Brief report
An emerging opportunistic infection: Fatal astrovirus (VA1/HMO-C) encephalitis in a paediatric stem cell transplant recipient

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Running title: Fatal astrovirus (VA1/HMO-C) encephalitis

Abstract

Neuroinvasive astrovirus (VA1-HMO-C) is an emerging life-threatening infection in immunocompromised hosts. We describe an 8-month-old child who died of VA1/HMO-C encephalitis following bone marrow transplantation. The diagnosis was only made post-mortem using RNA deep sequencing of the brain. Repeat analysis of the post-mortem brain tissue using PCR specific primers for VA1/HMO-C was positive. Astrovirus VA1/HMO-C should be included in the evaluation of patients with similar encephalitis.

Keywords: Unexplained encephalitis, astrovirus VA1-HMO-C, deep brain sequencing, BMT
Introduction

Encephalitis in immunocompromised patients is a diagnostic challenge as the aetiology of the global brain dysfunction varies depending on the timing, nature and intensity of immunosuppression. The classical clinical features, laboratory findings and cerebrospinal fluid (CSF) pleocytosis may be lacking in these patients. (1). Moreover, encephalitis remains unexplained in up to 60% of patients despite a comprehensive list of microbiological evaluation with cultures and molecular techniques using real time polymerase chain reaction (PCR) for viruses, bacteria, parasites and fungi.(2, 3)

Deep sequencing can be used as a non-selective method for pathogen discovery and has been increasingly used as a diagnostic tool to investigate causes of encephalitis in immunocompromised patients. Sequencing of mRNA is one such approach and is widely used in transcriptome analysis but can also be used to detect mRNA from microorganisms. This technique involves the isolation of messenger RNA from a clinical sample which following amplification of synthesised cDNA. The messenger RNA is analysed using next generation sequencing technology that allows for unbiased and sensitive identification of transcripts present. Subsequent bioinformatic analysis enables the subtraction of human transcripts present and remaining sequences investigated by searching nucleotide sequence databases for homology to identify a potential pathogen. The advantages of this RNA sequencing are 1) no prior knowledge about the type of pathogen is required 2) a sequence is generated for each fragment of cDNA thus allowing identification of mixed populations 3) RNA sequencing enables us to identify RNA viruses and RNA transcripts of bacteria, fungi and DNA viruses.

Here, we describe an 8-month-old child who died of fatal astrovirus (VA1/HMO-C) encephalitis following a haematopoietic stem cell transplant for acute myeloid leukaemia (AML) with high-risk cytogenetics, t (10;11) in first remission. The diagnosis of neuroinvasive astrovirus was made on deep sequencing of her post-mortem brain biopsy.

Case report

The child presented with pancytopenia and hepatosplenomegaly at age of 4 months. The diagnosis of acute myeloid leukaemia was based on marrow studies, which indicated chromosomal translocation 10 and 11. She achieved remission which was consolidated using standard chemotherapy schedules including fludarabine, a nucleoside analogue associated with T-cell immune
The treatment was complicated by *Enterobacter* neutropenic fevers and enteritis due to human astrovirus (HAsV) infection, followed by persistent asymptomatic astrovirus detection in her stools. Since t(10;11) is associated with a high risk of disease relapse, she had a fully HLA-matched unrelated donor bone marrow transplant in first remission at 8 months old. She was conditioned with busulfan (dose was adjusted to target area under curve (AUC) at 80mg/L/h), cyclophosphamide (120mg/kg), melphalan (140mg/m²) and alemtuzumab (1m/kg over 5 days) and received ciclosporin alone for post-transplant graft-versus-host prophylaxis. The total nucleated cell dose and CD34 cell dose were 2.16 x 10⁹/kg and 5.6 x 10⁹/kg respectively. Neutrophil engraftment was demonstrated on Day +17 and donor chimerism was 100%. Early transplant course was complicated by severe mucositis, culture negative neutropenic fevers, veno-occlusive disease and grade 1 cutaneous graft-versus-host disease that responded to topical steroid. Nutrition rehabilitation was complicated by protracted human astrovirus positive diarrhoea, which was finally resolved upon discharge home on Day +94. Her stool had been negative for astrovirus since Day +92. Her ciclosporin was tapered off by Day +100.

