Viral, Bacterial, Metabolic, and Autoimmune Causes of Severe Acute Encephalopathy in Sub-Saharan Africa: A Multicenter Cohort Study

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Objectives To assess whether viral, bacterial, metabolic, and autoimmune diseases are missed by conventional diagnostics among children with severe acute encephalopathy in sub-Saharan Africa.

Study design One hundred thirty-four children (6 months to 18 years) presenting with nontraumatic coma or convulsive status epilepticus to 1 of 4 medical referral centers in Uganda, Malawi, and Rwanda were enrolled between 2015 and 2016. Locally available diagnostic tests could be supplemented in 117 patients by viral, bacterial, and 16s quantitative polymerase chain reaction testing, metagenomics, untargeted metabolomics, and autoimmune immunohistochemistry screening.

Results Fourteen (12%) cases of viral encephalopathies, 8 (7%) cases of bacterial central nervous system (CNS) infections, and 4 (4%) cases of inherited metabolic disorders (IMDs) were newly identified by additional diagnostic testing as the most likely cause of encephalopathy. No confirmed cases of autoimmune encephalitis were found. Patients for whom additional diagnostic testing aided causal evaluation (aOR 3.59, 90% CI 1.57-8.36), patients with a viral CNS infection (aOR 7.91, 90% CI 2.49-30.07), and patients with an IMD (aOR 9.10, 90% CI 1.37-110.45) were at increased risk for poor outcome of disease.

Conclusions Viral and bacterial CNS infections and IMDs are prevalent causes of severe acute encephalopathy in children in Uganda, Malawi, and Rwanda that are missed by conventional diagnostics and are associated with poor outcome of disease. Improved diagnostic capacity may increase diagnostic yield and might improve outcome of disease. *(J Pediatr 2023; ■:113360)*.

Severe acute encephalopathy is a common reason for hospital admissions in children living in sub-Saharan Africa and is associated with high morbidity and mortality.[1] Cerebral malaria and bacterial meningitis are regarded classically as the most common causes, but recent decreases in malaria transmission and upscaling of *Haemophilus influenzae* type b and pneumococcal vaccination programs have lowered their respective incidence rates.[2-5] Although this resulted in a significant reduction in the number of admissions for severe acute encephalopathy, there was a concomitant 2-fold increase in admissions of children with encephalopathy of undetermined cause in a Kenyan hospital.[4] This suggests that a proportion of cases previously may have been misdiagnosed, that alternative causes are more prevalent than previously thought, or both.

Viral central nervous system (CNS) infections, inherited metabolic disorders (IMDs), and autoimmune diseases are likely to occur in sub-Saharan Africa but have not been studied in detail.[4,6] Moreover, although bacterial CNS infection can be detected using conventional diagnostics, the high prevalence of anti-

CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
EBV	Epstein–Barr virus
HHV	Human herpes virus
HSV	Herpes simplex virus
IMD	Inherited metabolic disorder
OMIM	Online Mendelian Inheritance in Man
qPCR	Quantitative polymerase chain reaction

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biotic use before hospital admission may result in many cases being missed. Recent developments of untargeted laboratory methods allow screening for a wide range of causes while using limited sample volumes, such as viral metagenomics (for known and unknown viruses), 16s ribosomal RNA sequencing (for typical and nontypical bacteria), untargeted metabolomics (IMDs), and rat-brain immunocytochemistry (for autoimmune encephalitides). Although these advanced assays are not meant to be used as standard diagnostic assays, their use permits detailed evaluation of the prevalent causes, which may guide the development and implementation of simpler routine diagnostic assays. The aim of this proof-of-concept study was to study the prevalence of viral, bacterial, metabolic, and autoimmune causes that are missed by conventional diagnostics in hospitalized children with severe acute encephalopathy in sub-Saharan Africa.

Methods

Study Sites

This observational study was performed between 2015 and 2016 in 4 hospital sites: 3 national referral centers (Queen Elizabeth Central Hospital in Blantyre, Malawi; Mulago Hospital in Kampala, Uganda; and University Teaching Hospital of Kigali in Kigali, Rwanda) and 1 regional hospital (Ruhengeri Referral Hospital in Musanze Town, Rwanda).

Inclusion and Exclusion Criteria

All children (6 months to 18 years) presenting to the hospital with severe acute encephalopathy, defined as nontraumatic coma or convulsive status epilepticus, were eligible for inclusion. Nontraumatic coma was defined as a Blantyre coma scale ≤2 for at least 1 hour.[7] Convulsive status epilepticus was defined as any convulsion lasting for >30 minutes as observed by a clinician or not responding to at least 2 doses of a first-line anticonvulsant drug.[8] Children with a history of trauma or restoration of consciousness upon correction of hypoglycemia were excluded. At the Malawi study site, children with clinically suspected bacterial meningitis (either proven by cerebrospinal fluid [CSF] Gram stain or culture or suspected based on CSF leukocyte count >1000/mm^3) were not included.[1] In this proof-of-concept study, we aimed to include 50 children per country.

Ethical Considerations

Study approval and material transfer agreements (Rwanda: 055/CMHS IRB/2016, Uganda: SOMREC REF 2015-111 and UNCST HS1893, Malawi: P.10/13/1474) were obtained from the institutional and national review boards from all 3 countries. Procedures of consent in emergency situations were used. Thus, verbal consent was requested from the parent or guardian of unstable patients to allow blood sample collection during stabilization of the patient without delaying standard of care management. Poststabilization, full written informed consent was obtained.

Clinical Assessment

A detailed history and physical examination were performed upon hospital admission and stabilization of the child. Presence of pre-existing neurologic impairment and epilepsy was screened using previously validated questionnaires.[9,10] Children were monitored at least 3 times daily during hospital admission (neurologic examination, recording of vital signs) and screened for major neurologic sequelae on hospital discharge using a checklist containing various possible sequelae. All clinical data were collected by trained study physicians and nurses and recorded on predefined case record forms.

Sample Collection and Routine Diagnostics

Blood and CSF samples were collected before the administration of antibiotics at the research site. All samples were immediately analyzed or stored at −80°C until analysis. In Uganda and Malawi, a standardized array of conventional diagnostic testing was performed on all included patients according to study protocol including CSF analysis (culture, cell count and microscopy, glucose, and protein), blood analysis (malaria rapid test and microscopy, HIV test, blood culture, full blood count, renal and hepatic function tests, ammonia level, lactate and glucose levels), urinalysis (dipstick), and other relevant examinations (eg, malarial retinopathy). In Rwanda, no study-related routine diagnostic tests were performed locally, but the results of all diagnostics requested by the treating physician were collected. Neuroimaging studies were not available routinely at any of the study sites.

Additional Diagnostics

Full details of the additional diagnostics can be found in Appendix 1.

Screening for Viral Encephalopathies. CSF and pooled plasma-CSF samples were analyzed by viral metagenomics to detect common, uncommon, and novel viruses as previously described.[11] In addition, because viral metagenomics has an overall lower sensitivity than targeted assays, specific quantitative polymerase chain reaction (qPCR) assays were performed on CSF for the following viruses: enterovirus, human parechovirus, cytomegalovirus (CMV), Epstein–Barr virus (EBV), influenza viruses A and B, herpes simplex viruses I and II (HSV-1 and 2), varicella-zoster virus, human herpes viruses 6 and 7 (HHV-6 and 7), mumps virus, measles virus, adenovirus, West Nile virus, and rabies virus.

Screening for Bacterial CNS Infections. Patients with meningeal signs and/or at least 1 abnormal result of CSF analysis (≥5 leukocytes/mm^3, protein >40 mg/dL, glucose <2.5 mmol/L, or CSF/blood glucose ratio <0.4) were tested for *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* DNA by qPCR, along with prokaryotic 16s ribosomal RNA qPCR and sequencing.

Screening for IMDs. CSF and plasma samples were analyzed separately by untargeted metabolomics.[12,13] The Rwandan samples were not analyzed by metabolomics, as they were stored in RNAlater, which was not compatible with the analysis method. A bioinformatic pipeline was used to score probability scores for more than 100 IMDs on the metabolomics results.[14]

Screening for Autoimmune Encephalitides. Immunohistochemistry on rat-brain sections was performed using CSF samples to screen for neuronal antibodies.[15,16] Samples with indeterminate or positive results by immunohistochemistry were tested for known autoimmune antibodies by cell-based assays, and, if negative, were tested more extensively by live neuron staining.[17,18]

Diagnostic Evaluation

Each patient was evaluated for the presence of a list of diseases as potential causes of severe acute encephalopathy (summarized in **Table I**; available at www.jpeds.com, details in **Appendix 1**). Stringent criteria were used for the results of the untargeted assays to minimize the risk of detecting findings unrelated to encephalopathy. A distinction was made between patients with a clinical meningitis/encephalitis (eg, fever, neck stiffness, and pleiocytosis) vs a laboratory-confirmed CNS infection/autoimmune encephalitis (eg, detection of a bacterium or autoimmune antibody). Each disease was stratified into suspected, probable, and confirmed causes (**Table I** and **Appendix 1**). The disease with the highest causality tie was considered as the most likely cause of encephalopathy. When multiple diseases were present within the highest hierarchy, these diagnoses were considered to coexist. Patients without any disease were classified as unknown.

