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Case Report

Acute meningoencephalitis caused by adenovirus serotype 26

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Adenoviridae are rare causes of meningoencephalitis in both immunocompetent and immunocompromised hosts. In this article the authors report a case of adenoviral meningoencephalitis caused by serotype 26 and its identification, not described previously, in cerebrospinal fluid (CSF) by PCR and brain tissue by immunohistochemical staining. *Journal of NeuroVirology* (2006) **12,** 235–240.

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Introduction

Adenoviridae are ubiquitous viruses that cause clinically important disease of the respiratory, gastrointestinal, and urinary tracts, and conjunctivae in immunocompetent hosts and disseminated infections with high rates of mortality in immunocompromised hosts (Baum, 2000). Rarely, they cause meningoencephalitis in both immunocompetent and immunocompromised hosts. Meningoencephalitis typically occurs in the setting of pulmonary or disseminated disease and is usually caused by serotypes 2, 3, 5, 6, 7, and 12 (Baum, 2000). It can be difficult to diagnose and often requires biopsy. Here we report a case of adenoviral meningoencephalitis caused by serotype 26 and its identification, not described previously, in cerebrospinal fluid (CSF) by PCR and brain tissue by immunohistochemical staining.

Case

In May 2003, a 38-year-old woman with a history of medulloblastoma of the cerebellum was admitted to the neurology service with a 24 h history of worsening confusion, problems with speech, difficulty with balance, low grade fevers, and progressive somnolence. Five months previously, a ventriculoperitoneal (VP) shunt was placed and four months prior, the medulloblastoma was resected. Four days before admission she completed her fifth round of total central nervous system irradiation.

Her admission temperature was 38°C and her other vital signs were normal. She answered questions slowly but appropriately. She was oriented only to person and had no focal neurological deficits. Routine admission labs were unremarkable as was a chest radiograph. Routine cultures of blood and urine were obtained. CSF was sampled from a lumbar puncture and from her VP shunt (Table 1). The patient was placed on empiric broad-spectrum antimicrobials for presumptive encephalitis (acyclovir, vancomycin, and cefepime), and her maintenance dose of dexamethasone was increased because of concern for radiation induced cerebral edema. An MRI of the brain with contrast from admission was unchanged from previous scans (post-operative changes) and an electroencephalogram (EEG) showed diffuse slowing without seizure activity. Cultures of blood, urine and cerebrospinal fluid showed no growth, and HSV, CMV, and VZV PCRs and viral culture of the CSF were negative.

The patient's neurological status declined rapidly and she was intubated for airway protection on hospital day 4. Following the negative CSF cultures, the antibacterial agents were stopped, but acyclovir was continued. The patient's hospital course was complicated by a left-sided pneumothorax and a saddle

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Table 1 Results of CSF evaluation from admission and hospital days 6 and 10. HD, hospital day; LP, lumbar puncture. To convert values for glucose to millimoles per liter, multiply by 0.05551

CSF sample	*Admit LP*	*Admit shunt*	*HD 6 LP*	*HD 10 LP*
Glucose (mg/dl)	91	76	108	111
Total Protein (mg/dl)	81	56	100	199
Total cells (cells/mm^3)	5	0	6	40100
Nucleated cells (cells/mm^3)	3	0	2	100
PMN (%)	1	0	2	21
Lymphocytes (%)	66	65	71	36
Micro results	HSV, CMV, and VZV PCRs negative		HSV PCR neg, arbovirus panel neg.	HSV PCR neg.

pulmonary embolism. Due to the development of partial seizures, an MRI of the brain (Figure 1), EEG, and lumbar puncture (Table 1) were repeated on hospital day 6. The EEG now showed epileptiform activity and the MRI showed hyperintense signaling on FLAIR sequencing in both temporal lobes (right side greater than left). Despite therapeutic doses of phenytoin and levetiracetam, subclinical status epilepticus was demonstrated by EEG and lorazepam was started. On hospital day 10, CSF was obtained (Table 1) and an MRI of the brain was repeated; it showed progression of the hyperintense signal abnormality on FLAIR sequencing.

