Translating metagenomics into clinical practice for complex paediatric neurological presentations

Justin Penner
Jane Hassell
Julianne R Brown
Kshitij Mankad
Nathaniel Storey, Laura Atkinson, Nisha Ranganathan, Alexander Lennon, Jack C D Lee
Dimitrios Champsas
Angelika Kopec, Divya Shah
Cristina Venturini
Garth Dixon, Surjo De, James Hatcher
Kathryn Harris
Kristian Aquilina
Maaike A. Kusters
Karyn Moshal, Delane Shingadia
Austen JJ Worth
Giovanna Lucchini
Ashirwad Merve, Thomas S Jacques
Alasdair Bamford
Marios Kaliakatsos
Judith Breuer
Sofia Morfopoulou

PII: S0163-4453(23)00450-4

DOI: https://doi.org/10.1016/j.jinf.2023.08.002

Reference: YJINF6024

To appear in: *Journal of Infection*
Accepted date: 3

Please cite this article as: Justin Penner, Jane Hassell, Julianne R Brown, Kshitij Mankad, Nathaniel Storey, Laura Atkinson, Nisha Ranganathan, Alexander Lennon, Jack C D Lee, Dimitrios Champsas, Angelika Kopec, Divya Shah, Cristina Venturini, Garth Dixon, Surjo De, James Hatcher, Kathryn Harris, Kristian Aquilina, Maaike A. Kusters, Karyn Moshal, Delane Shingadia, Austen JJ Worth, Giovanna Lucchini, Ashirwad Merve, Thomas S Jacques, Alasdair Bamford, Marios Kaliakatsos, Judith Breuer and Sofia Morfopoulou, Translating

metagenomics into clinical practice for complex paediatric neurological presentations, *Journal of Infection,* (2023)
doi:https://doi.org/10.1016/j.jinf.2023.08.002

Translating metagenomics into clinical practice for complex paediatric neurological presentations

Justin Penner[1], Jane Hassell[*2], Julianne R Brown[*3], Kshitij Mankad[4], Nathaniel Storey[3], Laura Atkinson[3], Nisha Ranganathan[3], Alexander Lennon[3], Jack C D Lee[3], Dimitrios Champsas[2], Angelika Kopec[3], Divya Shah[3], Cristina Venturini[6], Garth Dixon[3], Surjo De[3], James Hatcher[3], Kathryn Harris[5], Kristian Aquilina[6], Maaike A. Kusters[7,9], Karyn Moshal[1,9], Delane Shingadia[1,9], Austen JJ Worth[7], Giovanna Lucchini[8], Ashirwad Merve[9, 10], Thomas S Jacques[9, 10], Alasdair Bamford[1,11] , Marios Kaliakatsos[2], Judith Breuer[3,12†], Sofia Morfopoulou[12,13†]

1. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Infectious Diseases, London, UK
2. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Neurology, London, UK
3. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Microbiology, Virology, and Infection Prevention & Control, London, UK
4 . Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Radiology, London, UK
5. Barts Health NHS Trust, Department of Virology East & Southeast London Pathology Partnership, London, UK
6. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Neurosurgery, London, UK
7. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Immunology, London, UK
8. Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Haematology and Bone Marrow Transplant, London, UK
9 Histopathology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
10 Developmental Biology and Cancer Department, Great Ormond Street Institute of Child Health, University College London, London, UK
11 UCL Great Ormond Street Institute of Child Health, London, UK
12. Infection, Immunity and Inflammation Department, GOS Institute of Child Health, University College London, London, UK
13. Section for Paediatrics, Department of Infectious Diseases, Faculty of Medicine, Imperial College London, London, UK

*,† contributed equally to the manuscript

Corresponding Author: Dr Sofia Morfopoulou
sofia.morfopoulou.10@ucl.ac.uk
GOS Institute of Child Health
University College London

Dear Editors,

We read with interest the article published by Xu et al. on the improved diagnostic yield of metagenomic sequencing combined with conventional testing in patients with haematologic

disorders[1]. Similarly, paediatric meningoencephalitis diagnosis is complex, with multiple infective and non-infective aetiologies, while more than half of cases remain undiagnosed despite advancing diagnostic technologies[2]. Immunocompromised children are at higher risk of neurological compromise, a result of difficult-to-diagnose and sometimes novel pathogens, which can cause neuroinflammation with low pathogen burden. To address this challenge, we implemented routine clinical metagenomics (CMg) for children with complex presentations where the diagnosis of central nervous system (CNS) infection, neuro-inflammation, or neurotoxicity was uncertain. In parallel with the CMg service (**Supplementary Figure 1**), a neuro-infection multidisciplinary team (MDT) (**Supplementary Figure 2**) was established to discuss the investigation (**Supplementary Table 1**), management, and outcomes of children with complex/undiagnosed CNS pathology.