Effect of Additional Diagnostics

The yield of additional diagnostics was evaluated by comparing the original diagnosis (based on locally available data), and final diagnosis (based on locally available data and additional diagnostic testing). The original diagnosis was altered, broadened, or unmodified, when additional diagnostics identified a disease with a respectively higher, similar, or lower causal hierarchy than originally diagnosed.

Results

In total, 134 patients were included (**Figure 1**). Patient characteristics are shown in **Table II**.

Results of Standard Local Diagnostic Testing

Seventy-three (54%) patients were positive for malaria by rapid test or microscopy, of whom 42 (57%) had malarial retinopathy. Six patients had a positive blood culture (2 coagulase-negative *Staphylococcus*, 1 *H influenzae*, 1 *Salmonella* Typhi, 1 *Candida albicans*, and 1 *Klebsiella pneumoniae*). Two patients had a bacterium identified by CSF microscopy or culture; one was positive for *N meningitidis* and one for *H influenzae*. On the basis of these data, 46 (34%) patients had a confirmed cause, 49 (37%) had a probable cause, 23 (17%) had a suspected cause, and 16 (12%) had an unknown cause.

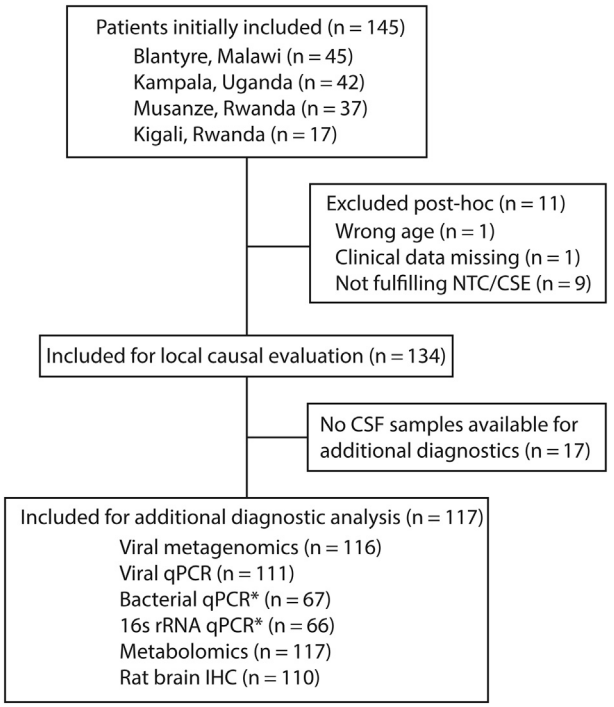

Figure 1. Patient inclusion. *Subset of patients with meningeal signs and/or at least 1 abnormal result in their CSF analysis: ≥5 leukocytes/mm^3, protein >40 mg/dL, glucose <2.5 mmol/L, or CSF/blood glucose ratio <0.4. *CSE*, convulsive status epilepticus; *NTC*, nontraumatic coma.

Results of Additional Diagnostics

Sufficient samples were available from 117 of 134 (87%) patients for additional testing. Full details are presented in **Appendix 1**.

Viral Encephalitides. Twenty patients tested positive for a virus in blood or CSF: 4 for EBV, 4 for HIV-1, 3 for CMV, 3 for HHV-6, 2 for HHV-7, 2 for hepatitis A virus, 2 for parvovirus B19, 1 for rabies virus, 1 for enterovirus, and 1 for a novel orthobunyavirus named Ntwetwe virus (coinfections were present in 3 patients; **Table III**; available at www.jpeds.com). All viruses except hepatitis A virus were detected in CSF.

Rabies virus, enterovirus, Ntwetwe virus, and CMV were classified as confirmed CNS infections (see disease-specific causality criteria in **Appendix 1**). The 2 patients with hepatitis A had no clinical or laboratory signs of liver failure, excluding a hepatic encephalopathy. Because hepatitis A was only found in blood, it was considered a suspected miscellaneous diagnosis. For all patients who

Table II. Patient characteristics

	Total
Total inclusions	134
Inclusion criteria	
Nontraumatic coma and convulsive status epilepticus	39/134 (29%)
Only nontraumatic coma	70/134 (52%)
Only convulsive status epilepticus	25/134 (19%)
Clinical characteristics	
Age, mo, median (IQR)	43 (6-168)
Male	83/134 (62%)
Symptom onset, d, median (range)	3 (0-28)
Referred from another center	112/133 (84%)
HIV status	
Positive	4/134 (3%)
Unknown	25/134 (19%)
Medical history*	
Previous nontraumatic coma or convulsive status epilepticus episode	7/126 (6%)
Epilepsy†	12/88 (14%)
Previous neurologic impairment†	31/128 (24%)
Previous medication use during current illness	
Antibiotic	47/134 (34%)
Antimalarial	54/134 (40%)
Traditional medicine	15/134 (11%)

Values are presented as number of positives of total known (percentage of the total known) unless stated otherwise.
*Patients could be included into multiple of the following categories.
†Epilepsy and previous neurologic impairment were scored using previously validated questionnaires.[9,10]

were HIV-1 positive, their viral load was greater in blood than CSF, classifying them as suspected viral CNS infections. Two patients positive for EBV had serological proof of an acute infection and therefore were considered as having confirmed viral CNS infections, whereas the other 2 were classified as probable. One patient with parvovirus B19 had a nearly 300-fold greater viral load in CSF than plasma, indicating a confirmed viral CNS infection. The other patient positive for parvovirus B19 had a lower viral load in CSF, classifying it as a probable CNS infection.

Bacterial CNS Infections. Eleven patients tested positive for a putative pathogenic bacterium in CSF: 5 for *H influenzae*, 3 for *S pneumoniae*, and 1 each for *N. meningitidis*, *Moraxella* sp., *Kocuria* sp., and *Bacillus* sp. (a coinfection was present in 1 patient). These patients included the 2 patients who were already positive for *N meningitidis* and *H influenzae* by local diagnostics. *H influenzae*, *S pneumoniae*, and *N meningitidis* were considered confirmed bacterial CNS infections, whereas *Bacillus* sp., *Moraxella* sp., and *Kocuria* sp.—because they are uncommonly associated with encephalopathy–were considered probable causes. Three patients (2%) tested positive for a probable nonpathogenic bacterium and were excluded as causes of encephalopathy.

Inherited Metabolic Disorders. For 15 patients, the metabolic profile in plasma and/or CSF was indicative of an IMD, of which 8 remained after evaluation by a metabolic disease expert: 2 for 5-oxoprolinase deficiency (Online Mendelian Inheritance in Man [OMIM] 26005, 1 probable, 1 suspected), 2 for dopamine transporter defect syndrome (OMIM 613135, both probable), 1 for citrullinemia type I (OMIM 215700, suspected), 1for phenylketonuria (OMIM 261600, confirmed), 1 for ribose 5-phosphate isomerase deficiency (OMIM 608611, probable), 1 for Canavan disease (OMIM 271900, suspected) and 1 for fructose-1,6-bisphosphatase deficiency (OMIM 229700, probable, 1 patient had 2 potential IMDs, Table III). The age range of patients with a metabolic profile indicative of a an IMD (median 2.25 years, IQR 0.77-3.35) was significantly lower (Mann–Whitney U test, $P = .008$) than patients without (median 3.83 years, IQR 2.00-6.00). Forty-one patients had increased probability scores for an IMD, which were considered to be confounded by a non-IMD condition (eg, prolonged sample storage and liver failure) and thus excluded as causes of encephalopathy.

Autoimmune Encephalitides. Nine patients showed neuropil staining by immunohistochemistry suggestive of a suspected autoimmune encephalitis, yet none was positive for known autoantibodies by CBA or stained live hippocampal neurons.