A brain biopsy was performed on hospital day 17 for progressive neurological deterioration. The biopsy findings were consistent with a viral infection and showed scattered cortical neurons with large nuclei, smudged chromatin, intranuclear inclusions, and a lymphocytic infiltrate (Figure 2). Ganciclovir and foscarnet were started and acyclovir was discontinued. Four days following the biopsy, adenovirus grew from the brain tissue viral culture.

Viral cultures of blood, stool, urine, and sputum were negative as was sputum direct fluorescent antibody (FA) staining for adenovirus. Her neurological function, including brain stem reflexes, continued to deteriorate and the patient expired on hospital day 24. Permission for an autopsy was granted and multiple studies were performed including immunohistochemical (IHC) stains, electron microscopy, *in situ* hybridization, PCR, and viral serotyping.

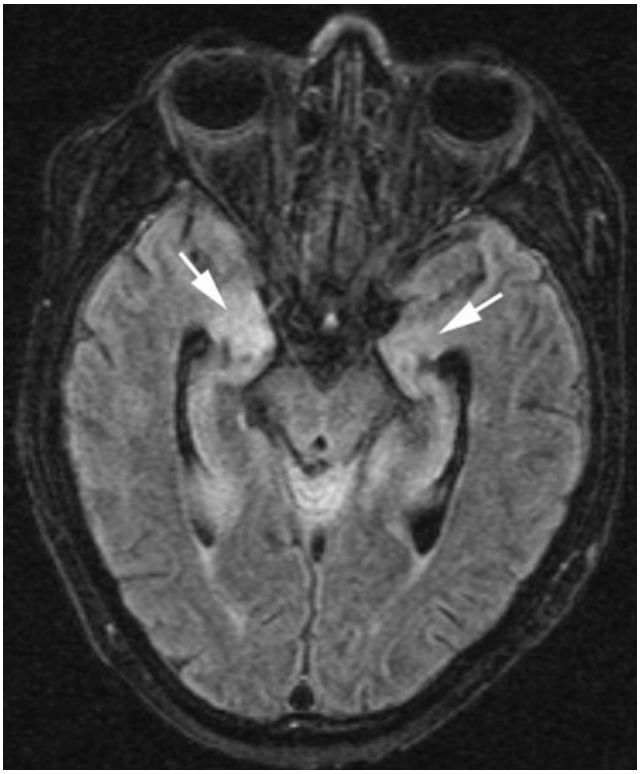

Figure 1 MRI of the brain from hospital day 10 showing hyperintense signaling in both temporal lobes on FLAIR sequencing (arrows).

Methods

Immunohistochemical stains

Four micron-thick sections of formalin-fixed and paraffin-embedded biopsy and autopsy brain materials were cut and placed on positively charged glass slides (Fisher Scientific, Pittsburg, PA). Slides were deparaffinzied in xyline for 10 min, and then rinsed in 4 changes of 100% alcohol. The endogenous peroxidase activity of brain tissue was blocked with 6 ml 30% H2O2/194 ml methanol for 25 min. Slides were rehydrated in 95%, 80% and 70% alcohol and water. Heat antigen retrieval was performed in citrate buffer (pH 6.0) for 20 min. The subsequent staining was done with the Autostainer (DAKO, USA). The slides were sequentially incubated for 30 min with the anti-adenovirus (Blend) mouse monoclonal antibody, which reacts with all 51 serotypes of adenovirus, at 1:2000 dilution, and Envision system (DAKO) with intervening washes with Tris-buffered saline with Tween 20 (DAKO). The viral antigen was then visualized with Liquid DAB Substrate-Chromogen System (DAKO).

PCR

The presence of adenovirus DNA was investigated in a sample of brain obtained by biopsy, as well as in samples of cerebrospinal fluid, whole blood, and urine. DNA was extracted from each sample using

