



Central Nervous Cryptococcosis and Chronic Hepatitis C: Two Case Reports and Review of the Literature

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Abstract

Hepatitis C Virus (HCV) could be a risk factor for neuropsychiatric illness and a predisposing factor for meningoencephalitis caused by opportunistic organisms such as *Cryptococcus neoformans*. Nonetheless, the association between virological cure and resolution of Brain-Blood Barrier (BBB) damage and neuroinflammation has remained unclear. Understanding this association may help develop strategies to reduce neuropsychiatric comorbidity in Chronic Hepatitis C (CHC) patients and improve outcomes in addiction medicine, specifically Medication-Assisted Therapy (MAT) retention. Cannabis may be a direct source of inoculation of *Cryptococcus species* and other fungi in the lungs and subsequent hematogenous dissemination.

Keywords: Blood Brain Barrier (BBB); Chronic Hepatitis C (CHC); Cryptococcal Meningitis (CM); Cerebrospinal Fluid (CSF); Hepatitis C Virus (HCV)

Introduction

We have recently noticed an increase in the incidence of Central Nervous System (CNS) cryptococcosis in chronically infected HCV patients. This paper aims to shed light on the association between HCV neuroinvasion and cryptococcal infection. Chronic hepatitis C can cause BBB disruption and neuroinflammation, with neuroinvasion occurring regardless of the level of fibrosis score [1-3]. In fact, brain microvascular endothelial cells [1], astrocytes and microglia become minor replication sites for HCV [4,5]. Additionally, viral compartmentalization in CSF is associated with a higher plasma viral load, suggesting that increased viral burden in the periphery, may facilitate invasion of the CNS [6]. The CNS HCV 'reservoirs' have the potential to predispose the brain to hematogenous seeding from transient bacteremias, fungemias or viremias. Whether HCV-associated neurocognitive deficits improve after HCV eradication remains unclear. After HCV clearance, some studies have reported significant improvement in attention and working memory, but other findings showed no improvement in neurocognitive performance [7-11].

OPEN ACCESS

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Received Date: 25 Aug 2020

Accepted Date: 25 Sep 2020

Published Date: 28 Sep 2020

Citation:

Al Akhrass F, Madison Tackett S, Mikhael E, Akhter S, Hanif SNM. Central Nervous Cryptococcosis and Chronic Hepatitis C: Two Case Reports and Review of the Literature. Clin Surg. 2020; 5: 2959.

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Case Series

Case 1

A 25-year-old male was admitted to the hospital on 01/10/2011 for chronic headaches and acute horizontal double vision. He had bifrontal positional headaches and intermittent fevers for three months. Two weeks prior to admission, he started having blurred, double vision. The patient was an active Person Who Injects Drugs (PWID) and frequent marijuana smoker. On examination, he was afebrile and hemodynamically stable. Examinations of the heart, lungs, and abdomen were normal. Neurological examination revealed normal (2.5 mm), symmetrical and reactive pupils; however, left-sided abducens nerve (CN VI) palsy was present. He had no other cranial nerve palsies, focal motor or sensory abnormalities, or neck rigidity. Fundoscopy showed bilateral papilledema suggestive of increased Intracranial Pressure (ICP). Laboratory assessment showed a White Blood Cell (WBC) count of 18,900/mm³ (Polymorphonuclear leukocytes [PMNs] 67.2% and lymphocytes 26%), hemoglobin level of 11.8 g/dL, platelet count of 376,000/mm³, blood urea nitrogen/creatinine level of 15/1.1 mg/dL, glucose level of 88 mg/dL, Total Bilirubin level (TB) of 0.9 mg/dL, Alanine Transaminase (ALT) of 95 U/L, Aspartate Aminotransferase (AST) of 22 U/L, albumin of 2.4 mg/dL. The Prothrombin Time (PT) was 11.7 sec (international normalized ratio, INR 0.99). Serum ammonia, thyroid functions, thyroid peroxidase antibodies, Antinuclear Antibodies (ANA), and immunoglobulin levels were normal. A chest X-ray was unremarkable. Abdominal CT scan revealed no evidence of cirrhosis or hepatosplenomegaly. Pre- and post-contrast Magnetic Resonance Imaging