Combining Local and Additional Diagnostic Evaluation

Fourteen viral encephalopathies (12 viral CNS infections and 2 cases of hepatitis A classified as miscellaneous, 12% of studied cases), 8 bacterial CNS infections (7%), 4 IMDs (3%), and no autoimmune encephalitides were newly diagnosed, had the highest level of causality, and were accordingly considered the most likely cause of encephalopathy (Table III; 1 patient had a combined viral and bacterial CNS infection). Accordingly, for 25 of 117 (21%) patients, the results of additional diagnostics modified (altered or broadened) the original diagnosis (Figure 2). The number of confirmed cases increased from 44 to 56 (a 27% increase).

Outcome

Of the 134 included patients, 91 (68%) survived without major neurologic sequelae at the time of discharge, 23 (17%) survived with major neurologic sequelae, and 20 (15%) died. Patients with a modified (broadened or altered) cause of encephalopathy (aOR 3.59, 90% CI 1.57-8.36, Table IV), and especially those with an altered cause of encephalopathy (aOR 3.82, 90% CI 1.49-10.15), were at increased risk of poor outcome (major neurologic sequelae or death). Viral CNS infection (aOR 7.91, 90% CI 2.49-30.07) and IMD (aOR 9.10, 90% CI 1.37-110.45) were associated with an increased risk of poor outcome, whereas cerebral malaria was associated with a decreased risk of poor outcome (aOR 0.34, 90% CI 0.13-0.86). Patients with a confirmed final diagnosis had an increased risk of poor outcome (aOR 3.63, 90% CI 1.50-9.27).

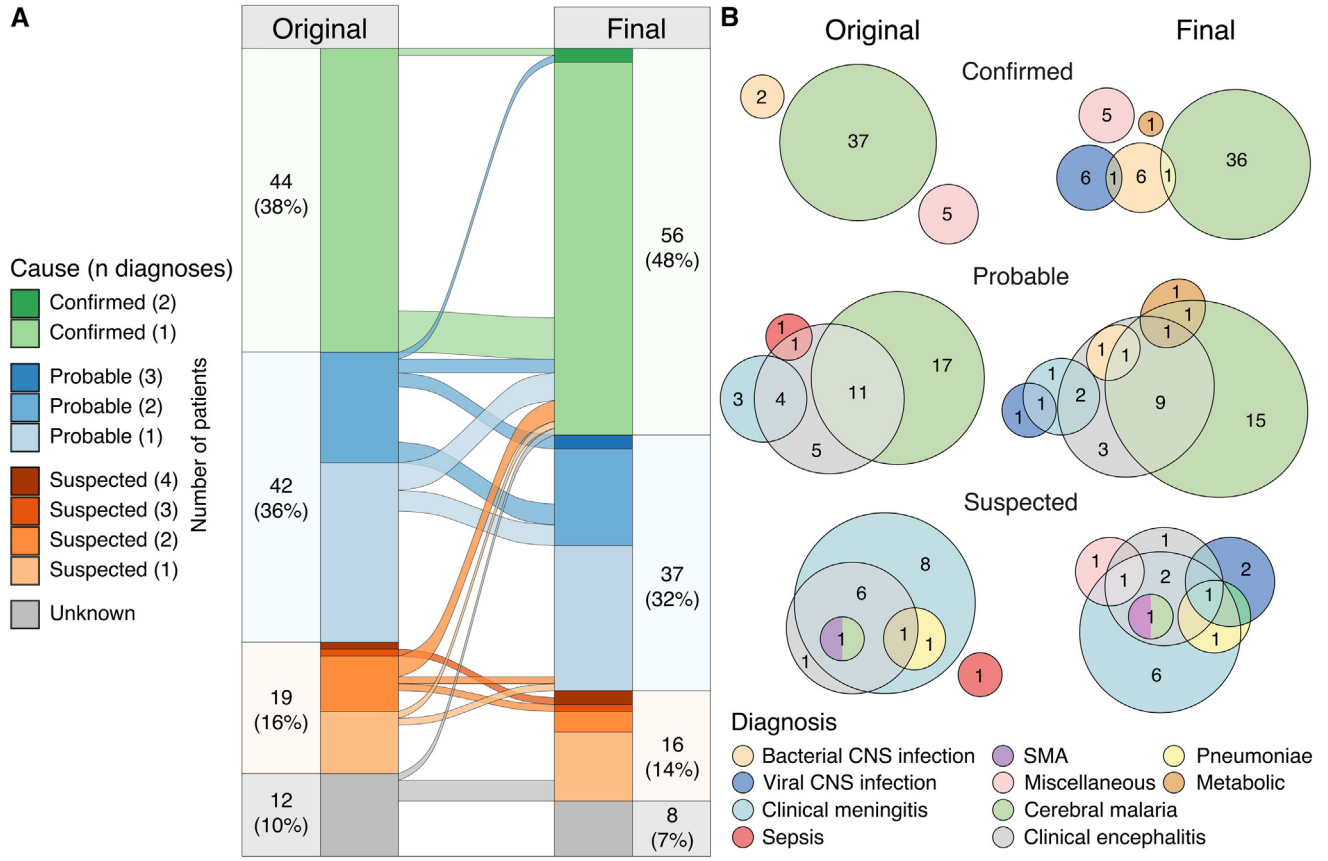

Figure 2. **A,** Effect of additional diagnostics on the distribution of causal certainty. The *height* of each colored bar represents the number of patients within a specific causality tier; *different shades* of the same color correspond to the number of diagnoses present within the highest causality tier of each patient. **B,** Effect of additional diagnostics on the distribution of overlapping diagnoses within each causal tier. The *size of the ellipses* indicates to the number of patients with a specific diagnosis. *Overlapping ellipses* describe patients with more than 1 diagnosis. Five patients had a confirmed miscellaneous diagnosis: chromosomal abnormality (R05), snake bite (R09), brainstem glioma (U05), severe electrolyte disturbance with metabolic acidosis caused by diarrhea (U88), and sickle cell stroke (U08). *SMA*, severe malarial anemia.

Discussion

We studied the extent to which bacterial, metabolic, and autoimmune encephalopathies are missed by conventional diagnostic testing in hospitalized children with severe acute encephalopathy in sub-Saharan Africa. Additional testing of 117 cases revealed an alternative cause of encephalopathy in 23% of patients. Viral encephalopathies (12%) were most often newly identified, followed by bacterial (7%) and metabolic (3%) causes. No case of autoimmune encephalitis was found.

Viral encephalopathies are increasingly being recognized as prevalent causes of severe acute encephalopathy in children in sub-Saharan Africa.[1,19-22] In our study, no single virus predominated, but rather a diverse group was found. These included several definitive causes of encephalopathy (eg, rabies virus and enterovirus), several indeterminate ones (eg, EBV and parvovirus B19), and even a novel virus (Ntwetwe virus), which was considered the most likely cause of disease.[23,24] Additional analyses for viruses that can only be considered causal in specific circumstances—serologic analysis and viral load comparisons in CSF and blood—suggested that some were likely to be causal (eg, 2 cases of primary EBV infection and 1 patient with a greater parvovirus B19 load in CSF than plasma), whereas others were most likely incidental findings. A previous study from Malawi detected an even greater prevalence (26%) of viral encephalopathies by conventional PCR testing.[1] Adenovirus infections accounted for one-third of cases in that study, whereas adenovirus infections were not detected in our and another recent study from Malawi.[21] Moreover, in another study, from Kenya, HSV-1 was detected in 9% and 12% of the children with malarial and nonmalarial encephalopathy, respectively.[22] In contrast, we did not detect any HSV-1 infections, and neither did a study from Uganda.[20] These differences may be explained by regional and temporal variability, which will need to be explored by future studies. Moreover, they underscore the need for routine viral diagnostics for children with severe acute encephalopathy, which is nonexistent currently.

Table IV. Outcome of severe acute encephalopathy

Groups	Total	Outcome of encephalopathy			Association with poor outcome
		No sequelae, No. (%)	Sequelae, No. (%)	Died, No. (%)	aOR* (90% CI)
Patients					
All	134	91 (68)	23 (17)	20 (15)	
Interrogated by additional diagnostics	117	85 (73)	18 (15)	14 (12)	
Result of additional diagnostics					
Diagnosis modified	25	11 (44)	9 (36)	5 (20)	**3.59 (1.57-8.36)**
Diagnosis altered	17	7 (41)	7 (41)	3 (18)	**3.82 (1.49-10.15)**
Diagnosis broadened	8	4 (50)	2 (25)	2 (25)	1.75 (0.49-6.32)
Final diagnosis					
Viral CNS infection	12	3 (25)	6 (50)	3 (25)	**7.91 (2.49-30.07)**
IMD	4	1 (25)	1 (25)	2 (50)	**9.10 (1.37-110.45)**
Bacterial CNS infection	10	6 (60)	3 (30)	1 (10)	1.17 (0.34-3.81)
Cerebral malaria	65	55 (85)	4 (6)	6 (9)	**0.34 (0.13-0.86)**
Clinical encephalitis	23	16 (70)	4 (17)	3 (13)	0.91 (0.36-2.17)
Clinical meningitis	16	11 (69)	3 (19)	2 (12)	0.74 (0.24-2.11)
Miscellaneous	7	3 (43)	2 (29)	2 (29)	2.56 (0.66-10.68)
Final causal certainty					
Confirmed	56	39 (70)	9 (16)	8 (14)	**3.63 (1.50-9.27)**
Probable	37	26 (70)	6 (16)	5 (14)	0.90 (0.41-1.95)
Suspected	16	12 (75)	3 (19)	1 (6)	0.52 (0.16-1.57)
Unknown	8	8 (100)	0 (0)	0 (0)	0 (NA-NA)

NA, not available.
Values in bold are statistically significant.
*Adjusted for research site. Poor outcome was defined as major neurologic sequelae or death.