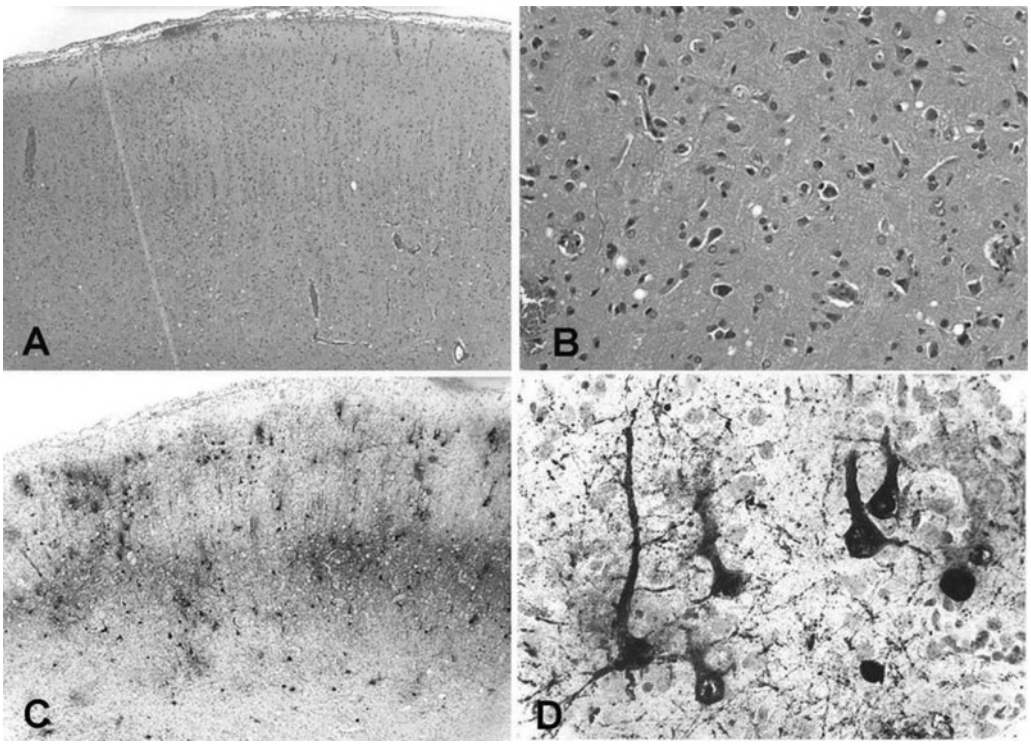

Figure 2 Hematoxylin and eosin (H & E) and immunohistochemical (IHC) staining for adenovirus of brain biopsy specimen from hospital day 17. (**A**) low power H & E showing increased cellularity of the cortical parenchyma and perivascular cuffing. (**B**) high power H & E showing cortical neurons with large nuclei, smudged chromatin, and eosinophilic cytoplasm (smudge cells). (**C**) low power IHC staining showing diffuse staining of the cortex for adenovirus. (**D**) high power IHC staining showing intense staining of cortical neurons for adenovirus.

QIAamp spin columns (Qiagen Inc, Chatsworth, CA) according to the manufacturer's directions. PCR was performed using a real time PCR method including primers and probe described by Heim *et al* (2003). This assay amplifies a conserved region of the adenovirus hexon gene, and is reported to detect all 51 adenovirus serotypes. The assay was modified to run on a Light Cycler instrument (Roche Molecular Systems, Indianapolis, IN). In each assay, 10 μL of purified DNA was included in a 20 μL reaction volume. Negative controls consisted of aliquots of water which were processed using QIAamp columns at the same time as the patient samples.

Results

Sections of the brain and meninges biopsy material from hospital day 17 (Figure 2) show mild-to-moderate infiltration of mononuclear cells, predominantly lymphocytes and histiocytes, in the leptomeninges, and increased cellularity in the brain parenchyma secondary to microglial proliferation and formation of microglial nodules, and lymphocytic infiltration. There is perivascular lymphocytic cuffing. Scattered cortical neurons have large nucleated cells with smudged chromatin and eosinophilic cytoplasm, consistent with "smudge cells" that are characteristic of adenoviral infection (Haura *et al*, 2002). Rare granular nuclear inclusions are identified. In some areas there is tissue necrosis with cells having bizarre, large hyperchromatic nuclei. The immunohistochemical (IHC) stain for adenovirus demonstrates strong immunoreactivity in the nucleus, perikarya and dendritic processes of neurons, and in the cell bodies and elaborate short cytoplasmic processes of rare astrocytes (Figure 2). Electron microscopy shows numerous non-enveloped hexagon-shaped viral particles in the nucleus and cytoplasm of neurons (Figure 3) and processes of neurons or glial cells (not shown). Taken together, these findings are consistent with adenoviral meningoencephalitis.