(MRI) and angiography (MRA) showed diffuse dural enhancement in the periventricular white matter, hyperintense basal ganglia, cerebral edema and communicating hydrocephalus. Patient had no evidence of aneurysm, venous sinus thrombosis, mass or midline shift on imaging. Lumbar pressure was obtained and showed an elevated intracranial pressure of 50 cm of H₂O. The CSF analysis revealed a 918/mm³ WBC count (lymphocytes 100%), 109 mg/dL protein level, 14 mg/dL glucose level, and negative bacterial direct antigen. The CSF India ink staining was positive. Cryptococcal antigen (CrAg) levels were extremely elevated in the blood and CSF with titers of 1:1280 and 1:560, respectively, suggestive of invasive cryptococcal infection with CNS cryptococcosis. Polymerase Chain Reaction (PCR) results for tuberculosis, enterovirus, Herpes Simplex Virus (HSV 1 and 2), Human Herpes Virus 6 (HHV6), Varicella Zoster (VZV), Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), HIV, West Nile Virus (WNV) and Borrelia burgdorferi were negative. Furthermore, CSF paraneoplastic antibodies, fluorescent treponemal antibodies and arbovirus serological testing were non-reactive. The patient was immediately administered liposomal Amphotericin B (lAmB) in combination with Flucytosine (5-FC) for 4 weeks with transition to fluconazole 800 mg for 3 months, followed by a tapered dose of fluconazole for 12 months. The patient received a lumbar drain on 1/31/11 to continuously monitor Intracranial Pressure (ICP), sample the CSF, and instill AmB deoxycholate (incremental dose of 0.1 mg to 0.5 mg; 1/26/11 to 1/30/11). That same day, a Ventriculoperitoneal (VP) shunt was placed and was subsequently complicated by *Escherichia coli* peritonitis and bacteremia on 02/08/2011. He was treated with four weeks of intravenous ceftriaxone followed by removal of the VP shunt on 03/12/2011. The patient's neurological symptoms, including left CN VI palsy, and sepsis resolved. Given the fact that he was doing significantly better with no residual neurological deficits, external ventricular device was not considered, and patient was discharged home on oral fluconazole for 12 months. The patient had CHC, genotype 1a, viral load of 234 IU/mL × 10⁵ IU/mL, fibrosis score F1, treatment naïve, co-infected with hepatitis B, HBe antigen reactive, without delta. Later in 2012, he was treated with Pegylated interferon, boceprevir and ribavirin for 6 months, resulting in HCV virological cure and hepatitis B surface antigen seroconversion in 2013. The patient had no neurological recurrences up to two years following the discontinuation of fluconazole.

Case 2

A 42-year-old male was admitted to the hospital on 2/2/19 for chronic headache, fever of 38.5°C, and mental status changes. He had CHC, genotype 1a, viral load of 5.68 IU/mL × 10⁵ IU/mL, fibrosis score F1, treatment naïve, mono-infection. He denied alcohol consumption but admitted to smoking marijuana daily. He had cognitive dysfunction, bifrontal positional headaches, and intermittent fevers for two months. His symptoms did not improve with analgesics. He was hemodynamically stable, and examinations of the heart, lungs, and abdomen were normal. The patient had no nuchal rigidity and no focal neurological deficits, but his reaction time was delayed along with slurred speech. Interestingly, he had an erythematous, macular, non-blanchable, non-pruritic, painful rash over the shins. Laboratory assessment showed a WBC of 7,400/mm³ (PMNs 59.1%, lymphocytes 0.2%, and monocytes 9.4%), hemoglobin level of 11.8 g/dL, platelet count of 227,000/mm³, blood urea nitrogen/creatinine level of 18.2/0.89 mg/dL, glucose level of 10³ mg/dL, TB of 0.9 mg/dL, ALT of 76 U/L, AST of 82 U/L. The prothrombin time was 12.7 sec (international normalized