Multiple bacterial CNS infections were missed by conventional diagnostic testing (CSF microscopy and culture). Local testing identified a bacterial CNS infection in only 2 patients, whereas bacterial infection was found in an additional 8 patients by bacterial and 16s qPCR. Likewise, a recent study from Uganda found that the addition of bacterial (and viral) qPCRs to routine diagnostics greatly increased the diagnostic yield.[20] This is likely explained by the administration of antibiotics before hospital admission—as was reported for the majority of children—or alternatively, by detecting nontypical bacteria that may only be detected by 16s qPCR and for which standard antibiotic therapy may be insufficient (eg, *Bacillus* sp., which often is resistant to ceftriaxone[25]). Failure to adequately treat these cases will likely result in poorer outcome. Considering that bacterial CNS infections were commonly missed, empiric antibiotic to treat meningitis may be warranted in patients without an initially confirmed cause of encephalopathy.

Testing for IMDs often has not been possible for patients in sub-Saharan Africa because of the lack of diagnostic capacity or newborn screening programs.[26] Using a novel approach of targeted metabolomics combined with an automated diagnostic algorithm, 4 (3%) patients had an IMD as the most likely cause of encephalopathy, including 1 case of a confirmed and fatal phenylketonuria. This highlights the need to build diagnostic capacity and study the epidemiology of IMDs in sub-Saharan Africa.

Similarly, the prevalence of autoimmune encephalitides in sub-Saharan Africa largely remains unknown. Eight percent of patients showed neuronal staining by rat brain slide immunohistochemistry, which may be reflective of antineuronal antibodies. However, despite extensive additional testing, neither known nor novel autoantibodies could be detected by cell-based assays or live hippocampal neuron testing. It has been hypothesized that early and broad exposure to microbes—more prevalent in sub-Saharan Africa than high-income countries—may reduce the risk of development of autoimmune diseases.[27] Our lack of cases may support this hypothesis, but larger studies are required to determine the true prevalence.

The World Health Organization case definition of cerebral malaria has likely contributed to a considerable proportion of misdiagnoses in the past. This case definition only requires the presence of coma persisting for at least 1 hour and *Plasmodium falciparum* parasitaemia in absence of alternative causes.[28] Because diagnostics to exclude such causes usually are not available, comatose children with asymptomatic malarial infection—present in nearly-one third of schoolchildren in Uganda and Malawi in high-prevalence areas[29,30]—can be misclassified as cerebral malaria.[31] Indeed, in our study, 5 of 28 patients with probable cerebral malaria (World Health Organization criteria for cerebral malaria) had a likely alternative cause of encephalopathy. In contrast, an alternative diagnosis was only found in 1 of 37 patients with confirmed cerebral malaria (requiring the presence of malarial retinopathy as marker for intracerebral malarial pathology[31]). This highlights the specificity of malarial retinopathy, which can be screened for by ophthalmoscopy, and suggests additional testing may not be required routinely for these patients.

The outcome of severe acute encephalopathies in children in sub-Saharan Africa remains unacceptably poor; 15% of our population died and 17% developed major neurologic sequelae. These outcomes are in line with a study from Uganda (18% mortality)[20] and somewhat better than a study from Kenya (25% mortality).[4] Enhanced etiologic understanding may improve the outcome by optimizing therapeutic management. Patients for whom additional diagnostics

aided causal evaluation were at increased risk of poor outcome, and several of these diseases may have been treatable if identified expeditiously. In addition, patients with a viral or metabolic cause were at increased risk of poor outcome, as treatments for these diagnoses are not routinely available. Nonetheless, antiviral medication has become increasingly available in sub-Saharan Africa. Although these antiviral agents may only be effective in a limited number of viral infections, improvements are currently being made to increase the therapeutic capacity, especially in the case of arboviral infections.[32] Improvement also can be made in terms of prevention and early detection of certain diseases. Vaccination and vector control may decrease virus transmission, and implementation of neonatal screening programs, which are practically not available in sub-Saharan Africa, can detect IMDs from a young age, which often could lead to relatively low-cost and uncomplicated patient management such as dietary interventions for phenylketonuria.

Performance of most of the additional diagnostic assays used in this study are not feasible routinely, but their results can guide prioritization and development of routine tests. For example, availability of qPCRs covering several pathogens (herpesviruses, enterovirus, rabies virus, parvovirus B19, *H influenzae*, *S pneumoniae*, and *N meningitidis*) would have allowed detection of nearly all CNS infections identified in this study. Although qPCR laboratories may only be available in a few places, tabletop multiplex qPCR assays exist that require minimal hands-on-time, have rapid turnaround time, and can be used in most hospitals in sub-Saharan Africa.[33] Furthermore, developments in small-scale handheld devices, eg, MinION-based metagenomic sequencing for broad pathogen detection[34] and RNA transcript-based point-of-care tests to differentiate between viral and bacterial infection,[35] will likely revolutionize diagnostic capabilities in the future.

This study has several limitations. First, although the results of the metabolic disease diagnostic algorithm were scrutinized by an expert in metabolic diseases, insufficient sample volumes were available for validation of the untargeted metabolomics results. As a result, except for the confirmed case of phenylketonuria, the other IMDs could not be diagnosed with certainty. Second, children with clinically suspected bacterial meningitis were excluded in the Malawian study site, which likely lowered the prevalence of bacterial meningitis in our cohort. Third, our sample size did not allow us to generate robust predictive models to identify which children should be evaluated by additional diagnostics, yet our preliminary results may be indicative for future studies. Fourth, we did not investigate all potential causes, such as poisonings or vasculopathies, which we estimated to be less prevalent than the causes we targeted. Moreover, we may have missed several viral CNS infections, which are best detected by serology such as flaviviruses.[36] Fifth, there was a delay between study subject inclusion and sample analysis. This was caused by a combination of factors, including difficulties in sample shipment, optimization and interpretation of experimental additional diagnostic assays, and processing delays caused by the coronavirus disease 2019 pandemic. Still, although changes in the etiology may have occurred since, the proof-of-concept nature of this study remains relevant.

Our results highlight that the etiologic landscape of acute encephalopathy is more heterogeneous than previously assumed. Future larger studies should aim to identify regional differences in the etiology within sub-Saharan Africa, identify clinical and laboratory diagnostic predictors, and define diagnostic algorithms for which this proof-of-concept study may serve as a stepping stone. Similar endeavors recently have been undertaken in Asia to study encephalitis in the Greater Mekong region[37] and India.[38] ■

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Appendix 1

Viral Polymerase Chain Reactions (PCRs)

All samples were analyzed according to routine clinical diagnostic protocols at the Amsterdam University Medical Centres. To summarize, samples were spiked with in interval RNA and DNA virus control. Nucleic acids (NAs) were extracted using the MagNA Pure 96 DNA and Viral NA Small Volume kit using 100 μL of cerebrospinal fluid (CSF). Extracted NAs were reverse transcribed to a total volume of 50 μL, of which 5 μL was used per quantitative (q)PCR on a LightCycler 480. Samples with a cycle threshold value ≤40 and validated by an independent molecular microbiologist were considered positive.

Viral Metagenomics

Full details are described in Edridge et al.[1] To summarize, samples were centrifuged and subjected to a DNase treatment to remove cellular material and free-floating DNA. NAs were extracted and reverse transcription was performed using nonribosomal primers followed by second-strand synthesis. The resulting double-stranded DNA was digested using Mse1 restriction enzyme and ligated to an adapter, preamplified by PCR, pooled at equimolar concentrations, and sequenced. The output was analyzed by multiple bioinformatic pipelines specialized in metagenomic classification.