From 2014-2022, 195 clinical samples from 178 patients were processed for CMg investigations. To determine the CMg impact on clinical management, we evaluated 60 GOSH MDT-managed patients (mean age=7y, range=0.25-17). 52% were male, 23% previously healthy, 60% immunocompromised at baseline (**Table 1**). Demographic details, neurological and neuroradiological features are shown in **Table 2**.

Most patients had one CMg sample; five (#6,10,44,54,59) had both CSF and brain biopsy CMg (**Table 2**, **Supplementary material** for RNA degradation and turnaround times). Pathogen detection rates were highest for brain tissue (29%) compared to CSF (15%) (**Supplementary Figure 3A**, Fisher's exact test p=0.24). Microbes of unknown clinical significance were more frequent in CSF (12%) compared to brain (3%) (**Supplementary Figure 3A**). A greater diversity of pathogens was identified in brain biopsies (**Supplementary Table 2**).

Of 18/60 (30%) cases with a positive CMg result, in 14 (23%) we identified the causative pathogen (**Tables 2, Supplementary Table 2**), while in four we detected microorganisms of unknown clinical significance (#7,42,48,57; **Supplementary Table 2**). Of 14 patients where the pathogen was identified, seven had previously undetected pathogens (#1,4,10, 29, 33,41,45; **Table 2**). Two patients had pathogens identified in brain tissue not previously known to cause encephalitis: Coronavirus OC43 (#4)[3] and mumps vaccine strain (MVS; #45)[4], while Avian orthoavulavirus (#41) and Astrovirus VA1/HMO-C (#1)[5] had been associated with encephalitis once before[6,7]. Cases #10 (Astrovirus VA1/HMO-C), #41 (Avian orthoavulavirus), #45 (MVS) and #29 (HSV-1), although confirmed PCR positive in brain, were PCR negative in all other specimens tested, including CSF. #29 had been diagnosed with and treated for HSV-1 encephalitis 10 months earlier (CSF PCR positive). In seven cases (#3,9,11,14,39,49,59; **Table 2**), CMg confirmed a pathogen previously identified elsewhere, as the cause of encephalitis. CMg identified the pathogen in 12/36 (33%) immunocompromised patients in contrast to 2/24 (8%) immunocompetent patients (**Supplementary Figure 3B,** Fisher's p=0.03).

Based on CMg results, the MDT recommended management changes in 74% (42/57), with three results (#1,4,51) only available post/peri-mortem, precluding intervention (**Table 2**). This included 24/33 (73%) of the immunocompromised and 18/24 (75%) of immunocompetent patients (**Supplementary Material**). Overall, 45/57 are alive in follow-up, including eight where new, specific antimicrobials were started following pathogen detection. Nonetheless, mortality was high (15/60; 12/57 with antemortem diagnosis), including eight despite MDT informed modification to management.

Here we show in a retrospective case-series, that integrating CMg in a clinical neuro-infection MDT improves diagnoses and informs potentially lifesaving management decisions, particularly

for immunocompromised hosts who are at higher risk of unusual CNS pathogens, missed by standard investigations.

The most evident benefit of CMg is the identification of unexpected pathogens that have never or rarely been reported to cause meningoencephalitis. Neurotropic astroviruses and coronavirus OC43 have since been identified in additional cases of undiagnosed encephalitis[8,9] . Interestingly, four cases (#41,45,10,29) were positive for the causative agent only in brain tissue. While treatment options remain few, particularly for viral encephalitis, the potential use of repurposed antivirals with broad spectrum anti-RNA activity is being actively investigated. Where known pathogens were found, CMg still informed treatment. In #9, who had aspergillus in non-CNS tissue and group-B streptococcus in blood culture prior to developing encephalitis, CMg detected only aspergillus in brain, facilitating targeted antifungal treatment. Importantly, negative CMg, may increase confidence in an inflammatory aetiology, resulting in recommendations to start or escalate immunomodulation/immunosuppression.