In situ hybridization (ISH) for adenovirus was performed on the brain biopsy material and the medulloblastoma. Signals were detected within the cortical neurons and rare astrocytes, but not in tumor cells (data not shown). The ISH study confirmed the result of IHC staining.

An autopsy was performed. Grossly, the brain was markedly swollen without herniation. Coronal sections showed diffuse dark discoloration and petechial hemorrhage in the cortical ribbon and underlying white matter of bilateral temporal lobes, insular cortex, lower lateral aspect of right frontal lobe, and

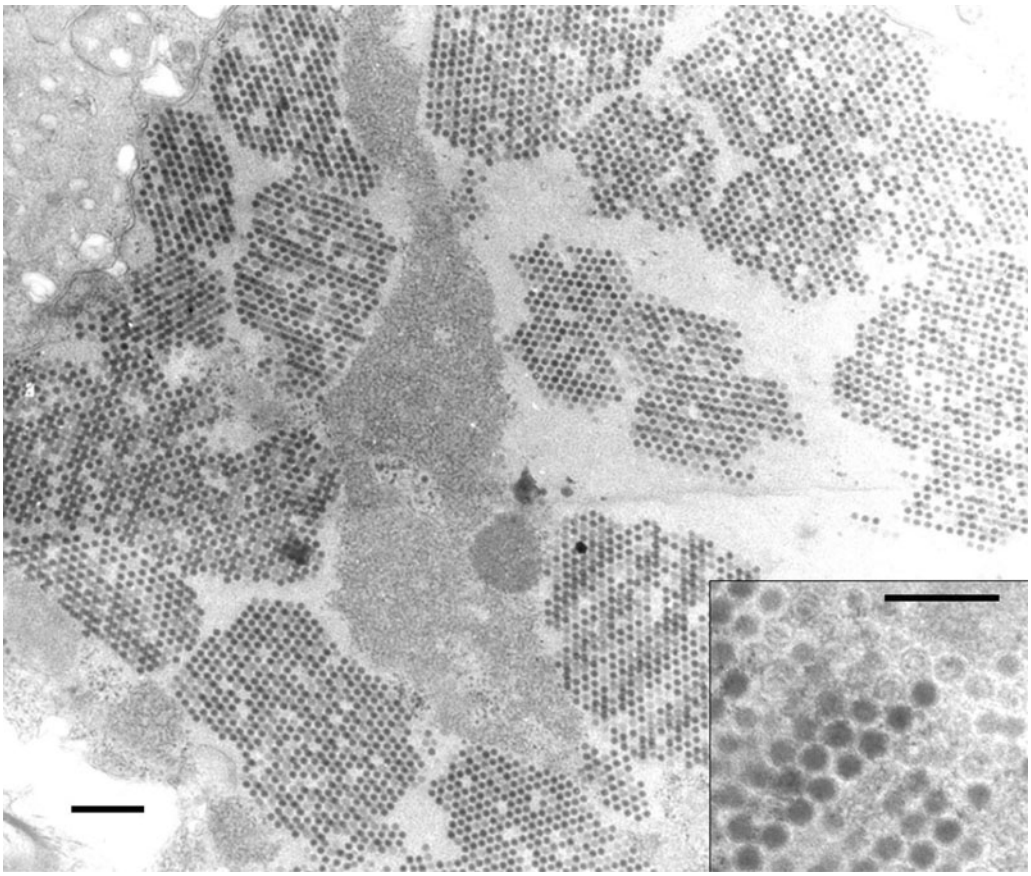

Figure 3 Electron microscopy of brain tissue obtained at biopsy on hospital day 17 showing hexagon-shaped viral particles in the nucleus and cytoplasm of an infected neuron. Bar = 500 nm. Inset bar = 300 nm.

bilateral occipital lobes. Bilateral hippocampi, basal ganglia and thalami had similar changes. The spinal cord appeared unremarkable. Microscopically, characteristic smudged cells and extensive tissue necrosis was identified in all brain regions, midbrain and brain stem. However, the spinal cord appeared less involved.