Bacterial qPCRs

CSF were assessed by qPCRs targeting *ctrA* (*N meningitidis*), *pia* (*S pneumoniae*), and *siaT* (*H influenzae*) at the Netherlands Reference Laboratory for Bacterial Meningitis using in house developed modifications on DNA extracted (MagnaPure) from a noncentrifuged fraction of 100 μL CSF as previously described.[2]

16s qPCR and Sequencing

An in-house developed qPCR was first performed to screen for presence of sufficient bacterial DNA for sequencing. When positive, a second PCR using dual priming oligonucleotides[3] was performed, of which the amplicon underwent Sanger Sequencing. Amplicons were characterized by Blast on the NCBI Genbank database.

Untargeted Metabolomics

Metabolomics was performed at the genomic facility of the UMC Utrecht using an aliquot of 7.5 μL of EDTA plasma and CSF. Samples from 10 South Sudanese without severe acute encephalopathy were added to each run for comparison. The concentration of each metabolite was expressed as a z score compared with the median concentration in the controls.

Autoimmune Screening

Rat brains were fixed with paraformaldehyde, cryoprotected, snap frozen, and cut into sagittal sections. Sections were incubated with patients' CSF (1:2). The staining was visualized with diaminobenzidine and slides were counterstained with hematoxylin.[4,5]

Commercial cell-based assay (Euroimmun) was used to detect anti–N-methyl-D-aspartate receptor, anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, anti-γ-aminobutyric acid B receptor, anti-leucine-rich glioma-inactivated 1, anti-contactin-associated protein-like 2. An in-house cell-based assay with live cells was used to detect anti–N-methyl-D-aspartate receptor, anti-γ-aminobutyric acid (GABA) A receptor, anti-GABA B receptor, anti-immunoglobulin-like cell adhesion molecule 5, and anti-dipeptidyl-peptidase–like protein 6.

Samples scored questionable or positive on IHC, but without a known antibody, were tested more extensively. Neuronal cultures and staining protocol were performed as described before.[6,7] In short, living hippocampal neurons of at least 14 days in vitro were incubated with patients' CSF (1:2) and were subsequently fixed and stained with a fluorescently labeled secondary antibody.

Examples of Causality Model

Example #1. Based on locally available diagnostic data, a patient fulfills the diagnostic criteria of cerebral malaria (CM, probable), severe malarial anemia (SMA, suspected), and meningitis (probable), but no other diagnoses. As there is no confirmed diagnosis, the original cause of encephalopathy is CM and meningitis with a probable causal certainty. Using additional diagnostics, *H influenza* is detected in CSF, complying to the criteria of a confirmed bacterial CNS infection. As such, the causal certainty is "altered" from probable to confirmed, and the final cause of encephalopathy is bacterial CNS infection.

Causality model example 1—altered

Example 1: Altered	Diagnoses present	
Causal certainty	Original (local data only)	Final (local + additional diagnostics)
Confirmed		Bacterial CNS infection
Probable	CM, meningitis	CM, meningitis
Suspected	SMA	SMA

Example #2. Based on locally available diagnostic data, a patient complies to the diagnostic criteria of CM (suspected, probable), but no other diagnoses. As there is no confirmed diagnosis, the original cause of encephalopathy is CM with a probable causal certainty. Using additional diagnostics, an IMD (suspected and probable) is diagnosed based on the metabolomics CSF score and interpretation of the patient status. Because the highest causal certainty remains probable, the original cause of encephalopathy is broadened, and now includes both CM and IMD.

Causality model example 2—broadened

Example 1: Broadened	Diagnoses present	
Causal certainty	Original (local data only)	Final (local + additional diagnostics)
Confirmed Probable Suspected	CM	CM, IMD

Argumentation for Causality Model

The model was designed to include (1) different types of causes (eg, infectious and noninfectious diseases); (2) diagnoses with different likelihoods of being the cause of encephalopathy (eg, pneumonia was scored lower than central nervous system [CNS] infection); and (3) diagnoses that normally can only be considered by exclusion of alternative ones (eg, cerebral malaria).

Cerebral Malaria. We used the World Health Organization (WHO) diagnostic criteria of cerebral malaria[8] for a probable cause of encephalopathy in this study, but in addition required the presence of malarial retinopathy for a confirmed diagnosis. Retinopathy—a sign of malarial retinal vasculopathy that is a strong marker of more extensive cerebral vasculopathy, which is hallmark in pathologic confirmed cerebral malaria—has been demonstrated to increase the diagnostic sensitivity of cerebral malaria.[9] For noncomatose children with status epilepticus (who were also included in this study), we determined that the presence of malaria infection would support a diagnosis of suspected cerebral malaria, as this could be an early stage of a more comatose phenotype.

Clinical Meningitis/Encephalitis. Meningitis and encephalitis are descriptions of clinical syndromes characterized by inflammation of the brain (encephalitis) or meninges (meningitis). These syndromes may be caused by a wide range of infectious (eg, bacterial, viral, fungal, and parasitic) and noninfectious (eg, autoimmune, malignancy, and oncologic) causes. This is reflected by the commonly used diagnostic criteria, which classify a suspected and probable meningitis/encephalitis on the basis of clinical symptoms and laboratory analysis (eg, CSF white blood cell counts and protein and glucose concentrations) without identification of the underlying cause.[10,11] Identification of the underlying cause has important clinical value, as it may direct treatment (eg, antibiotic, antiviral, or steroid treatment) and may provide prognostic information. Moreover, applying the previously described criteria, nearly all patients in this study comply to a syndromic meningitis (74% suspected, 8% probable) or encephalitis (60% suspected, 34% probable), suggesting these diagnoses hold relatively low diagnostic information in such a population. Consequently, for this study, we consider that moving from a syndromic diagnosis (eg, a suspected or probable encephalitis/meningitis) to a confirmed diagnosis (eg, a viral/bacterial CNS infection or autoimmune encephalitis) is of considerable improvement, and classified these as an "altered" diagnosis.

Viral and Bacterial CNS Infection. Commonly used diagnostic criteria for a confirmed encephalitis and meningitis (eg, from WHO) only consider pathogens commonly associated with the syndrome (eg, *Streptococcus pneumoniae* and HSV-1), but do not specify pathogens with possible but lower causal associations (eg, Epstein–Barr virus [EBV] and Human herpes virus [HHV]-6). To allow these pathogens to be considered, we used a similar causal certainty classification for each specific pathogen based on previously established guidelines (Granerod et al[12]). For pathogens not included in this list, we evaluated whether these pathogens have been associated with CNS disease in other literature, and, if so, considered them as probable causes of encephalopathy. Because using a pathogen-based causality model alongside a clinical model was incompatible (eg, a probable syndromic meningitis and a suspected CNS pathogen would be hard to combine), we separated syndromic diagnosis (eg, meningitis and encephalitis) and ones in which a causal factor was identified (eg, viral/bacterial CNS infection and autoimmune encephalitis).

Pneumonia and Sepsis. Pneumonia and sepsis are frequently described as causes of encephalopathy in etiologic cohort studies in African children. However, from a pathophysiological perspective, these syndromes generally are unlikely to result in deep coma or status epilepticus. Only when the circulation or respiration is thus far hampered that it leads to brain stem impairment could these syndromes result in deep coma. Thus, we decided to include both syndromes, but only in severe forms as a suspected cause of encephalopathy, so that whenever an alternative etiology would be present, it would overrule the sepsis/pneumonia. In contrast, a microbiological confirmed sepsis with some specific pathogens (eg, *N. meningitidis* or rabies virus) also may be a sign of a CNS infection (according to WHO criteria a bloodborne pathogen in a child with a clinical presentation of meningitis would be classified as confirmed meningitis). Therefore, patients with a pathogen—commonly known to cause CNS infection—detected in the bloodstream were classified as a probable cause of encephalopathy.

Diagnostic Definitions

General note: the causal certainty classification (suspected, probable or confirmed) reflects the certainty of a specific diagnosis as being the cause of encephalopathy. In other words, while a specific diagnosis may be confirmed (eg, sepsis confirmed by a positive blood culture), this is regarded as a "probable" cause of encephalopathy.