ratio, 0.99). Serum ammonia, thyroid functions, thyroperoxidase antibodies, Antinuclear Antibodies (ANA), and immunoglobulin levels were normal. A chest X-ray was unremarkable. Pre-contrast and post-contrast brain MRI showed extensive leptomeningeal enhancement throughout the brain with dilated lateral and third ventricles suggestive of non-communicating hydrocephalus but no evidence of sinusitis, mastoiditis, orbital abnormalities, brain abscess, mass or midline shift. Abdominal CT scan revealed no evidence of cirrhosis or hepatosplenomegaly. We examined the CSF and began administration of empiric acyclovir, ceftriaxone, and vancomycin for suspected meningitis. The CSF analysis revealed a 122/mm³ WBC count (lymphocytes 93%), 814 mg/dL protein level, 23 mg/dL glucose level, and negative bacterial direct antigen. The CSF light microscopy with Gram, Ziehl Neelsen and India ink staining were negative. Polymerase Chain Reaction (PCR) results for tuberculosis, enterovirus, HSV1 and 2, Human Herpes Virus 6 (HHV6), VZV, EBV, CMV, HIV, WNV and Borrelia burgdorferi were negative. Furthermore, CSF paraneoplastic antibodies, fluorescent treponemal antibodies, and arbovirus serologies were non-reactive. Further studies showed a CSF/serum IgG ratio of 91 with 8 oligoclonal bands, indicative of severe BBB disruption. Cryptococcal antigen levels were extremely elevated in the blood and CSF, with titers of 1:2560 and 1:1280, respectively, suggestive of invasive cryptococcal infection with CNS and cutaneous cryptococcosis. On 02/07/19, the patient was administered liposomal amphotericin B in combination with flucytosine for two weeks. He was discharged home in good condition on high dose fluconazole 800 mg daily for 10 weeks followed by a tapered fluconazole regimen for 12 months. The CSF bacterial, viral, fungal and mycobacterial cultures resulted in no growth. Furthermore, cultures of the skin lesions were negative. On March 12th, 2019, he presented again with nausea, vomiting, and worsening mental status. Repeat lumbar puncture showed increased ICP with an opening pressure of 53 cm of H₂O. CSF analysis continued to show hypoglycorrachia (glucose of 18 mg/dL), hyperproteinorrachia (protein of 760 mg/dL), and lymphocytic pleocytosis (cell count of 58 with lymphocytic predominance of 52%). The cell blood count, comprehensive metabolic panel, and PT/INR were normal. The patient was instituted on lAmB, 5-FC, and dexamethasone 10 mg IV every 8 h. Because of worsening mental status and persistent non-communicating hydrocephalus, the patient received a right frontal External Ventriculostomy Device (EVD) in the ICU to mitigate intracranial hypertension, continuously monitor ICP, sample the CSF and instill lAmB. After one week, the EVD was removed and the patient was transferred in acceptable condition to the medicine ward. Nonetheless, 10 days later (04/01/2019), the patient became more lethargic and was found to have multiple small, shallow genital ulcers on exam. Repeat MRI was suggestive of parenchymal edema with worsening hydrocephalus. Herpes Simplex-2 Virus, verified by PCR, was isolated from genital and CSF specimens. Patient was transferred again to the ICU on 04/03/2019 and a new EVD was placed immediately. A 3 week-course (04/01/2019 to 04/21/2019) of IV acyclovir 10 mg/kg every 8 h was given in conjunction with cryptococcal induction therapy. A second course of intrathecal lAmB was given for 7 days (04/09/2019 to 04/16/2019). Mental status had drastically improved and EVD was removed. The skin rash on the legs and the genital lesions resolved. Induction therapy was given from 03/12/2019 to 04/23/2019 (6 weeks) and therapy was transitioned to high dose fluconazole 800 mg daily PO. Patient received physical rehabilitation from 05/02/2019 to 05/20/2019 and was then discharged home. Because of persistent headache and

