Astrovirus VA1/HMO encephalitis after allogeneic hematopoietic cell transplantation: significant role of immune competence in virus control

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Abstract

Neurotrophic human Astrovirus (HAstV) VA1/HMO can cause severe encephalitis in immunocompromised patients. Advanced diagnostic tools are often required for diagnosis. Previous studies have described cases of acute HAstV VA1/HMO encephalitis that developed after allogeneic hematopoietic cell transplantation (HCT) with a fatal outcome. Here, we describe a rare case of a patient that survived severe progressive HAstV VA1/HMO encephalitis following HCT. The halt in clinical deterioration coincided with recovery of lymphoid competence, which indicated that the neuroinvasive HAstV infection was controlled by the reconstituting immune system.

Introduction

Viral complications frequently occur after administering lymphotoxic agents in the context of cancer treatments. Moreover, viral infections often occur during the T-lymphopenia and immunosuppressive phase after allogeneic hematopoietic cell transplantation (HCT). Viral infections are a major cause of treatment-related morbidity and mortality.[1] Classical human Astrovirus (HAstV) causes gastroenteritis[2]; however, some recently described HAstV genotypes, including HAstV-VA1/HMO, have shown neurotrophic properties.[3] Interestingly, acute neurotropic HAstV-VA1/HMO infections were mainly observed in immunocompromised patients, and severe immuno-incompetence typically led to fatality.[4-7] Importantly, diagnosing these neurotrophic viruses is challenging; modern methods, like metagenomics, are often required.[8, 9] Hence, HAstV encephalitis may frequently be undiagnosed. Without effective anti-viral agents that target novel HAstV genotypes,[10] adaptive immune responses are likely the only and primary protection against HAstV-induced encephalitis. Here, we describe a patient with severe immunosuppression that survived HAstV-VA1/HMO-induced acute encephalitis. Clinical stabilization occurred with lymphoid recovery after HCT. This case study provides support for the swift tapering of immunosuppression in patients with HAstV encephalitis.

Case

A 15-year-old patient with high-risk, acute myeloid leukemia (M2, FLT3-ITD pos/NPM1wt) in complete remission underwent an allogeneic HCT in May 2016 from a 10/10 HLA-matched unrelated donor. Due to tricuspid valve insufficiency, reduced conditioning was administered, with busulfan (AUC 90 mg*h/L) and fludarabine (140mg/m^2). Anti-thymocyte globulin (Grafalon 45mg/kg) was given for in vivo T-cell depletion. Post-HCT methotrexate (d+1,+3,+6) and cyclosporine A (targeted concentration 100-200ug/L) were given for graft versus host disease (GVHD) prophylaxis. Neutrophil engraftment was observed on day 22 as well as complete donor chimerism. A bone-marrow evaluation on day 28 showed complete remission. On day

60 post-HCT, an increasing Epstein Barr virus (EBV) plasma load (max 30000 copies/ml) was successfully treated with two doses of rituximab (375mg/m^2/dose).