The lungs showed diffuse hemorrhagic consolidation and bilateral pulmonary artery organizing thromboemboli. Microscopic sections of the lung tissue showed diffuse alveolar damage in varying stages, from acute inflammation to hyaline membranes. Intranuclear inclusions typical for adenoviral infection (smudge cells) were not present and immunohistochemical stains and *in situ* hybridization for adenovirus were negative on histological examination of the lung. There was no microscopic evidence of adenovirus induced abnormalities in cardiac, liver, spleen, bone marrow, or genitourinary specimens. As there was no histopathological evidence of adenoviral infection, IHC staining was not performed on these specimens.

PCR testing documented evidence of adenovirus DNA in brain tissue obtained at biopsy, CSF from hospital day 6, and blood from hospital day 23 (Figure 4). Urine from hospital day 23 and a CSF sample obtained approximately one month prior to the index hospitalization did not reveal adenoviral DNA by PCR.

Serotyping performed at the Centers for Disease Control and Prevention using adenovirus

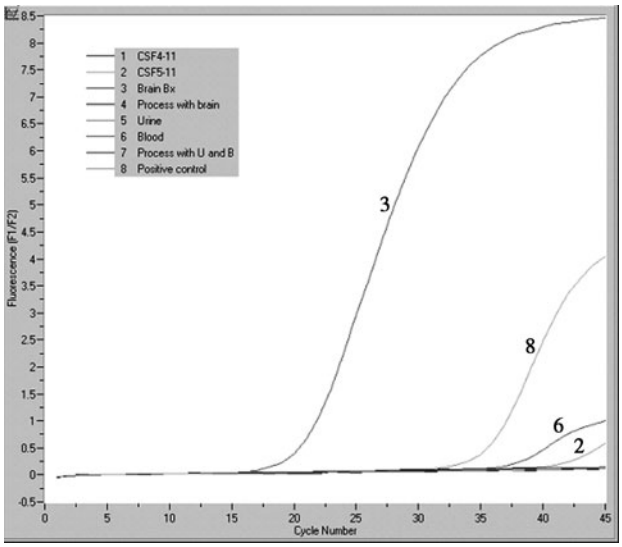

Figure 4 Light Cycler output showing the detection of adenoviral DNA in the brain biopsy specimen from hospital day 17 (3), CSF from hospital day 6 (2), and blood from hospital day 23 (6).

species-specific primers to the fiber gene and sequences of the hexon gene hypervariable region revealed the isolate to be human group D adenovirus, serotype 26.

Discussion

Human adenoviruses are ubiquitous, non-enveloped viruses with linear double-stranded DNA. Adenoviruses are categorized into subgenuses (A–F) and serotypes (1–51). The types of infection and severity of disease are related to the serotype of the virus and the age and immune status of the patient (Baum, 2000; Hierholzer, 1992).

Adenoviruses are capable of three types of interactions with cells (Baum, 2000; Hierholzer, 1992). During lytic infection, the virus undergoes a full replicative cycle and infectious virions are released when the infected cell lyses. Adenoviruses are also capable of becoming latent. The mechanism of latency is not well established, but it is thought cell multiplication is greater than cell death, resulting in an inapparent infection. Subgenus C can become latent in the adenoid tissue of children, and subgenus B causes latent infection in the kidney tissue of both adults and children. Adenoviruses are also associated with oncogenic transformation in cell lines and animal models.

Meningoencephalitis is a rare manifestation of adenoviral infections (Ryan *et al*, 2002; Studahl *et al*, 1998). It is most commonly associated with respiratory epidemics of serotype 7 in otherwise healthy children (Huttunen, 1970; Sakata *et al*, 1998; Simila *et al*, 1970; Yamadera *et al*, 1998; Antoine *et al*, 1987), though cases have been reported with serotypes 2, 3, 5, 6, and 12 in this patient population (West *et al*, 1985; Kelsey, 1978; Chatterjee *et al*, 2000; Janner *et al*, 1990; Cardosa *et al*, 1999; Baum, 2000). The patients have a meningoencephalitis in addition to pneumonia. The diagnosis is often presumed based on clinical evidence of meningoencephalitis and serological studies and/or culture results of peripheral sites (Huttunen, 1970; Sakata *et al*, 1998; Simila *et al*, 1970; Yamadera *et al*, 1998; Janner *et al*, 1990; Antoine *et al*, 1987). Less often it is actually confirmed by culture or biopsy of the CSF or brain (Osamura *et al*, 1993; West *et al*, 1985; Kelsey, 1978; Horoupian *et al*, 1984; Chou *et al*, 1973; Schnurr *et al*, 1995; Chatterjee *et al*, 2000; Cardosa *et al*, 1999). Mortality rates of 26% to 39% have been reported (Kelsey, 1978; Simila *et al*, 1970). In immunocompromised patients adenoviral meningoencephalitis occurs as part of a disseminated infection (Hierholzer, 1992; Janner *et al*, 1990; Shields *et al*, 1985; Bordigoni *et al*, 2001; Carter *et al*, 2002). The serotypes involved are those typically associated with more severe disease in immunocompetent patients, such as serotypes 2, 3, 5, and 7.