MDT management changes could be broadly grouped into four categories: pathogen directed therapy, immunomodulation, targeted therapy for non-infective/non-inflammatory pathology, re-evaluation of goals of care. Antemortem CMg results, led to the above decisions being applied to 73% (24/33) of immunocompromised patients. However, not all MDT-recommended changes were due to CMg results alone, and co-ordinated MDT communication was essential for contextualisation, particularly required when microorganisms were found at low levels or did not fit with the clinical presentation (TTV, HHV-6&7, **Supplementary Material**).

Our analysis is limited by its retrospective single-centre nature with heterogenous presentations. MDT outcomes are subjective, although we attempted to overcome this with two independent clinicians analysing patient data. Shifting MDT expertise as knowledge increased, and CMg diagnostic advancements over time, may have altered decision-making and limited standardisation.

In summary, we report a clinical MDT service that has successfully integrated CMg into the diagnostic pathway for complex/undiagnosed CNS presentations where infection remained unconfirmed despite extensive conventional diagnostics. CMg patient selection and MDT diagnostic stewardship was essential. Based on our findings, the practice for all GOSH patients with undiagnosed meningoencephalitis now includes early evaluation at the MDT, and routine recommendation of CSF CMg. Brain biopsy, appropriately stored to allow CMg if required, is carried out more confidently where diagnostic dilemmas remain, supported by the procedure's safety record in children[10]. While unbiased testing is expensive and limited to centres with laboratory capacity and CMg expertise, it overcomes the multiple pathogen testing constraints that small paediatric specimen volumes impose. Crucially, both positive and negative CMg results are of great utility in clinical decision making, particularly in immunocompromised individuals. Increased funding for referral CMg centres that can process large sample numbers with quicker turnaround-time and lower CMg cost, especially in the context of high healthcare costs for undiagnosed CNS pathology, could foster further knowledge translation and should encourage routine CMg adoption into clinical practice.

Declaration of Interest
Nothing to disclose

Ethics
Metagenomic analysis were carried out by the routine diagnostic service at Great Ormond Street Hospital. Additional PCRs, Immunohistochemistry on samples received for

metagenomics (not discussed in the paper in detail) are part of the Great Ormond Street Hospital (GOSH) protocol for confirmation of new and unexpected pathogens. The use for research of anonymised laboratory request data, diagnostic results and residual material from any specimen received in the GOSH diagnostic laboratory was approved by UCL Partners Pathogen Biobank under ethical approval granted by the NRES Committee London-Fulham (REC reference: 17/LO/1530).

Demographic and clinical information on cases included in this study were obtained according to the clinical audit with registration number: 3583.

The case IDs in the paper are anonymised IDs that cannot reveal the identity of the study subjects and are not known to anyone outside the research group, such as the patients or the hospital staff.

Acknowledgements

We would like to recognise the large group of junior doctors, clinical nurse specialists, laboratory scientists, and specialist physicians that contribute their expertise to the GOSH neuro-infection MDT. We would like to specifically thank Drs Louis Grandjean, Seilesh Kadambari, Nigel Klein, Ashwin Pandey, James Soothill, Claire Booth, Winnie Ip, Catherine O'Sullivan, Reem Elfeky, Waseem Qasim, John Hartley, Cheryl Hemingway, and Yael Hacohen for their contributions. In addition, we would like to acknowledge the patients and their families for their valued contributions to the clinical MDT and the advancement of translational diagnostics.

S.M is funded by a W.T. Henry Wellcome fellowship (206478/Z/17/Z). J.B receives funding from the National Institute of Health Research UCL/UCLH Biomedical Research Centre. TJ is grateful for funding from the Brain Tumour Charity, Children with Cancer UK, Great Ormond Street Hospital Children's Charity, Olivia Hodson Cancer Fund, Cancer Research UK and the National Institute of Health Research.

All research at Great Ormond Street Hospital NHS Foundation Trust and UCL Great Ormond Street Institute of Child Health is made possible by the NIHR Great Ormond Street Hospital Biomedical Research Centre.

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Contributors

JP, SM, JB, AB, MK conceived and designed the study, JP, JB & SM wrote the manuscript, JP, NR, DC, GD collated the patients' clinical data, JP, JHassell assessed and categorised the MDT outcomes, JRB developed the wet-lab CMg methods, JRB, LA, AL, JCDL, AK, DS processed samples and generated the CMg data, SM developed the computational methods, SM, CV, NS analysed the CMg data, SM, NS, JRB, LA, SD, JHatcher, KH and JB interpreted the CMg data, MK, AB, DS, KMoshal developed the initial framework for the neuro-infection MDT, MK and JHassell are the MDT neurology leads, AB is the infectious diseases MDT lead, JHatcher the microbiology MDT lead, MAK and AJJW the clinical immunology MDT leads, AM and TJ the histopathology/neuropathology MDT leads, JB the clinical virology MDT lead, KA the

neurosurgery MDT lead, and GL the BMT MDT lead. KMankad is the neuroradiology MDT lead, interpreted radiological findings and collated radiological figures.