Acute GVHD stage II was detected in skin and mucosa on day 90 post-HCT. Methylprednisolone treatment (1 mg/kg/day) provided good clinical effect. At 5 months post-HCT, limited chronic GVHD was detected in the skin, mucosa, and gut. Cyclosporine A and methylprednisolone treatment provided an initially good response, but it was steroid dependent. At 11 months post-HCT, lymphocyte counts remained very low (Figure 2B, time point: -4 months), and regular immunoglobulin substitution was required. At that time, the patient developed bilateral diplopia and impaired vision. An ophthalmology examination only revealed low tear production. Brain magnetic resonance imaging (MRI, Figure 1A) showed no pathology; a broad cerebrospinal fluid (CSF) examination showed no sign of leukemia relapse or infection by a virus (HSV1, HSV2, CMV, VZV, AVD, EBV, BK virus, JC virus, enterovirus, parvovirus B19, morbillivirus, HHV6, HHV7, tick-borne encephalitis), bacteria (both culture and 16S rDNA tests), fungus (both culture and 18S rDNA tests), or protozoa (toxoplasma, entamoeba). An autoimmune encephalitis evaluation showed negative results. However, CSF pleocytosis was detected. The symptoms were initially interpreted as retrobulbar neuritis with negative MRI findings. Methylprednisolone pulses (1 g/day) were administered for 3 days. Rather than improvement, deterioration ensued. At 13 months post-HCT, a brain and orbital MRI examination again showed no pathology. The clinical neurological symptoms progressed to ataxia, tiredness, photosensitivity, and speech difficulties, perhaps suggesting retrobulbar neuritis progression; thus, we administered plasmapheresis and high-dose immunoglobulins (2 g/kg). At 14 months post-HCT, another MRI examination showed changes in white matter signals of unknown origin in the pons and thalamus, and demyelination. Initially, a brain biopsy was not performed, due to the high risk of damaging vital neurological pathways by sampling lesions in the pons and thalamus. However, the complications progressed to severe neurological symptoms, including dysarthria, lower-extremity weakness, and abnormal eye movements. A new CSF examination again showed no signs of leukemia relapse or infection. However, another MRI performed 15 months post-HCT (Figure 1A) revealed further progression of the signal changes in the pons and thalamus, new signal changes in the basal ganglia and cerebellum, and abnormal meningeal enhancement in the mesencephalon. Still, the patient continued to display severe lymphopenia (Figure 2B).

Finally, a brain biopsy was acquired from the hypothalamic region, 15 months post-HCT, 4 months after the first neurological symptoms, and 2 months after clear progression of neurological deterioration. A microbial evaluation of all the aforementioned pathogens showed negative results. A histological examinations of the biopsy revealed inflammatory and regressive processes; fragmentation of myelin and microvesicular inclusions in several neurons, but no glial inclusions typical of JC-virus-induced progressive multifocal leukoencephalopathy (Figure 1B). Metagenomic analysis of the biopsy (supporting information) finally revealed the presence of astrovirus VA1/HMO RNA (Figure 2A).

Methylprednisolone was tapered off, without GVHD progression or an immediate increase in peripheral blood lymphocytes; the CD3 count remained below 0.4×10^9/L. In the weeks to months following immunosuppression withdrawal, the patient stabilized clinically. An MRI performed 14 months after the onset of neurological symptoms and 8 months after the biopsy (Figure 1A) showed no signs of ongoing inflammation and lesion regression in the pons, but persistent changes in the thalamus and cerebellum. In addition, the brain stem and cerebellar volumes were reduced, which indicated atrophy. At that time, the lymphocyte count had fully recovered (Figure 2B). Currently, at >2 years after the brain biopsy, the patient remains alive without further neurological deterioration. However, severe neurological impairments have persisted, including spasticity, ataxia, and dysarthria.

Discussion

This case study described encephalitis due to an infection of HAstV with a VA1/HMO genotype. Both the VA1/HMO and MLB genotypes were recently found to be overrepresented among patients with HAstV encephalitis[3]. Previously, only two case studies described neuroinvasive infections with classical HAstV, one in a healthy patient,[11] and one associated with HCT, which caused death.[12] Additionally, five cases of acute encephalitis (1 MLB and 4 VA1/HMO) in severely immunocompromised patients were fatal.[4-7, 13] In tho-

se cases, anti-viral agents did not resolve the ongoing infections, consistent with previous observations[10]. Two other case reports described neuroinvasive HAstV infections (1 MLB[14] and 1 VA1/HMO[15]) in immunocompromised patients that did not result in death. However, one patient had X-linked agammaglobulinemia with no additional immunosuppression and developed slow-progressing encephalitis, which lasted 4 years.[15] In the other case, no microbial evidence of HAstV neuroinvasion was presented[14]. Additionally, two cases of HAstV-induced encephalitis in immunocompetent patients were described, and both survived the infection.[11, 13] These observations clearly indicated that HAstV encephalitis primarily, but not exclusively, affected immunocompromised patients and that immunocompetence was required to control the infection.