There is a paucity of data on effective therapeutic agents for human adenoviral infections. Agents that are active against adenovirus *in vitro* that have been used with some success clinically include ganciclovir, ribavirin, and cidofovir (Hierholzer, 1992; Duggan *et al*, 1997; Bordigoni *et al*, 2001; Carter *et al*, 2002; Safrin *et al*, 1997). Randomized studies to determine the most effective agents are difficult to perform given the rare nature of severe, life-threatening adenoviral infections.

To the best of our knowledge this is the first reported case of acute adenoviral meningoencephalitis with isolated central nervous system involvement. Evidence of actively replicating virus in the central nervous system demonstrated by electron microscopic visualization of virions in neurons has only been reported one other time in the literature (Chou *et al*, 1973). In addition, this is the first case to demonstrate adenoviral proteins by IHC staining of brain tissue as well as adenoviral DNA in the CSF by PCR.

Serotype 26 has not been previously reported to cause meningoencephalitis and the only two known case reports were both AIDS patient with diarrhea (Hierholzer *et al*, 1988; Lord *et al*, 2000). In addition subgenus D adenoviruses are thought to be less pathogenic as they rarely cause disease in immunocompetent hosts other than keratoconjunctivitis (serotypes 8, 19, and 37) and are most commonly associated with diarrhea in AIDS patients (Hierholzer, 1992; Hierholzer*et al*, 1988).

The mode of acquisition in this case is not clear. While latent adenovirus infections can reactivate when the immune system is compromised, subgenus D adenoviruses are not thought to be capable of latent infections. *In situ* hybridization performed on the original tumor did not identify latent virus.

It is possible that this patient had an asymptomatic infection at a peripheral site that spread to the CNS because of disruption of the blood brain barrier and localized immunosuppression caused by the CNS radiation therapy. This patient did have alveolar damage seen at autopsy but we do not believe it was associated with the adenoviral infection for several reasons. In patients with adenoviral meningoencephalitis associated with pneumonia, the pneumonia typically precedes the CNS disease. This patient did not have respiratory tract symptoms, the chest radiograph was normal on admission, and adenovirus was not detected in the sputum by culture or direct FA staining. The lung tissue at autopsy was not consistent with adenovirus infection, and the IHC staining and *in situ* hybridization were negative for adenovirus. We feel the pathological findings were most consistent with prolonged exposure to elevated levels of oxygen. Adenovirus DNA was detected by PCR in the blood, but the virus was not isolated from the blood by culture. This may have been due to the extensive disease in the CNS with release of adenoviral DNA into the circulation. Also, despite the fact that adenovirus can be excreted in the stool for prolonged periods after a gastrointestinal infection and the isolate in this case has only been known to cause

diarrhea, adenovirus was unable to be cultured from this patient's stool.

In conclusion, this patient had acute adenoviral encephalitis with predominant temporal lobe involvement that progressed despite treatment with acyclovir. This case demonstrates that adenoviral infection should be included in the differential diagnosis of progressive encephalitis in immunocompromised patients and adenoviral DNA can be detected in the CSF early in the course of the disease. Given that our patient was in remission from an oncologic perspective, earlier diagnosis may have prompted the use of antiviral agents with potential therapeutic benefit. Consequently, progressive illness in patients with a clinical syndrome consistent with encephalitis and a negative work-up for the most likely agents should prompt early brain biopsy and CSF evaluation for adenovirus.

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