References

1 Xu C, Chen X, Zhu G, *et al.* Utility of plasma cell-free DNA next-generation sequencing for diagnosis of infectious diseases in patients with hematological disorders. *Journal of Infection* 2023; **86**: 14–23.

2 Glaser CA, Honarmand S, Anderson LJ, *et al.* Beyond viruses: clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis* 2006; **43**: 1565–77.

3 Morfopoulou S, Brown JR, Davies EG, *et al.* Human Coronavirus OC43 Associated with Fatal Encephalitis. *New England Journal of Medicine* 2016; **375**: 497–8.

4 Morfopoulou S, Mee ET, Connaughton SM, *et al.* Deep sequencing reveals persistence of cell-associated mumps vaccine virus in chronic encephalitis. *Acta Neuropathol* 2017; **133**: 139–47.

5 Brown JR, Morfopoulou S, Hubb J, *et al.* Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients. *Clinical Infectious Diseases* 2015; **60**: 881–8.

6 Quan PL, Wagner TA, Briese T, *et al.* Astrovirus Encephalitis in Boy with X-linked Agammaglobulinemia. *Emerg Infect Dis* 2010; **16**: 918.

7 Winter S, Lechapt E, Gricourt G, *et al.* Fatal encephalitis caused by Newcastle disease virus in a child. *Acta Neuropathol* 2021; **142**: 605–8.

8 Lum SH, Turner A, Guiver M, *et al.* An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient. *Transplant Infectious Disease* 2016; **18**: 960–4.

9 Nilsson A, Edner N, Albert J, Ternhag A. Fatal encephalitis associated with coronavirus OC43 in an immunocompromised child. *https://doi.org/101080/2374423520201729403* 2020; **52**: 419–22.

10 Bai HX, Zou Y, Lee AM, Lancaster E, Yang L. Diagnostic Value and Safety of Brain Biopsy in Patients with Cryptogenic Neurological Disease: A Systematic Review and Meta-analysis of 831 Cases. *Neurosurgery* 2015; **77**: 283–95.

Table 1. (a) Patients' demographic and clinical characteristics. (b) Sampling type and CMg findings

a.

Demographic and Clinical Characteristics	N	Percentage %
Patients	60	
Age		
Median/Range	7 / 0.25-17 y	
0-1	3	5%
1-5	24	40%
6-10	15	25%
11-15	16	27%
>=16	2	3%
Sex		
Female	29	48%
Male	31	52%
Outcome		
Hospitalized	60	100%
Died	15	25%
Health at baseline		
Immunocompromised/suppressed	36	60%
Primary Immune deficiency	16	35%
Malignancy	9	20%
Post HSCT/CART/thymus transplant	11	24%
Known rheumatological/inflammatory conditions	3	7%
Previously Healthy	14	23%

b.

Sampling type and CMg report	N	Percentage %
Patients	60	
CNS Sampling		
CSF	21	35%
CNS Tissue	35	58%
CSF and CNS Tissue	4	7%
CMg report		
Positive - Pathogenic	14	23%
Positive – non pathogenic	4	7%
Negative	42	70%

CART: chimeric antigen T-cell receptor; CMg: clinical metagenomics; CNS: central nervous system; CSF: cerebrospinal fluid; HSCT: haematopoietic stem cell transplant;

