE in order to modulate long term neurodisability.

The overall influenza immunisation rate during 2017 in Australia was low at 33%,[32] however a recently observed rate of vaccine receipt among children was even lower at 17.1%.[33] Four age-specific quadrivalent influenza vaccines containing two strains of Influenza A (H1N1 [Michigan] and H3N2 [Hong Kong]) and two strains of influenza B (Brisbane and Phuket) were available in 2017. Children older than six months were eligible to be vaccinated and the vaccine was provided free to children with neurological disease.[34] In our cohort just one child had a documented influenza vaccination, although a third of children were eligible for free immunisation and the remainder could have received an immunisation at the cost of around $20–50 AUD. We emphasise that the severe syndromes and adverse outcomes observed here should be considered preventable. Following high rates of influenza related morbidity in 2017(including these cases), New South Wales and other Australian states have introduced universal funded seasonal influenza immunisation to all children aged 6 months to 5 years.[35]

Our series has limitations. We describe children with severe influenza-associated neurological complications but did not include children with mild neurological complications. Children with pre-existing epilepsy may not have always been tested for influenza and may be under-represented. The collection of clinical data was retrospective, and some electronic data were incomplete. Seasonal influenza immunisation status was not always clearly recorded, although we reviewed the Australian immunisation register to verify vaccination status where possible. Influenza sub-typing from NPA samples and CSF influenza PCR testing was not routinely performed. In the SE group, CSF studies, including CSF neopterin were performed infrequently and MRI brain infrequently requested. Due to this we were unable to use this group as a direct control for the finding of elevated CSF neopterin the IAE group. Serial CSF neopterin to assess treatment and clinical progress were not performed.

5. Conclusion

This is the first series to demonstrate that elevation of CSF neopterin, a marker of CNS inflammation, occurs commonly in children with IAE. CSF neopterin may be a useful diagnostic marker for IAE while its role as a prognostic marker

requires further evaluation. MRI diffusion restriction was associated with a poor outcome in IAE. Short-term outcomes of children with neurological complications of influenza, especially within the IAE group, were alarming, with nearly two-thirds of children having a poor outcome despite receipt of ICU support, anticonvulsants, first-line immunotherapy and, in some, anti-viral treatment. Given the severity of influenza associated neurological complications, we recommend a "treat and test" approach to the use of oseltamivir in children presenting with acute encephalopathy/encephalitis during the influenza season. Finally, seasonal influenza vaccination should be universally provided to children and those at risk of severe influenza, with better education and awareness to increase uptake in the paediatric population.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have stated that they had no interests, which might be perceived as posing a conflict or bias. This manuscript has been contributed to, seen, and approved by all the authors. All the authors fulfill the authorship credit requirements. No honorarium grant or other form of payment was received for the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2018.09.009.

