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Case report

Human pegivirus detected in a patient with severe encephalitis using a metagenomic pan-virus array

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ABSTRACT

We have used a metagenomic microarray to detect genomic RNA from human pegivirus in serum and cerebrospinal fluid from a patient suffering from severe encephalitis. No other pathogen was detected. HPgV in cerebrospinal fluid during encephalitis has never been reported before and its prevalence in cerebrospinal fluid needs further investigation.

1. Why this case is important

Metagenomic methodologies are excellent complement in cases where a diagnosis is difficult to establish with conventional laboratory tests and its usage is ever increasing. Metagenomic approaches reveal presence of both pathogenic and commensals in patient samples where the focus is on identifying an underlying ethological agent for a specific disease condition. We report a case of severe encephalitis where the only microbe detected in the CNS was human pegivirus (HPgV), hitherto only known to cause asymptomatic infections in humans. One previous report describes the detection of HPgV in brain tissue and CSF [1]. In both cases it is uncertain if HPgV is pathogenic but it is noteworthy to detect a virus at a high viral load in the CNS. In other cases, HPgV infections have been associated with beneficial outcomes in patients dually infected with HPgV and HIV or Ebola [2–5].

2. Case description

A 25-year old Danish female was admitted to the hospital for abdominal pain, vomiting, dizziness and lower extremity pain. She was working as a bartender on a cruise ship, was sexually active but had no travel history outside Scandinavia or exposure to blood transfusions, intravenous drugs or close contact to animals albeit recently received a tattoo. Her past medical history included radiofrequency catheter ablation for Wolf–Parkinson–White syndrome and she awaited elective cholecystectomy due to prior gallstone attacks. Upon admission she was alert and circulatory stable with a fever of 38.5 °C, Glasgow coma score of 15 and a BMI of 20. Routine blood test showed haemoglobin of 8.0 mmol/L, white blood cell count of 2.5×10^9/L with lymphocytopenia, normal platelet count, c-reactive protein (CRP) of 33 mg/L, alanine aminotransferase 270 U/L, lactate dehydrogenase 281 U/L, alkaline phosphatase 110 U/L and bilirubin of 8 μmol/L. An acute laparoscopic cholecystectomy was performed but no pathology of the gall bladder was found. Immediately following the surgery she developed increasing abdominal pain and fever and she underwent an explorative laparoscopy, again with normal findings. Post-operatively the patient complained of headache and diplopia, which both disappeared within 24 h and she was discharged from the hospital. However, she was readmitted the following day with fever, headache, sudden behavioral change, photosensitivity and ataxia. She presented with somnolence and neck stiffness on physical examination (Glasgow coma score 14). Vital signs were within normal limits but she had a fever of 38.0 °C. Peripheral blood showed CRP of 17 mg/L, white blood count 3.2×10^9/L. Cerebrospinal fluid (CSF) examination on day 6 of illness revealed

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Table 1
Diagnostic assays performed on spinal fluid and serum samples. All tests were negative.

	(q)PCR		Serology		Culture[a]	Other
	Spinal fluid	Serum	Spinal fluid	Serum	Spinal fluid	Spinal fluid
Fungi					x	
Parasites, Schistosoma				x		
Bacteria	x				x	
Borrelia burgdorferi	x		x			
Leptospira ssp.[b]	x	x		x		
Mycobacterium tuberculosis			x			
Neisseria meningitidis	x					
Streptococcus pneumoniae	x					
Treponema pallidum[c]				x		
Virus						
Adenovirus	x					
Coronavirus NL63	x					
Coronavirus OC43	x					
Coronavirus 229E	x					
Enterovirus	x	x				
Epstein-Barr virus		x		x		
Hepatitis A virus[d]				x		
Hepatitis B virus[e]				x		
Hepatitis C virus		x				
Hepatitis E virus[f]				x		
Herpes simplex 1	x		x	x		
Herpes simplex 2	x		x	x		
Human immunodeficiency virus				x		
Human herpesvirus 6A	x	x				
Human herpesvirus 6B	x	x				
Human herpesvirus 7	x	x				
Influenza A	x					
Influenza B	x					
Metapneumovirus	x					
Morbillivirus[f]	x			x		
Parainfluenza	x					
Parechovirus	x					
Mumpsvirus[f]	x			x		
Respiratory syncytial virus	x					
Rhinovirus	x					
Rubella virus[f]	x			x		
Tick-borne encephalitis[f]	x			x		
Varicella zoster	x		x	x		
Autoimmune synaptic encephalitis[g]						x

[a] Culture is for aerobic bacteria.
[b] qPCR is for *L. interrogans*, *L. alexanderi*, *L. borgpetersenii*, *L. fainei*, *L. kirschneri*, *L. noguchii*, *L. santarosai*, *L. weilli*. Microagglutination analysis for antibodies against 15 different Leptospira serotypes. Analysis for Leptospira was performed after patient recovery on samples collected during the acute phase of illness.
[c] Tested for Wassermann antibody and phospholipid-antibody.
[d] Patient where negative for HAV-IgM.
[e] Patient is negative for HBsAg.
[f] Serology included tests for IgG and IgM.
[g] Tests for autoimmune synaptic encephalitis included NMDAR1 IgG and IgA, Glutamate receptor 1 and 2 IgG and IgA, CNTNAP2 IgG, LGI1 IgA, GABA-B receptor 1 IgG, Glutamatedecarboxylase <75 kIU/L. Total IgG in serum was 7.55 g/L and for albumin 39.70 g/L.

pleocytosis of 150 cells/mm^3 (99% lymphocytes) and increased protein concentration of 3.5 g/L, indicative of a viral infection. An MRI of the brain revealed leptomeningeal enhancement over the right hemisphere together with parenchymal changes, consistent with meningoencephalitis. She was treated with aciclovir for suspected viral encephalitis and with meropenem for possible bacterial infection.

Over the following days the patient worsened with mental status deterioration and progressed into coma and was transferred to an intensive care unit for mechanical ventilation. Repeated lumbar puncture on day 9 disclosed an increase of mononuclear cells to 333 leukocytes/mm^3 (99% lymphocytes) with a protein concentration of 2.8 g/L. She received dexamethasone, methylprednisolone and later prednisolone. Serum and CSF were tested for relevant pathogens, all returned negative (Table 1). Because of the lack of a specific diagnosis serum and CSF were sent to Statens Serum Institut, Copenhagen, where the specimens were run on a Lawrence Livermore pan-microbial array. This array contains 360000 probes against all sequenced bacteria and viruses present in the NCBI database as of 2010 [6,7]. The only positive signal was for human pegivirus (HPgV) (Acc. nr. GSE67021), and two separate diagnostic laboratories subsequently confirmed HPgV RNA in both serum and CSF [8,9]. The Ct value for HPgV during the acute phase in serum and spinal fluid was 23.4 and 32.1, respectively. An RNAseq library was prepared from serum and sequenced on an Illumina platform to obtain type information. The reads mapped to the entire reference genome (Acc. nr. NC_001710) with a mean sequence depth of 48, a pariwise identity of 89.7% (nt) and 98,3% (aa), respectively. The assembled sequence (Acc. nr. KP259281) clustered within genotype 2 [10]. After eight days with severe neurological symptoms the patient gradually recovered and was discharged from the hospital four weeks later for rehabilitation. Five weeks after discharge she was still viremic for HPgV in serum but viral load had decreased 21 times (Ct 27.8).