Table 2. Patients' detailed clinical information and results

Case	Age Band (y)	Sex	Past Medical History	Immune suppression	Main Neurological Features	Radiological Findings	Main Microbiological Findings	CMg Sample	CMg Findings	Change in Targeted Anti-infective Therapy	MDT change in Patient Management	Died
1*	1-5	M	CID, status post HSCT	Yes	Enc, dystonia, DR, oromotor dyskinesia	Progressive global atrophy with bilateral subdural effusions	Adenovirus, EBV (blood)	Brain biopsy	Astrovirus VMA1/HMO-C	No	NA, Results perimortem	Yes
2*	11-15	F	Undefined neurologic disorder	No	Tremor, dystonia, bradykinesia, DA, head titubation	Progressive white matter lesions	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy	No
3*	6-10	M	NF1, HM status post HSCT (x2)	Yes	HA, FP	Large left basal ganglia lesion	EBV (blood)	Brain biopsy	EBV	Yes, Treated EBV CNS PLTD	Yes, Targeted EBV PTLD treatment	No
4*	1-5	M	SCID, status post HSCT	Yes	Enc, dystonia, truncal hypotonia, tremor	Diffuse leptomeningeal infiltration with brain swelling	PJP pneumonia, Rotavirus (stool)	Brain biopsy	Coronavirus OC43	No	NA, Results post-mortem	Yes
5*	6-10	F	Healthy	No	Enc, HA, vision abn, increased ICP, DA, PN	Enhancing nerves – cranial and cauda equina	EBV viraemia	Brain biopsy	ND	No	Yes, Targeted therapy/immunomodulation for atypical Miller Fisher syndrome	No
6	11-15	M	HM, status post CAR-T	Yes	Sz, progressive HP and FP	Diffuse confluent white matter signal abn	Adenovirus, CMV (blood)	Brain biopsy, CSF	ND	No	Yes, Immunomodulation therapy for treatment-related side effect	No
7	11-15	M	HM	Yes	Sz, HA	PRES-like changes	BKV (blood, urine; equivocal in CSF)	CSF	HHV-7	No	Yes , Targeted therapy for atypical PRES	Yes
8	11-15	F	Healthy	No	HP, foot drop	Diffuse right parietal lesion with bilateral perilesional oedema	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy	No
9	11-15	F	HM, status post sepsis	Yes	Sz	Multiple focal lesions, some ring enhancing, some microhaemorrhagic	GBS (blood), aspergillus fumigatus (BKA & gastrectomy tissue)	Brain biopsy	Aspergillus fumigatus	Yes (antifungal)	Yes, Targeted anti-infective treatment	No

10	1-5	F	CID	Yes	DR, enc, dystonia, hypotonia	Bilateral basal ganglia and thalamic signal abn with atrophy	Parainfluenza, rhino/enterovirus (NPA), norovirus (stool), H. flu/stenotrophomonas pneumonia	Brain biopsy, CSF	Brain Biopsy - astrovirus VA1/ HMO-C; CSF - NP	Yes (Nitazoxanide, ribavirin, favipiravir)	Yes, Targeted salvage antivirals and subsequent change in goals of care	No
11	1-5	M	CID	Yes	Enc, bulbar abn	Subdural effusions, signal abn with diffusion restriction in fornices, cerebellar peduncles, dentates and periventricular white matter	RSV (NPA) and Adenoviraemia (Blood, NPA, CSF)	CSF	Adenovirus, TTV	Yes (brincidofovir)	Yes, Targeted CNS antiviral therapy & change in goals of care	No
12	11-15	M	Alopecia	No	HP, DA, enc	Multifocal cortical and subcortical lesions with leptomeningeal enhancement	ND	Brain biopsy	ND	No	Yes, Targeted malignancy treatment in the absence of infection	Yes
13	11-15	M	Healthy	No	Bilateral progressive leg weakness	Multiple small enhancing and restricting white matter lesions	ND	Brain biopsy	ND	No	No	No
14	1-5	M	HM, status post CAR-T therapy	Yes	Enc, sz, muscle wasting, hyperreflexia	Periventricular white matter T2-hyperintensity and atrophy	adenovirus (blood)	CSF	Adenovirus	No	Yes, Change in goals of care	Yes
15	<1	M	SMA type-1, status post gene therapy (Nusinersen®)	No	Hypotonia, feeding abn	Diffuse subcortical and deep grey matter diffusion restricting signal abn	ND	Brain biopsy	ND	No	No	Yes
16	6-10	M	CNS-M	Yes	Enc	Progressive atrophy with deep and cortical grey matter lesions with multiple infarcts	ND (Presumptive Histoplasmosis)	Brain biopsy	ND	Yes (antifungal)	Yes, Targeted anti-infective treatment	No
17	1-5	F	Healthy	No	Rhombo-enc, truncal hypotonia, HP	Diffuse brain stem and cerebellar inflammation	Parechovirus (stool & NPA)	Brain biopsy	ND	No	Yes, Targeted malignancy treatment in the absence of infection	No
18*	1-5	M	DID	Yes	Enc, DA, sz	Extensive grey and white matter signal abn	ND	Brain biopsy	ND	No	Yes, Proceed to HSCT in absence of active infection	No
19*	1-5	F	lysosomal storage disease, status post HSCT, VP shunt	Yes	HP, DR, feeding abn	Multiple enhancing and diffusion restricting mass lesions	Aspergillus Fumigatus (CSF), brain biopsy (18S)	Brain biopsy	ND	Yes (intrathecal antifungal)	Yes, Salvage anti-infective therapy, change in goals of care	Yes
20*	1-5	M	Healthy	No	Refractory febrile sz, enc	Progressive diffuse signal abnormality of the cerebral	Adenovirus (NP)	Brain bio	ND	No	Yes, Immunomodu	No