Cerebral malaria

- Suspected (expert panel)
  - Malaria positive (RDT or slide)
- Probable (WHO criteria[8])

- Malaria positive AND
- Blantyre coma scale ≤2 for 1 hour AND
- Correction of hypoglycemia and convulsions

- Confirmed (Taylor et al[9])
  - Probable cerebral malaria AND
  - Presence of at least ONE of the following types of retinopathy:
    - Hemorrhages
    - Peripheral whitening
    - Vascular changes

Severe malarial anemia (adapted from WHO criteria[8])

- Suspected
  - Hemoglobin concentration <5 g/dL OR
  - Hematocrit <15% in children <12year (<7g/dL and <20% for older) OR
  - Undocumented pre-transfusion hemoglobin and hematocrit and transfused

Clinical meningitis (adapted from WHO criteria[11])

- Suspected
  - Fever AND
    - Altered consciousness OR
    - Meningeal signs (ONE of below):
      - Neck stiffness
      - Kernig sign
      - Brudzinski
      - Raised/pulsating fontanelle
      - Headache
- Probable
  - Turbid CSF appearance OR
  - >100 cells/mm^3 OR
  - >10 cells AND
    - protein >100 mg/dL OR
    - glucose <40 mg/dL
- Confirmed (nonexistent)
  - Patient with a confirmed meningitis (which requires pathogen identification according to WHO criteria) were classified as viral or bacterial CNS infection. Argumentation for this can be found in **Argumentation for causality model**.

Clinical encephalitis (adapted from Venkatesan et al[10])

- Altered mental status >24 hours (any decrease in BCS or MGCS >24 hours upon admission or history of unconsciousness for >24 hours) AND
- At least 2 (suspected) or 3 (probable) of the following criteria:
  - Fever
  - Generalized or partial seizures
  - New onset of focal neurologic findings
  - WBC count ≥5 cells/mm^3
  - Electroencephalogram signs consistent with encephalitis

- Confirmed (nonexistent)
  - According to Venkatesan et al.,[26] a confirmed encephalitis can be established if: (1) pathologic confirmation of brain inflammation consistent with encephalitis is shown; (2) a microbiological cause is confirmed; (3) an autoimmune cause is identified. As no pathologic data was available, patients with a confirmed encephalitis were classified as a viral or bacterial CNS infection or an autoimmune encephalitis depending on laboratory results. Argumentation for this can be found in **Argumentation for causality model**.

CNS infection (microbiologically confirmed)

- General (for pathogens that are not included in the pathogen-specific criteria, see below)
  - Probable (expert panel)
    - Pathogen detected in CSF, which is only rarely associated with CNS infections in literature
  - Confirmed (adapted from several studies[10-12])
    - Pathogen detected in CSF which is considered a well-known cause of CNS infections
    - List of pathogens (detected in this study, Granerod et al[12])
      - *N meningitidis*
      - S pneumonia
      - H influenza
      - Rabies virus
      - Enterovirus
- CMV (adapted from Granerod et al[12])
  - Confirmed
    - DNA detected in CSF AND
    - Proof of host immunosuppression (eg, HIV positive)
  - Probable
    - DNA detected in CSF
- EBV and HHV-7 (adapted from Granerod et al[12])
  - Suspected
    - DNA detected in CSF
  - Confirmed
    - DNA detected in CSF AND
    - Serological evidence of primary infection
- HHV-6 (adapted from Granerod et al[12])
  - Suspected
    - DNA detected in CSF
  - Probable
    - DNA detected in CSF AND
    - Evidence of primary CNS infection
  - Confirmed
    - DNA detected in CSF AND
    - Evidence of primary CNS infection AND
    - Exclusion of chromosomal integration
- HIV-1 (adapted from Granerod et al[12])
  - Suspected
    - RNA detected in CSF
  - Probable
    - RNA detected in CSF

■ Viral load greater in CSF than in plasma
- Parvovirus B19 (expert panel)
  - Suspected
    - ■ DNA detected in plasma
  - Probable
    - ■ DNA detected in CSF
  - Confirmed
    - ■ DNA detected in CSF AND
    - ■ Viral load higher in CSF than plasma
- Hepatitis A associated encephalopathy (miscellaneous cause, expert panel)
  - Suspected
    - ■ DNA detected in plasma
  - Probable
    - ■ DNA detected in CSF
  - Confirmed
    - ■ Viral load higher in CSF than plasma

Pneumonia

- Suspected (severe pneumonia, WHO criteria[13])
  - ALL of the following:
    - ■ ONE of the following
      - Cough
      - Difficulty breathing
    - ■ ONE of the following
      - Lower chest wall indrawing
      - Fast breathing (≥ 50/min 2-11 months, ≥ 40/min ≥12 months)
    - ■ ONE of the following
      - Oxygen saturation <90%
      - Central cyanosis
      - Inability to drink/breastfeed
      - Vomiting
      - Altered consciousness
      - Convulsions

Sepsis

- Suspected (clinical, adapted from "severe sepsis"[14])
  - The presence of at least 2 of the following 4 criteria, 1 of which must be abnormal temperature or leukocyte count AND:
    1. Core temperature of >38.5°C or <36°C.
    2. Tachycardia:
       - a mean heart rate >2 SD above normal for age OR
       - <1 year: bradycardia (HR <10 percentile for age)
    3. Mean respiratory rate >2SD above normal age or mechanical ventilation
    4. Leukocyte count elevated or depressed for age OR >10% immature neutrophils
  - Suspicion of infection (including malaria) AND
    - ■ Cardiovascular dysfunction OR
    - ■ Two or more other organ dysfunctions (see below)
- Probable (microbiological, expert panel)
  - Bacterium detected in the bloodstream by blood culture which is unlikely a skin contaminant

Organ dysfunction (for calculation of syndromic sepsis)

- Cardiovascular
  - Decreased blood pressure <5th percentile or systolic blood pressure <2 SD below normal age
  - Two of the following
    - ■ Unexplained metabolic acidosis: base deficit >5
    - ■ Increased venous lactate >2 times upper limit (interpreted as 5.0 as normal upper limit usually is 2.0 or 2.5)
    - ■ Prolonged capillary refill
- Respiratory
  - >50% FiO_2 to maintain saturation 92%
- GCS
  - Modified Glasgow coma scale <11 (or when not measured Blantyre coma scale <4)
- Hematologic
  - Platelet count <80 000
- Renal
  - Serum creatinine ≥2 times upper limit of normal age
    - ■ Limits[15]:
      - <5 years: 78 μmol/L
      - 5-12 years: 106
      - >12 tears: 180
- Hepatic
  - Total bilirubin >4ml/dL (68.4 μmol/L) OR
  - ALT 2-fold increase for normal age
    - ■ Limits[15]:
      - 1-2 years: 56
      - 3-6 years: 58
      - 7-12 years: 72
      - ≥13 years: 74

Inherited metabolic disorder (Adapted from Haijes et al[16])

- Suspected
  - IMD probability score of >100 in CSF OR plasma based on metabolomics data
- Probable
  - ONE of the following
    - ■ IMD probability score of >100 in CSF AND plasma based on metabolomics data
    - ■ IMD probability score of >1000 in CSF OR plasma based on metabolomics data
- Confirmed
  - ALL of the following
    - ■ Suspected or probable IMD
    - ■ All patient data congruent with IMD assessed by metabolic disease expert

Autoimmune encephalitis (adapted from Graus et al[17])

- Suspected

- CSF rat-brain immunohistochemistry staining
- Confirmed
  - Suspected AND
  - Detection of known autoimmune antibody by cell-based assay OR positive live neuron staining

Supplemental Results

Viral qPCRs

Viral qPCR was performed on CSF from 111 patients, of whom13 (12%) were positive for 1 or more viruses: 5 for EBV, 3 for HHV-6, 3 for CMV, 1 for HHV-7, 1 for rabies virus, and 1 for enterovirus.

Bacterial qPCRs

Bacterial qPCR on CSF in a subset of 67 patients (see the Appendix 1) yielded 8 (12% of tested) positive samples: 5 for *H influenzae*, 3 for *S pneumoniae*, and 1 for *N meningitidis* (1 sample was positive for both *H influenzae* and *S pneumoniae*).

16s qPCRs and Sequencing

16s rRNA qPCRs, performed on the same subset of 66 patients as bacterial qPCR (minus 1 patient for whom insufficient sample was available), yielded 8 positive samples (12% of tested). A putative causative pathogen was identified in CSF of five patients: one each with *N. meningitides*, *H. influenzae*, *Bacillus* species, *Moraxella* species and *Kocuria* species. The first 2 bacteria also were detected by conventional bacterial qPCR. For the latter 3 bacteria, literature evidence was available suggesting a putative association with encephalopathy,[18-20] considering a probable causality. In the samples of the 3 remaining patients, Sanger sequenced identified the bacteria to belong to the *Pseudoduganella*, *Blastococcus*, and *Bosea* species. Because these bacteria have never been detected in patients with encephalopathy, these results were not considered to be causally associated.