The child became encephalopathic at Day+120 and subsequently developed uncontrolled dystonic movement. She needed ventilatory support for poor respiratory effort. There was no evidence of seizure activities on electroencephalogram. Cerebrospinal fluid analysis done on two occasions showed albuminocyttoplasmic dissociation (acellular fluid with protein 1.1g/L and glucose 2.7mmol/L; no blast was seen on cytospin) and real-time PCR was negative for a list of comprehensive neutrotropenic virus (table 1), *Toxoplasma gondii*, 18S fungal ribosomal RNA and 16S bacterial ribosomal RNA. Cerebrospinal fluid culture did not yield any positive results. Repeat brain images indicated progressive brain volume loss, very poor myelination and high signal within bilateral basal ganglia (Figure 1). Brain biopsy showed scattered glial cells, increased cellularity with large reactive astrocytes and calcified cells, perivascular chronic inflammatory cell infiltrate and meningeal chronic inflammatory cell infiltrate (Figure 2). Although brain biopsy showed marked inflammatory changes, the extensive microbiological evaluation failed to identify any infectious causative agents for her encephalopathy. She received additional donor cells to accelerate immune reconstitution and her total (CD3-positive) T cells rose from 4 x 10⁹/L at Day +130 to 391 x 10⁹/L at Day +150. Despite intensive supportive care the child died of irreversible global brain dysfunction on Day +196. Encephalitis due to
Astrovirus VA1/HMO-C was diagnosed after deep sequencing of post-mortem formalin fixed paraffin embedded (FFPE) brain biopsy tissue. Sequencing was performed as previously described by Brown et al with the exception that human ribosomal RNA was excluded by ribodepletion, instead of polyA RNA purification, to account for the degraded RNA in FFPE tissue. Astrovirus VA1/HMO-C was identified by the metaMix method. (4) Sequences generated had 97–98% nucleotide identity to Astrovirus VA1/HMOC-London1, previously identified by Brown et al (genbank accession KJ920196). (5) After knowing the exact aetiology of her encephalitis, retrospective analysis of her post-mortem brain biopsy using AsV-Contig real-time PCR assay, which uses specific PCR primers for Astrovirus VA1/HMO-C, was positive.

Discussion

Our case demonstrates that the diagnosis of unexplained encephalitis in immunocompromised patients remains a great challenge. Neuroinvasive astrovirus (VA1/HMO-C) has been reported as an emerging life-threatening infection in immunocompromised hosts. Our patient is the fifth patient with this astrovirus encephalitis reported to date. This astrovirus belongs to VA1 and HMO-C group of astroviruses which are highly divergent to classical human astrovirus (HasV 1–8). The previous reported cases are summarized in table 2. (5-8) These patients share a number of similarities: 1) they are immunocompromised hosts 2) the diagnosis was only made from deep sequencing of brain biopsy 3) rapid progression of the illness 4) the prognosis is poor. These cases have highlighted that astrovirus encephalitis has multifaceted presentations, ranging from acute encephalopathy to chronic neurological dysfunction. The first and second reported cases presented with progressive cognitive impairment and motor incoordination. In addition, the clinical features can be bizarre and misleading to other diagnoses. Psychiatric illness was suspected in first patient for suicidal and homicidal ideation while third patient presented with sudden onset of sensorineural deafness.

The diagnosis of astrovirus VA1-HMO-C encephalitis requires high index of suspicion. Negative human astrovirus (HasV) real-time-PCR of cerebrospinal fluid might potentially mislead. In fact, recent reports have shown that VA1/HMO-C astrovirus has been previously underrecognized in human. Burbelo et al found that high prevalence of anti-HMOAstV-C in adult healthy blood donor. Of
the 106 healthy adult US blood donors tested for immunoreactivity against the C-terminal capsid fragment of HMOASTV-C, 65% (n=69) were seropositive. (9) Similarly, this virus has been increasingly isolated in human stools in different countries, including Nigeria, Nepal, Pakistan, US and UK. (5, 10) This virus might be asymptomatic or cause mild illnesses in immunocompetent hosts but it potentially causes devastating diseases in immunocompromised patients, including encephalitis as reported. The mode of transmission in our patient was unclear as the child had no history of exposure to animals.

In conclusion, our patient had two astrovirus infections, the first one being classical human astrovirus gastroenteritis while the second one due to a VA1/HMO-C encephalitis. Our patient and reported cases illustrate the value of including testing for astrovirus VA1/HMO-C in the diagnosis of encephalitides in immunosuppressed patients. We suggest that deep sequencing should be performed promptly for patients with unexplained encephalopathy as the result is available within 6 days, allowing institution of specific therapy with intravenous immunoglobulin, antiviral therapy and withdrawal of immunosuppressive therapy, which potentially augment the outcome of the patients.

Author contributions:
Drafting article: Su Han Lum, Robert Wynn
Histopathogical examination of brain biopsy: Melanie Newbould
Analysis of brain biopsy tissue using deep sequencing: Julianne Brown, Sofia Morfopoulou
Analysis of brain biopsy using AsV-Contig real-time PCR assay: Andrew Turner, Malcolm Guiver, Emma Davies.
Critical revision of article: Robert Wynn, Malcolm Guiver, Julianne Brown, Judith Breuer, Denise Bonney, Timothy Martland

References:


Legends for figures and tables

Figure 1: MRI images on Day +120 (figure A, B, C) and Day +140 (figure D, E, F) showed progressive global volume loss with poor myelination.

Figure 2: Brain biopsy showed scattered glial cells with pleomorphic nuclei (arrow) and perivascular chronic inflammatory cell infiltrate.

Table 1: Reported cases of Astrovirus VA1-HMO encephalitis in the literature