						cortex, basal ganglia and thalami		psy			lation therapy for FIRES	
21*	11-15	M	DID	Yes	Occipital HA, vision abn, DR, repeat collapse, aphasia, hand tingling	Multifocal enhancing cerebral and cerebellar white matter lesions	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy for CNS HLH	No
22*	6-10	M	Undefined neurologic disorder	No	Enc, HA, swallowing abn, vision/hearing loss, bilat OA	Extra-axial, enhancing, lesions in frontal lobes with oedema	NP (presumptive CNS TB)	Brain biopsy	ND	No	Yes, Immunomodulation therapy for undiagnosed neuroinflammatory disorder	No
23	6-10	F	HM, status post HSCT	Yes	HP, FP, facial pain, elbow swelling	No intracranial abnormality. No intracranial abnormality. Diffuse subcutaneous oedema from shoulder to wrist with diffuse muscle signal changes	SARS-CoV-2 (NPA), CMV (blood), rhinovirus/enterovirus (NPA)	CSF	ND	No	Yes, Immunomodulation of treatment related side effect	No
24	6-10	M	Epilepsy partialis continua, undefined neurologic disorder	No	Deterioration in sz control	Confluent progressive frontal and temporal white matter lesions, some with established scarring	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy for undiagnosed neuroinflammatory disorder	No
25*	1-5	M	DID	Yes	Sz, hypertonia, DR, enc	Diffuse multifocal enhancing lesions. Leptomeningeal enhancement	EBV (blood), FluA (NPA), RSV (NPA)	Brain biopsy	ND	No	Yes, Immunomodulation therapy for CNS HLH	Yes
26*	11-15	F	Undefined vasculopathy of the CNS	No	Stroke, DR, sz, vision abn, fatigability	Mature cortical and subcortical infarcts	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy for undiagnosed neuroinflammatory disorder	No
27*	6-10	F	Inflammatory disorder of the CNS	No	Sz, HP, HA, cognitive regression, uveitis, chorioretinitis	Patchy signal abnormality with enhancement in the right midbrain, thalamus, PLIC and pre/post-central gyrus	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy	No
28*	11-15	F	Healthy	No	Sz, progressive HP	Left mesial temporal sclerosis	EBV (blood)	Brain biopsy	ND	No	Yes, Immunomodulation for atypical Rasmussen's	No
29*	1-5	F	Status post HSV encephalitis	No	Sz, lethargy, increased ICP	Confluent new signal change with leptomeningeal enhancement in both frontal lobes, the peri-rolandic regions and thalami.	ND	Brain biopsy	HSV	Yes (chronic HSV antiv	Yes, Immunomodulation for post-infectious	No