confusion following discharge, Ventriculoperitoneal (VP) shunt was placed on 07/11/2019 resulting in remarkable improvement in mental status. Due to persistently elevated serum cryptococcal antigen and potential fluconazole resistance, antifungal therapy was switched to voriconazole resulting in substantial improvement in cryptococcal antigen levels. Patient was treated as well for CHC, GT1a, F1 with sofosbuvir/ledipasvir for 12 weeks with virological cure in January 2020.

For the preparation of the intrathecal administration of lAmB, a vial containing 50 mg of AmBisome powder was diluted with 10 ml of sterile water to achieve a final concentration of 5 mg/ml in the vial. After removing at least 10 ml of CSF, 2 ml of the lAmB solution (10 mg) was extracted and mixed with 3 ml of sterile saline without preservatives in a 5-ml syringe and administered intraventricularly slowly over 2 min. The rest of the AmBisome vial was kept in a fridge at 2°C to 8°C and used as required for a maximum of 7 days, according to the stability information given by the manufacturer.

Discussion

Hepatitis C virus belongs to the family *Flaviviridae*, which includes well-known neurotropic viruses (e.g., yellow fever, dengue, and tick-borne encephalitis viruses). While the primary cellular reservoir for HCV infection is liver hepatocytes, infection is associated with extrahepatic symptoms including CNS abnormalities, cognitive dysfunction, fatigue, and depression [12]. It seems that HCV-associated CNS symptoms are a function of systemic disease (spontaneous bacterial peritonitis and other infections; GI bleed), impaired hepatic function (advanced liver disease with encephalopathy and hyponatremia) and brain infection [1]. Direct infection of the CNS by HCV seems likely. HCV RNA has been detected in post-mortem brain samples and CSF using PCR based methods [13-16]. Moreover, direct evidence showed that HCV in CSF cells may be actively replicating and not just passively adsorbed. Low level HCV replication in the CNS is suggested by more sophisticated molecular techniques, including the detection of the negative strand of HCV RNA, which is considered a replicative intermediate [14], and distinct viral quasispecies within the brain samples [14-16]. However, HCV RNA is detected at a 1000 to 10,000-fold lower level in brain compared to liver, indicating that the brain is a minor replication site at most.

Compartmentalization can occur in the CNS of chronically infected HCV subjects via viral genetic adaptation to the CNS environment [17]. Hepatitis C virus can form somewhat separate viral populations, driven to adapt to their particular environment when subjected to different selective pressures. Compartmentalization has been defined in different ways: as genetic heterogeneity between subpopulations, as the result of independent micro-evolution, as the result of restricted viral gene flow, and as the presence of distinct but phylogenetically related genotypes [1,6,13,15]. It is unclear if compartmentalized HCV populations possess distinct phenotypic characteristics, such as cellular tropism, drug resistance, and level of pathogenesis. Compartmentalization can occur in the CNS of chronically infected HCV subjects via viral genetic adaptation to the CNS environment. The presence of distinct HCV populations in the CSF indicates that independent HCV replication can occur in the CNS. HCV enters the CNS most likely *via* the migration of infected leukocytes across the BBB. It seems that HCV-infected leukocytes carry HCV into the CNS. Thus, HCV neuroinvasion could be related to trafficking of infected leukocytes through the BBB in a process similar