Viral Metagenomics

Viral metagenomics was performed on CSF from 102 and pooled plasma/CSF from 109 patients; at least 1 sample was evaluated by viral metagenomics from 116 patients. Excluding probable nonpathogenic viruses, 12 patients (10% of tested) were positive for a virus: 4 for HIV-1, 2 for human parvovirus B19, 2 for hepatitis A virus, 2 for HHV-6, 1 for HHV-7, and 1 for enterovirus. In addition, a novel orthobunyavirus, Ntwetwe virus, was detected in the CSF and blood of one patient, as we previously described,[14] which was classified as a confirmed cause of encephalopathy. For the 2 patients with hepatitis A, the virus was only detected in plasma. Both patients did not have signs of liver failure nor hyperammonemia (10 μmol/L for U36 and not determined for R33) which excluded hepatic encephalopathy. Because hepatitis A associated encephalopathy has been described as a potential but disputed cause of encephalopathy,[21] we classified these patients as suspected miscellaneous cause of encephalopathy. The patients positive for HIV-1, Parvovirus B19, and EBV were further interrogated for viral load quantification and serology to further elucidate a potential cause of encephalopathy.

For 1 of the 2 patients positive for parvovirus B19 (K02), the viral load in CSF (3.23×10^7 copies per mL) was 288-fold greater than plasma (1.12×10^5 copies per milliliter). This patient was positive for IgM and IgG in plasma but not in CSF. Because of the high viral load ratio, in combination with the subacute infection, as described in other literature, this was considered a confirmed CNS infection. The other patient positive for parvovirus B19 (M16) had a lower viral load in CSF than in plasma. Although this patient was IgM positive in both plasma and CSF, the lower viral load in CSF with the bloodstained appearance and high erythrocyte concentration in CSF suggests this was most likely caused by virus and antibody leakage from the bloodstream. This infection was accordingly considered a probable cause of encephalopathy. HIV-1 RNA could be detected in the plasma and CSF of all patients positive for HIV by metagenomics (M15, U27, U15, and U87). However, for each patient the viral load was greater in plasma than CSF, thereby classifying it as a suspected CNS infection according to our diagnostic criteria. For 2 patients positive for EBV (U41 and U76), serum was available for serology. Both patients were positive by IgM, thereby classifying them as a confirmed viral CNS infection.

Several nonpathogenic viruses, including anelloviruses, human endogenous retroviruses, Merkel cell polyomaviruses, human papillomaviruses, and GB C virus, frequently were detected. Because these viruses are generally considered nonpathogenic[22] and, in case of endogenous retroviruses, cannot be distinguished from human chromosomal DNA, their presence was not confirmed by (real-time)-PCR. We also detected a sequence belonging to a gemycircularvirus,[23] which recently has been associated to encephalopathy. However, we were unable to confirm the presence of this sequence on the original patient sample by an in-house developed PCR on this sequence, suggesting it may have been a contaminant.

Metabolomics

Metabolomics was performed on plasma and CSF samples from respectively 107 and 75 patients (65 had a paired sample). In total, at least one sample was analyzed from 117 patients. Of these 117 patients, 56 (47.9%) were initially classified as having an increased probability score for an IMD according to the pipeline.

Further analysis revealed many patients had an increased probability score for an IMD that belonged to one or more of five IMD disease clusters which were most likely secondary to a non-IMD condition (Table V).

Because several increased IMD probabilities were most probably caused by confounding non-IMD conditions (Table V), these IMDs were considered clinically relevant

unless the IMD score was factor >10 higher than the median of patients with an increased score for that IMD. After removal, 15 patients remained, who were further evaluated in detail by a metabolic disease expert (Table VI).

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Table I. Summary of diagnostic criteria

Diseases	Suspected	Probable	Confirmed	Reference
Cerebral malaria	Malaria positive	Suspected + BCS ≤2 for 1 h	Probable + malarial retinopathy	Taylor et al[31] WHO[28]
Bacterial/viral CNS infection	Pathogen-specific criteria	Pathogen-specific criteria	Pathogen-specific criteria	Granerod et al[24]
Meningitis (clinical diagnosis)	Fever + consciousness↓ or meningeal signs	Suspected + CSF inflammatory profile	NA	WHO[25]
Encephalitis (clinical diagnosis)	Consciousness↓+ 2 of: fever/seizures/focal/CSF WBC >5/EEG)	Consciousness↓+ 3 of: fever/seizures/focal/CSF WBC >5/EEG	NA	Venkatesan et al[26]
Sepsis	Clinical diagnosis	Suspected + microbiological confirmation*	NA	Goldstein et al[27]
Inborn metabolic disorder	Probability score* >100 in CSF or plasma	Probability score >1.000 in CSF or plasma or >100 in both	Suspected or probable + evaluation by IMD expert	Haijes et al[14]
Autoimmune	IHC positive	NA	Suspected + CBA or live neuron positive	Graus et al[28]
Severe malarial anaemia	WHO criteria	NA	NA	WHO[28]
Severe pneumoniae	WHO criteria	NA	NA	WHO[23]
Miscellaneous	Case-specific	Case-specific	Case specific	Expert opinion

BCS, Blantyre coma scale; *CBA*, cell-based assay; *CNS*, central nervous system; *CSF*, cerebrospinal fluid; *EEG*, electroencephalogram signs indicative of encephalitis; *IHC*, rat-brain immunohistochemistry; *IMD*, inherited metabolic disorder; *NA*, not available; *WBC*, white blood cell count; *WHO*, World Health Organization.
Full details on diagnostic criteria are described in the **Appendix 1**.
*Confirmed by pathogen identification (eg, culture, quantitative polymerase chain reaction).

Table III. Results of additional diagnostic assays and their effect on the original diagnosis based on locally available data

ID	Age	History	Type	Outcome	Local diagnostics	Original diagnosis	Viral		Bacterial		Metabolomics	Autoimmune		Effect	Final diagnosis
							qPCR	Metagen	qPCR	16s qPCR		IHC	CBA/LNS		
U46	5 y	Healthy	NTC/CSE	No seq	M+	Conf (CM)			*H inf*					Broadened	Conf (CM/bact)
M08	6 y	Healthy	NTC	No seq	M+	Conf (CM)						Pos	Neg	Unmodified	Conf (CM)
M15	7 y	Healthy	NTC	No seq	M+, HIV	Conf (CM)	EBV	HIV-1*						Unmodified	Conf (CM)
M43	3 y	Healthy	NTC	No seq	M+	Conf (CM)					S CTLN1			Unmodified	Conf (CM)
R08	13 y	Healthy	CSE	No seq	*N. men* CG	Conf (Bact)			*N men*	*N men*		Pos	Neg	Unmodified	Conf (bact)
U08	5 y	NI	NTC	Died	SCD stroke	Conf (misc)						Pos	Neg	Unmodified	Conf (misc)
U32	5 mo	Healthy	NTC/CSE	No seq	*H inf* CC	Conf (Bact)			*H inf*	*H inf*				Unmodified	Conf (bact)
U62	12 y	Healthy	NTC/CSE	Seq		Prob (men/enc)	HHV-7		*H inf*†			Pos	Neg	Altered	Conf (bact)
K01	14 y	Healthy	NTC/CSE	Seq		Prob (men)	CMV					Pos	Neg	Broadened	Prob (men, vir)
M16	14 mo	Healthy	NTC	Died		Prob (enc)	RABV†	ParB19‡						Altered	Conf (vir)
U27	7 mo	NI	NTC	Died	HIV, *C alb* BC	Prob (sepsis)	CMV†/EBV	HIV-1*			P DTDS/OPLAHD			Altered	Conf (vir)
M01	8 mo	Epi	NTC/CSE	Seq	*H inf* BC	Prob (enc/sepsis)			*H inf*			Pos	Neg	Altered	Conf (bact)
M06	19 mo	NI	NTC	Died	M-, *S typh* BC	Prob (CM/sepsis)					C PKU			Altered	Conf (IMD)
U76	20 mo	Healthy	CSE	No seq		Prob (men)	EBV*,¶			*Moraxella*				Altered	Conf (vir)
U41	11 mo	Healthy	NTC/CSE	Seq		Prob (men/enc)	EBV¶		*H inf/S pneu*					Altered	Conf (bact/vir)
M25	10 y	Healthy	NTC	No seq	M-	Prob (CM/enc)	HHV-6	HHV-6						Unmodified	Prob (CM/enc)
K13	13 y	NI	NTC	No seq		Prob (men)	EBV							Unmodified	Prob (men)
U01	10 mo	NI	NTC	No seq	M-	Prob (CM)					P RPID			Broadened	Prob (CM/IMD)
U91	4 y	Healthy	NTC	No seq		Prob (enc)				*Kocuria*				Broadened	Prob (enc/bact)
U33	7 y	Healthy	NTC/CSE	No seq	M-	Prob (CM/enc)				*Bacillus*				Broadened	Prob (CM/enc/bact)
R01	9 y	Healthy	NTC/CSE	No seq	M?	Prob (CM/enc)					P DTDS	Pos	Neg	Broadened	Prob (CM/enc/IMD)
U60	3 y	Healthy	CSE	Died		Prob (men/enc)					S OPLAHD			Unmodified	Prob (men/enc)
U15	5 y	Healthy	CSE	Seq	M?, HIV	Prob (men/enc)		HIV-1*						Unmodified	Prob (men/enc)
U87	4 y	Healthy	NTC/CSE	No seq	M?, HIV	Prob (CM/enc)		HIV-1*				Pos	Neg	Unmodified	Prob (CM/enc)
R27	2 y	Healthy	NTC	Seq		Susp (men)	CMV				S CD			Altered	Prob (vir)
U40	11 mo	NI, Epi	NTC	No seq		Susp (sepsis)	EV	EV						Altered	Conf (vir)
R11	9 mo	Healthy	CSE	No seq		Susp (pneu/men)			*S pneu*			Pos	Neg	Altered	Conf (bact)
U98	7 y	Healthy	NTC	No seq		Susp (men/enc)			*S pneu*					Altered	Conf (bact)
U80	3 y	Healthy	NTC/CSE	Seq		Susp (men/enc)		NTWV						Altered	Conf (vir)
U28	6 mo	Healthy	NTC/CSE	No seq		Susp (men/enc)					P FBP1			Altered	Prob (IMD)
R32	2 y	Healthy	NTC/CSE	Died		Susp (pneu/men/enc)	HHV-6	HHV-6						Broadened	Susp (pneu/men/enc/vir)
R33	6 y	Healthy	NTC	No seq		Susp (men/enc)		HAV§						Broadened	Susp (men/enc/misc)
K02	2 y	NI, Epi, SI	NTC/CSE	Seq		Unknown		ParB19‡						Altered	Conf (vir)
K05	13 y	Healthy	NTC	Seq		Unknown	HHV-6							Altered	Susp (vir)
R17	5 y	Epi, SI	CSE	No seq		Unknown		HHV-7						Altered	Susp (vir)
U36	3 y	Healthy	NTC	No seq		Unknown		HAV§						Altered	Susp (misc)