Our patient was severely immunocompromised. Apart from lymphodepletion during conditioning, pharmacological immunosuppression was given for GVHD prophylaxis after the HCT. Additionally, immunosuppression was intensified, first with rituximab for the EBV reactivation, and then with methylprednisolone, due to GVDH. Furthermore, the initially suspected retrobulbar neuritis was treated with methylprednisolone pulses. We speculate that the symptoms that led to the retrobulbar neuritis diagnosis were actually early signs of HAstV encephalitis. If true, the patient would have benefitted from discontinuation, rather than intensification of immunosuppression. This clearly illustrates the complexity of HCT, where sometimes immunosuppression must be balanced against an immune response that can effectively fight infections. In that context, a timely, proper diagnosis of suspected underlying conditions is crucial in determining the appropriate treatment. In the present case, diagnosis based on a metagenomic analysis facilitated the decision to withdraw all immunosuppression, which most likely contributed to patient survival.

In conclusion, we described a severely immunocompromised patient with acute HAstV encephalitis that survived, and the infection was controlled upon immune recovery. This study underlined the need for a timely, broad diagnostic workup, including metagenomics when necessary, for evaluating neurological signs without a clear cause.

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Disclosure of conflict of interest

We have no conflicts of interest to disclose.

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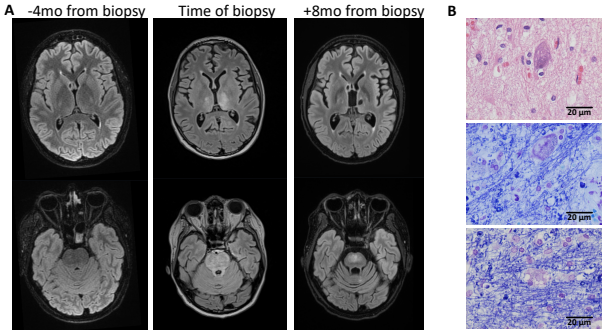
Figure legends

Figure 1. Brain MRI and histology show signs of encephalitis. (A) MRI (T2 FLAIR) at 3 different timepoints during neurological progression. (*left*) MRI at the first appearance of symptoms, 4 months prior to the biopsy; no pathology is apparent; (*center*) MRI at the time of the biopsy displays signal changes in the pons, thalamus, basal ganglia, and cerebellum; (*right*) MRI at 8 months after the biopsy shows reduced, but persistent signal changes in the pons, thalamus, and cerebellum, and signs of atrophy in the brain stem and cerebellum. (B) Histological images of brain biopsies acquired at different stages of viral-induced cell damage. (*top*) An enlarged, slightly swollen neuron with diffusely granular cytoplasm, surrounded by edematous extracellular matrix; hematoxylin-eosin stain; (*center*) two affected, enlarged neurons with densely granular cytoplasm, and on the left, dissolvement of chromatin and the cell border. Marked edema and dissolution is also visible in the surrounding matrix. Luxol Fast Blue/cresyl violet stain; (*bottom*) an affected neuron with prominent intracytoplasmic vacuoles and vaguely granular content. Marked edema is visible in the surrounding myelin sheaths and matrix. Luxol Fast Blue/cresyl violet stain.

Figure 2. Metagenomics and lymphocyte subset analyses. (A) Results of metagenomic analysis of the brain biopsy. (B) Peripheral blood lymphocyte concentrations at the indicated time points (time 0 = day of brain biopsy). Normal values (x10^9 cells/ml) are: total CD3 T-cells = 1.0-2.20; CD4 T-helper cells: 0.53-1.30; CD8 cytotoxic T-cells: 0.33-0.92; and CD19: B-cells 0.11-0.57.



A

Sample		Type of sequencing	Total number of raw reads x10^6	Hits and number of reads identified by Kraken	% of total filtered reads
Brain biopsy	freshly extracted	DNA	22		
		RNA	23	Astrovirus VA1: 3651	0.02
			26	Astrovirus VA1: 3772	0.02
	freshly extracted with Trizol	DNA	0.8		
		RNA	18	Astrovirus VA1: 1631	0.01
			7.4	Astrovirus VA1: 595	0.01

B