to that postulated for HIV-1 infection [13]. Subsequently, there could be a secondary spread of HCV to permissive cells within the brain. The most likely target would be the brain microglial cells, which are essentially tissue-resident macrophages of blood monocytic origin [13]. Immunohistochemical and molecular techniques have indicated that microglia and astrocytes are cellular targets for HCV infection [4,5]. Unique sequences incorporating the HCV Internal Ribosomal Entry Site (IRES) were detected in the CNS [18]. The IRES mediates viral protein translation and when these variants were incorporated into a reporter vector, there was a reduction in translational efficiency, which may represent a possible mechanism for CNS latency [18]. Furthermore, HCV is associated with neurological symptoms in the absence of confounding factors such as advanced liver disease, HIV co-infection, or prior interferon treatment [1,6,13,16,19]. Hepatitis C virus, but not hepatitis B virus, particles were found to induce loss of tyrosine hydroxylase-positive neurons in midbrain neuron-glia cocultures, suggesting a neurotoxic effect of HCV on the dopamine-producing cells [1]. HCV core protein has been shown to trigger activation of the Extracellular Signal-Related Kinase (ERK)/Signal Transducer and Activator of Transcription 3 (STAT3) system *via* Toll-Like Receptor 2 (TLR2) in the CNS [15]. Analysis of post-mortem brain tissue revealed increased phospho-ERK levels in HCV infected patients that was not seen in patients with HIV encephalitis or in HCV seronegative patients [15]. Using a neuronal cell line, HCV core protein was shown to activate ERK via TLR2 expression, which is thought to play a role in neurodegeneration [15]. In a murine model, HCV demonstrated ERK activation, dendritic shortening, reduced neuronal density, astrogliosis, and cytoskeletal disruption [15]. These findings are in concordance with those from neuroimaging studies of metabolite changes and microarchitectural breakdown [16,20]. HCV Non-Structural protein 3 (NS3) microglial immune activation (CD68) [5] parallels the findings from the clinical PET studies using PK 11195 [16,20].

Hepatitis C virus penetrates the blood brain barrier regardless of the severity of the fibrosis score. Astroglia cell lines do not express all the cell molecules required for classical HCV entry (tetraspanin, CD81, and tight junction proteins claudin-1 and occludin) [21] therefore attention has turned to the BBB. Viral compartmentalization in CSF is associated with a higher plasma viral load, suggesting that increased viral burden in the periphery may facilitate invasion of the CNS [6]. Fletcher and colleagues have shown that HCV can replicate in Brain Microvascular Endothelial Cell (BMEC) culture [1]. Microvascular endothelial cells of the human brain express receptors that allow entry of HCV, and the viral replication in the endothelial cells may subsequently disrupt the BBB. Infected BMECs displayed apoptosis, which might result in reduced endothelial barrier activity. In this way, peripherally derived cytokines, viruses, other pathogens and immune cells might gain access to the CNS across an HCV-disrupted blood-brain barrier, resulting in immune activation within the CNS. Concurrent HCV-related systemic inflammation, exposure of neurotoxin and a disrupted BBB could exert a greater detrimental power on the neuron. In addition, viral proteins might act directly to potentiate neurotoxicity, as suggested for HCV core protein by Paulino [20].

HCV infection is associated with peripheral and neuroinflammation, with elevated inflammatory biomarkers, such as Interleukin 6 (IL-6), Tumor Necrosis Factor (TNF), IL-1 β , and IL-2, found in the peripheral blood and CNS. However, we do not fully comprehend the causality between the HCV, peripheral