bact, bacterial CNS infection; *BC*, blood culture; *C alb*, *Candida albicans*; *CBA*, known autoantibody testing by cell-based assay; *CC*, CSF culture; *CD*, Canavan disease; *CG*, CSF gram stain; *CM*, cerebral malaria; *CSE*, convulsive status epilepticus; *CTLN1*, citrullinemia type I; *DTDS*, dopamine transporter defect syndrome; *enc*, encephalitis; *Epi*, epilepsy; *EV*, enterovirus; *FBP1*, fructose-1,6-bisphosphatase deficiency; *HAV*, hepatitis A virus; *H inf*, *Haemophilus influenzae*; *IHC*, rat-brain immunohistochemistry; *LNS*, live neuron staining; *men*, meningitis; *metagen*, metagenomics; *misc*, miscellaneous; *NI*, neurologic impairment, *N men*, *Neisseria meningitidis*; *No seq*, no major sequelae at discharge; *NTC*, nontraumatic coma; *OPLAHD*, 5-oxoprolinase deficiency; *ParB19*, Parvovirus B19; *PKU*, phenylketonuria; *Seq*, major sequalae at discharge; *M+/−/?*, malaria positive with malarial retinopathy positive/negative/unknown; *pneu*, pneumonia, *RABV*, rabies virus; *RPID*, ribose 5-phosphate isomerase deficiency; *SCD*, sickle cell disease; *S pneu*, *Streptococcus pneumoniae*; *S typh*, *Salmonella* Typhi; *vir*, viral CNS infection.

Only patients for whom additional diagnostics found a diagnosis (36 of 117 evaluated by additional diagnostics) are shown. All diagnostics were performed on CSF unless stated otherwise.

*Viral load comparison of plasma and CSF revealed a greater HIV plasma load.

†Pathogen responsible for modified cause of encephalopathy when coinfections were present.

‡The CSF/plasma viral load ratio was 0.003 for M16 and 288.47 for K02.

§Only detected in plasma without signs of hepatic encephalopathy, deemed as suspected miscellaneous (hepatitis A associated encephalopathy).

¶Proof of primary infection (IgM positive).

Table V. Association between inborn error of metabolism (IMD) scores and non-IMD conditions

Clusters	IMD included in cluster	Patients, No.	Non-IMD condition
1	Hypoxanthine guanine phosphoribosyltransferase deficiency Phosphoribosylpyrophosphate synthetase superactivity Xanthinuria type I and II Molybdenum cofactor deficiency	25	Prolonged sample storage
2	Proline dehydrogenase deficiency Ornithine aminotransferase deficiency	3	Bacterial CNS infection
3	GTP cyclohydrolase I deficiency Liver failure Pterin-4a-carbinalmine dehydratase deficiency Phenylketonuria DNAJC12 deficiency 6-pyrovoyltetrahydropterin synthase deficiency Dihydropteridine reductase deficiency	10	Liver failure
4	Frustose-1,6-biphosphatase deficiency Dihydropyrimidinase deficiency Succinic semialdehyde dehydrogenase deficiency Succinyl-CoA:3-oxoacid-CoA transferase Mitochondrial acetoacetyl-CoA thiolase deficiency Carbonic anhydrase Va deficiency Fasting or malnutrition 3-Hydroxyacyl-CoA dehydrogenase deficiency	11	Fasting
5	3-Methylcrotonylglycinuria 2-Methyl-3-hydroxybutric aciduria SLC5A6 deficiency Biotinidase deficiency or holocarboxylase deficiency	8	Unknown

CNS, central nervous system.

Table VI. Potential inborn errors of metabolism in children with severe acute encephalopathy from sub-Saharan Africa

Patients	IMD	Causal certainty	Reasoning
M06	PKU	Confirmed	Exceptionally high phenylalanine, phenyl lactic acid, and phenylpyruvic acid in plasma and CSF.
M11	HAOD	Excluded	Increased likelihood score based on elevated homogentisic acid, potentially increased by medication use. IMD would not explain clinical presentation.
M14	TMAU	Excluded	Biochemically and clinically unlikely.
M41	BTD, HCLSD	Excluded	Metabolite levels increased in large proportion of study population (for this patient only marginally higher than others).
M43	CTLN1	Suspected	CSF amino acid profile supports IMD, but normal plasma levels make the diagnosis less likely.
R01	DTDS	Probable	Substantially elevated homovanillic acid in plasma but CSF not available.
R17	DMGDHD	Excluded	Based on elevation of one metabolite with similar mass as GABA. IMD would not explain clinical presentation.
R19	PH	Excluded	Only oxalic acid but not glycolic acid elevated. IMD would not explain clinical presentation.
R27	CD	Suspected	Increased plasma n-acetyl aspartic acid. Also signs of disrupted citric acid cycle.
U01	RPID	Probable	Disruptions in multiple plasma metabolites, including a pentose, a sugar acid (possibly galactonic acid), a polyol and a disaccharide. However, similarity of m/z makes differentiation between sugars impossible without chromatography.
U27	DTDS, OPLAHD	Probable	Substantially elevated homovanillic and pyroglutamic acid in plasma and CSF without known dopamine or paracetamol use
U28	FBP1	Probable	Elevated plasma glycerol and lactate.
U60	OPLAHD	Suspected	Substantially elevated pyroglutamic acid in CSF but minor elevation in plasma with only non-excessive (1 tablet 3 hours before hospital presentation).
U62	GALKD, CPTIID	Excluded	Biochemically unlikely (GALKD). Metabolite disturbance only in CSF, not plasma (CPTIID).
U93	DTDS	Excluded	Only alterations in plasma, not CSF.

BTD, biotinidase deficiency; *CD*, Canavan disease; *CPTIID*, carnitine palmitoyltranferase II deficiency; *CSF*, cerebrospinal fluid; *CTLN1*, citrullinaemia type I; *DMGDHD*, dimethylglycine dehydrogenase deficiency; *DTDS*, dopamine transporter defect syndrome; *FBP1*, fructose-1,6-bisphosphatase deficiency; *GALKD*, galactokinase deficiency; *HAOD*, homogentisic acid oxidase deficiency; *HCLSD*, holocarboxylase deficiency; *OPLAHD*, 5-oxoprolinase deficiency; *PKU*, phenylketonuria; *RPID*, ribose 5-phosphate isomerase deficiency; *TMAU*, trimethylaminuria.