						Residual encephalomalacia and hemosiderin staining				iral suppression)	granulomatous condition + HSV antivirals	
30*	6-10	M	DID	Yes	Sz, enc	Multifocal cortical-subcortical ring enhancing lesions	EBV (blood)	Brain biopsy	ND	No	Yes, Targeted therapy for atypical PRES	Yes
31*	1-5	F	Healthy	No	Ataxia. DA, HP, hypertonia, speech regression, bulbar abn	Right cerebral atrophy	ND	Brain biopsy	ND	No	Yes, Immunomodulation therapy for undiagnosed neuroinflammatory condition	No
32*	11-15	F	Healthy	No	Ataxia, myoclonus, DR, oromotor apraxia	No abnormalities	ND	Brain biopsy	ND	No	No	No
33*	6-10	F	Healthy	No	Enc, HP	Hydrocephalus with diffuse leptomeningeal enhancement	ND	Brain biopsy	M. tuberculosis	Yes, (targeted TB treatment)	Yes , Targeted anti-infective treatment	No
34	11-15	F	Healthy	No	Sz, enc	No abnormalities	ND	CSF	ND	No	Yes, Immunomodulation therapy for FIRES	No
35	11-15	F	IID, status post HSCT (10 years prior)	No**	Gait disturbance, hand tremor	No abnormalities	ND	CSF	ND	No	No	No
36	1-5	M	TD, status post thymus transplant	Yes	Sz	No abnormalities	SARS-CoV-2 (NPA)	CSF	ND	No	Yes, Immunosuppression for post-infectious immune-dysregulation	No
37	6-10	F	HM	Yes	HP	Multi-territorial acute infarcts, with nodular leptomeningeal enhancement	ND	CSF	ND	No	No	Yes
38	11-15	F	Vein of Galen malformation, status post embolisation	No	Enc, HA	Focal diffusion restriction along the globus pallidus with subarachnoid haemorrhage	ND	CSF	ND	No	No	No
39	1-5	M	SCID, status post HSCT	Yes	Mycoclonus, enc	Meningoencephalitis with ventriculitis	CMV (blood)	CSF	CMV	Yes, (foscarnet, ganci	Yes, Diagnostic confirmation and targeted	No

										clovir, letermovir)	CMV treatment	
40	6-10	F	HM	Yes	HA	Right pontine diffusion restriction and left hippocampal swelling	ND	CSF	ND	No	Yes, Chemotherapy for CNS malignant spread after CNS infection ruled out	No
41	≥16	M	DID, status post HSCT (15 years prior)	Yes†	Descending paralysis, enc, myoclonus	Extensive cortical signal change with diffusion restriction in the left frontal, insular and parietal regions with further involvement of the left lentiform nucleus and the right peri-rolandic cortex and cerebellum	ND	Brain biopsy	TTV, Avian orthoavulavirus	Yes (ribavirin, nitazoxanide, favipiravir)	Yes, Directed antivirals with concurrent immune-suppression of CNS HLH	No
42	1-5	M	SCID, status post HSCT	Yes	Gait disturbance, ptosis, weakness	No abnormalities	Rhino/enterovirus (NPA), EBV (blood)	CSF	TTV	No	Yes , Targeted Myasthenia Gravis Treatment	No
43	<1	F	BMF syndrome	Yes	Abn movements, neck stiffness	Progressive atrophy and subdural effusions	SARS-CoV-2 (NPA)	CSF	ND	No	No	No
44	11-15	M	IBD, large vessel vasculitis	Yes	Sz, enc	Extensive white matter signal changes throughout with microhaemorrhages and nodular enhancement	CMV (blood), SARS-CoV-2 (NPA)	Brain biopsy, CSF	ND	Yes	Yes, Change in Immunosuppression regimen	No
45	1-5	M	SCID, status post HSCT	Yes	Sz, DR, speech delay, hearing/vision loss	Multifocal areas of enhancing signal abn in the grey and white matter involving the cerebral hemispheres bilaterally, particularly the basal ganglia and parietooccipital lobes	ND	Brain biopsy	Mumps virus (vaccine strain)	No	Yes, IVIG & reduction of immunosuppression	Yes
46*	<1	F	TD	Yes	Sz	Surface microhaemorrhages, cerebral underdevelopment	Rotavirus, norovirus (stool), BCGosis, Rhino/enterovirus (NPA)	CSF	ND	No	No	Yes
47	1-5	F	Healthy	No	Sz, increased ICP	Extensive craniospinal leptomeningeal enhancement, left MCA ischaemic infarcts	SARS-CoV-2 (NPA)	Spinal Meningeal Tissue	ND	Yes (targetted TB treatment)	Yes, Diagnostic confirmation and targetted anti-infective treatment	No
48	1-5	M	CID, status post HSCT (X2)	Yes	Increased ICP	No abnormalities	ND	CSF	HHV6(B)	No	No	No