inflammation, and neuroinflammation. It might therefore be expected that CNS symptoms correlate with levels of peripheral cytokines but the studies to date have given mixed results. Inflammatory cytokines, including interferons, are known to enter the brain in a variety of disease states and induce fatigue and other neurological symptoms [2]. Recent studies show that inflammatory cytokines, including IL-1 β and TNF- α , promote HCV particle entry into target cells [3], which may result in increased viral load in patients with high levels of circulating pro-inflammatory cytokines. These cytokines disrupt BBB integrity and may facilitate viral invasion of the brain [3]. HCV infection affects 3.5 million people in the United States and 71 million people worldwide [22,23]. Chronic hepatitis C virus infection has been associated worldwide with hepatocellular carcinoma, liver failure, and cirrhosis [24]. Chronic HCV infection not only affects the liver but is also a risk factor for extrahepatic diseases such as diabetes, chronic kidney disease, atherosclerosis, coronary artery disease, Parkinson disease, stroke, and neurocognitive impairment [25-31]. In this population, health outcomes and quality of life are greatly affected by a range of common comorbidities, including various types of psychological and cognitive disorders [29-31]. Studies have shown that approximately one-third of patients with chronic HCV infection experience depression and anxiety, whereas other findings indicate that neuropsychiatric dysfunction occurs in up to 50% of patients [31]. Symptoms of fatigue and "brain fog" are also commonly reported by individuals with chronic HCV infection [31]. Neurocognitive impairment, one of the most common extrahepatic manifestations of HCV, can lead to subtle changes in processing speed, memory, attention, fatigue, and cognitive performance [30]. Approximately 20% of non-cirrhotic patients with HCV demonstrated cognitive deficits pertaining to attention and concentration, psychomotor speed, mental flexibility and visual scanning and tracking, whereas roughly one-half of patients required an excessive amount of time to complete the given task and nearly 30% of patients made a significant number of errors [30]. In addition to increased rates of psychiatric illness and cognitive impairment, psychosocial stressors, including uncertainty regarding disease course and treatment, limited social support, and lack of coping skills affected the mental and overall health of patients with CHC [30]. Stigma associated with the disease may further contribute to anxiety, decreased intimacy, social isolation, discrimination, and reduced treatment seeking and adherence.

Neuropsychiatric and cognitive deficits in patients with CHC often occurred independently of liver fibrosis or presence of hepatic encephalopathy and does not correlate with specific HCV genotypes [30-32]. These alterations occur in the absence of structural brain damage or signal abnormalities on conventional brain Magnetic Resonance Imaging (MRI) [32]. These deficits were observed in the absence of HIV co-infection, substance abuse, or depression [30]. Although the underlying mechanisms have yet to be elucidated, the brain is a suitable site for HCV replication, where the virus may directly exert neurotoxicity [31]. Other potential mechanisms that may explain the pathogenesis of neuropsychiatric disorders in chronic HCV infection include derangement of metabolic pathways of infected cells, alterations in neurotransmitter circuits, autoimmune disorders, and cerebral or systemic inflammation [31].

In MRI studies, CHC was associated with cerebral inflammatory response, cognitive impairment, and neuropsychiatric symptoms, even when the liver disease was mild and in the absence of hepatic encephalopathy [33-35]. In another iodine 123-labeled β -carboxymethoxy-3- β -(4-iodophenyl) tropane single-Photon

Emission Computed Tomography (PET) study, serotonin and dopamine transmission was disrupted in patients with HCV infection, including those whose virus had been cleared [35].

Whether HCV-associated neurocognitive deficits improve after HCV eradication remains unclear. After HCV clearance, some studies have reported significant improvement in attention and working memory, but other findings showed no improvement in neurocognitive performance. Interferon-based antiviral therapy may reduce extrahepatic manifestations such as cognitive dysfunction, cardiovascular events, and stroke in patients with HCV infection [7-11] in addition to its positive outcome on the hepatic disease. In a study published in 2017, a subgroup of patients with Sustained Virologic Response (SVR) after treatment with pegylated alfa interferon and ribavirin showed significant improvement in cognitive function [7]. Based on neuroimaging data, these changes were linked to improvements in white matter integrity in the posterior corona radiata and the superior longitudinal fasciculus. However, interferon-based therapies have been associated with high rates of mental and cognitive dysfunction and related disability [8]. Emerging results of studies focused on Direct-Acting Antivirals (DAAs) may provide a clearer picture of the effects of HCV eradication on these outcomes. A recent double-blind placebo-controlled trial involving 750 patients with HCV compared outcomes among those treated with sofosbuvir/velpatasvir to patients receiving placebo. Patients in the active treatment group demonstrated improvements in general health, emotional well-being, and fatigue at 4 weeks and at the end of treatment [11]. The reduced incidence of extrahepatic manifestations by antiviral therapy could be associated with the clearance of HCV in patients. The underlying mechanism of the protective potential of antiviral therapy could be complicated and may involve the modulation of anti-inflammatory cytokines [36]. Another recent study found that Parkinson Disease (PD) incidence appeared to be lower in patients who were receiving interferon-based antiviral therapy for chronic HCV infection. The results seem to support the theory that HCV infection is a risk factor for developing PD. Antiviral therapy has shown potential in lowering this risk [37].