REFERENCES

1. Goenka A, Michael BD, Ledger E, et al. Neurological manifestations of influenza infection in children and adults: results of a National British Surveillance Study. *Clin Infect Dis* 2014;**58**:775–84.
2. Britton PN, Blyth CC, Macartney K, et al. The spectrum and burden of influenza-associated neurological disease in children: combined encephalitis and influenza sentinel site surveillance from Australia, 2013–2015. *Clin Infect Dis* 2017;**65**:653–60.
3. Paksu MS, Aslan K, Kendirli T, et al. Neuroinfluenza: evaluation of seasonal influenza associated severe neurological complications in children (a multicenter study). *Childs Nerv Syst* 2017;**34**:335–47.
4. Britton PN, Dale RC, Elliott E, Festa M. Pilot surveillance for childhood encephalitis in Australia using the paediatric active enhanced disease surveillance (PAEDS) network. *Epidemiol Infect* 2016;**144**:2117–27.
5. Morishima T, Togashi T, Yokota S, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;**35**:512–7.
6. Muhammad Ismail HI, Teh CM, Lee YL, National Paediatric HNSG. Neurologic manifestations and complications of pandemic influenza A H1N1 in Malaysian children: what have we learnt from the ordeal? *Brain Dev* 2015;**37**:120–9.
7. Zeng H, Quinet S, Huang W, et al. Clinical and MRI features of neurological complications after influenza A (H1N1) infection in critically ill children. *Pediatr Radiol* 2013;**43**:1182–9.
8. Health Do. Influenza season in Australia. A summary from the national influenza surveillance committee. In: Health Do, editor. *Australian Government Department of Health*, **2017**; 2017. www.health.gov.au.
9. Zurynski Y, McIntyre P, Elliott E, Group PI. Paediatric active enhanced disease surveillance: a new surveillance system for Australia. *J Paediatr Child Health* 2013;**49**:588–94.
10. Sejvar J, Kohl K, Bilynsky R, et al. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 2007;**25**:5771–92.
11. Dale R, Brilot F, Fagan E, Earl J. Cerebrospinal fluid neopterin in paediatric neurology: a marker of active central nervous system inflammation. *Dev Med Child Neurol* 2008;**51**:317–23.
12. Bonita R, Beaglehole R. Recovery of motor function after stroke. *Stroke* 1988;**19**.
13. (ATAGI) ATAGoI. The Australian immunisation handbook (updated February 2018). In: (ATAGI) ATAGoI, editor. 47 *Influenza*. 10th ed. Australian Government Department of Health; 2017. http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook10-home~handbook10part4~handbook10-4-7-4-7-7.
14. Wang GF, Li W, Li K. Acute encephalopathy and encephalitis caused by influenza virus infection. *Curr Opin Neurol* 2010;**23**:305–11.
15. van Riel D, Leijten LM, Verdijk RM, et al. Evidence for influenza virus CNS invasion along the olfactory route in an immunocompromised infant. *J Infect Dis* 2014;**210**:419–23.
16. Moon JH, Na JY, Kim JH, et al. Neurological and muscular manifestations associated with influenza B infection in children. *Pediatr Neurol* 2013;**49**:97–101.
17. Studahl M. Influenza virus and CNS manifestations. *J Clin Virol* 2003;**28**:225–32.
18. Wilking AN, Elliott E, Garcia MN, Murray KO, Munoz FM. Central nervous system manifestations in pediatric patients with influenza A H1N1 infection during the 2009 pandemic. *Pediatr Neurol* 2014;**51**:370–6.
19. Eisenhut M. Neopterin in the diagnosis and monitoring of infectious diseases. *J Biomarkers* 2013:1–10 [Article ID 196432].
20. Hagberg L, Cinque P, Gisslen M, et al. CSF fluid neopterin: an informative biomarker of CNS immune activity in HIV-1 infection. *AIDS Res Ther* 2010;**7**:1–12.
21. Kothur K, Wienholt L, Mohammad S, et al. Utility of CSF cytokine/chemokines as markers of active intrathecal inflammation: comparison of demyelinating, anti-NMDAR and enteroviral encephalitis. *PLoS One* 2016;**11**:e0161656.
22. Akins PT, Belko J, Uyeki TM, Axelrod Y, Lee KK, Silverthorn J. H1N1 encephalitis with malignant edema and review of neurologic complications from influenza. *Neurocrit Care* 2010;**13**:396–406.
23. Tsai JP, Baker AJ. Influenza-associated neurological complications. *Neurocrit Care* 2013;**18**:118–30.
24. Tachibana M, Mohri I, Hirata I, et al. Clasmatodendrosis is associated with dendritic spines and does not represent autophagic astrocyte death in influenza-associated encephalopathy. *Brain Dev* 2018 [In press, Corrected Proof, published online 26 July 2018].
25. Couch RB. Prevention and treatment of influenza. *N Engl J Med* 2000;**343**:1778–87.

26. Jefferson T, Jones M, Doshi P, Spencer E, Onakpoya I, Heneghan CJ. Oseltamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. *BMJ* 2014;**348**:1–18.
27. McDaneld LM, Fields JD, Bourdette DN, Bhardwaj A. Immunomodulatory therapies in neurologic critical care. *Neurocrit Care* 2010;**12**:132–43.
28. Iro MA, Martin NG, Absoud M, Pollard AJ. Intravenous immunoglobulin for the treatment of childhood encephalitis. *Cochrane Database Syst Rev* 2017. https://doi.org/10.1002/14651858.CD011367.pub2. CD011367.
29. Kawashima H, Morichi S, Okumara A, Nakagawa S, Morishima T. National survey of pandemic influenza A (H1N1) 2009-associated encephalopathy in Japanese children. *J Med Virol* 2012;**84**:1151–6.
30. Britton PN, Dale RC, Blyth CC, et al. Influenza-associated encephalitis/encephalopathy identified by the Australian childhood encephalitis study 2013–2015. *Pediatr Infect Dis J* 2017;**36**:1021–6.
31. Pillai SC, Hacohen Y, Tantsis E, et al. Infectious and autoantibody-associated encephalitis: clinical features and long-term outcome. *Pediatrics* 2015;**135**:e974–84.
32. Health D. *Influenza season in Australia. A summary from the National influenza surveillance committee*, vol. 2017. Department of Health; 2017.
33. Blyth CC, Macartney KK, McRae J, et al. Influenza epidemiology, vaccine coverage and vaccine effectiveness in children admitted to sentinel Australian hospitals in 2017: results from the PAEDS-FluCAN collaboration. *Clin Infect Dis* 2018 [epub ahead of print].
34. Flannery B, Reynolds SB, Blanton L, et al. Influenza vaccine effectiveness against pediatric deaths: 2010–2014. *Pediatrics* 2017;**139**:1–9.
35. Government N. *Seasonal influenza vaccination 2018*. 2018. http://www.health.nsw.gov.au/immunisation/Pages/seasonal_flu_vaccination.aspx.