49	1-5	M	HM	Yes	HP, enc, sz	Multiple ring enhancing lesions in left frontal and parietal lobes	aspergillus fumigatus (CSF 18S)	Brain biopsy	Aspergillus fumigatus	Yes (antifungal)	Yes, Exclusion of alternative/concurrent diagnosis (ie. toxoplasma)	No
50	6-10	M	sJIA	Yes	Sz	White matter changes in centrum semiovale	norovirus (stool), adenovirus (stool), entero/rhino (NPA)	CSF	ND	No	No	No
51	≥16	M	IBD, status post HSCT	Yes	limb weaknes s/altered sensatio n, areflexia	Global atrophy	ND	CSF	ND	No	NA, Results post-mortem	Yes
52	1-5	F	SCID, status post HSCT	Yes	Sz	Global atrophy	rhinovirus (NPA)	CSF	ND	No	No	No
53	6-10	F	CID	Yes	HP	No abnormalities	ND	CSF	ND	No	No	No
54	1-5	F	Undefined autoinflammatory disorder	Yes	Sz	Limbic enc	ND	CSF & Brain biopsy	ND (CSF), ND (Brain biopsy)	No	Yes, Change in immunomodulation therapy	No
55	1-5	M	SCID, status post HSCT	Yes	Enc	Global atrophy	BCGosis, PJP pneumonia	CSF	ND	No	No	No
56	1-5	M	IID	Yes	Sz, aphasia	Diffuse cortical/subcortical enhancing signal abn in temporal lobes	bacillus spp (blood)	CSF	ND	No	Yes, Change in immunomodulation therapy	No
57	6-10	F	Healthy	No	Enc, HP, Sz, hallucinations	Multifocal infarcts in right MCA and PCA territories	HHV6 (blood, CSF, saliva)	Brain biopsy	HHV6(B)	No	Yes, Treated for alternative diagnosis in the absence of infection (PRES)	No
58	6-10	F	Healthy	No	HP, ataxia, CN, DA	Global atrophy, focal pontine lesion	ND	Brain biopsy	ND	No	Yes, Treatment of alternative diagnosis (CNS malignancy)	Yes
59	11-15	M	DID	Yes	Limb weaknes s, hyporefl exia, sz	Extensive confluent white matter and thalamic signal abn with punctate enhancement	HHV6(B) (Blood, CSF), astrovirus (stool), rhino/enterovirus (NPA), parvovirus (brain biopsy), Ecoli, klebsiella, enterococcus (dural matter)	CSF & Brain biopsy	HHV6(B) & TTV (CSF), HHV6(B) (Brain biopsy)	Yes (foscarnet, IVIG)	Yes, Change in goals of care, salvage antiviral therapy	Yes
60	1-5	F	Healthy	No	Sz	Cerebellar hemispheric swelling with microhaemorrhages, and further restricted foci in the cerebral hemispheres	HHV6(B) (blood, brain)	Brain biopsy	ND	Yes (ganciclovir)	Yes, Change in goals of care, treatment of alternative diagnosis (CNS	No

											malignancy), salvage antiviral therapy	

Table 2: Demographic and clinical features (with radiological, microbiological and metagenomics results) of patients referred for metagenomics with related clinical management outcomes of patients managed in neuro-infection multidisciplinary team

*pre-2019 CMg pipeline **fully engrafted and off immunosuppression †poor immune reconstitution.
Abn: abnormalities; BKA: below knee amputation; BMF: bone marrow failure; CAR-T: chimeric antigen receptor t-cell therapy; CID: combined immunodeficiency; CMg: clinical metagenomics; CN: cranial neuropathy; CNS-M: central nervous system malignancy; DA: dysarthria; DID: disorder of immune dysregulation; DR: developmental/behavioural regression; Enc: encephalopathic; FIRES: febrile infection-related epilepsy syndrome; FP: facial palsy; GBS: group B streptococcus; HA: headache; HLH: haemophagocytic lymphohistiocytosis; HM: haematologic malignancy; HP: hemiparesis/hemiparalysis; HSCT: haematopoietic stem cell transplant; IBD: inflammatory bowel disease; ICP: intracranial pressure; IID: innate immune deficiency; MCA: middle cerebral artery; MDRTB: multi-drug resistant tuberculosis; MDT: multidisciplinary team; ND: nil detected; NF1: neurofibromatosis type-1; NPA: nasopharygeal aspirate; OA: optic atrophy; PCA: posterior cerebral artery; PJP: pneumocystis jirovecii pneumonia; PLIC: posterior limb of internal capsule; PN: peripheral neuropathy; PRES: posterior reversible encephalopathy syndrome; PTLD: post-transplant lymphoproliferative disorder; SCID: severe combined immunodeficiency; sJIA: systemic onsert juvenile idiopathic arthritis; SMA: spinal muscular atrophy; Sz: seizures; TB: tuberculosis; TD: thymic disorder; VP; ventriculoperitoneal

Declaration of Competing Interest
All authors declare they have nothing to disclose