Previous studies showed that interferon can enter the brain, [38] but ribavirin, a water-soluble molecule, can hardly penetrate the blood-brain barrier [39]. Once the HCV enters the neuron or glia cell, the advantage of antiviral therapy may be damped. This is yet another reason to further study the pharmaco-dynamics and -kinetics of DAAs and their ability to cross the blood-brain and blood-spinal cord barriers. Antiviral therapy may, therefore, reduce the opportunity of CNS damage caused by HCV, especially if they are introduced early in disease and probably in the acute infection.

Cryptococcus neoformans causes infection following inhalation through the respiratory tract. The organism disseminates hematogenously and has a propensity to affect the CNS. The basis for the tropism for the CNS is uncertain, but several hypotheses have been proposed: a) the CSF is a favourable growth medium for the organism as it lacks the factors present in serum, such as the alternative complement pathway, that inhibit cryptococcal growth [40,41]; b) dopamine levels in the CNS may promote cryptococcal virulence by serving as a substrate for melanin production by the organism [42]; c) production of mannitol by the organism may contribute to brain edema and inhibit phagocyte function [43]; d) *C. neoformans* appears to evade the host innate immune system and invade through the blood-brain barrier [44]. The inflammatory response in the brain to *C. neoformans* is generally milder than

that seen in bacterial meningoencephalitis. The inflammatory cell infiltrate is predominantly comprised of mononuclear cells with occasional polymorphonuclear leukocytes. In general, involvement of the brain is diffuse, but localized infections (i.e., cryptococcomas) can also occur.

Most patients with cryptococcal meningitis are immunocompromised. The most common forms of immunosuppression other than HIV include glucocorticoid therapy, solid organ transplantation, cancer (particularly hematologic malignancy), and other conditions such as sarcoidosis and hepatic failure. Other risk factors include the use of the tyrosine kinase inhibitor, ibrutinib [45], and the development of anti-Granulocyte-Macrophage Colony Stimulating Factor (anti-GM-CSF) antibodies [46]. Although many patients have risk factors for disease, in a multicenter retrospective study of 157 cases of CNS cryptococcosis in HIV-negative patients, 30 percent had no apparent underlying condition [47]. Among solid organ transplant recipients, CNS involvement and disseminated infection have been documented in 52 to 61 percent of patients with cryptococcal infection [48-50].

The clinical presentation of cryptococcal meningoencephalitis in HIV-seronegative patients is variable. Most patients present with signs and symptoms of subacute meningoencephalitis; fever is observed in approximately 50 percent of cases [47,51]. Typically, headache, lethargy, personality changes, and memory loss develop over two to four weeks. Patients may also present with disseminated disease.

Cryptococcal Meningitis (CM) continues to be tied to high rates of mortality, especially among HIV-infected patients. While over half of HIV-patients with CM from low- and middle-income countries die within 10 weeks of diagnosis, the mortality in developed countries is nearly 10% [52]. CM is still responsible for 15% of deaths in patients with AIDS [53]. The diagnosis of CM is based on the presence of *Cryptococcus* antigens in CSF by latex agglutination, India ink staining and/or fungal culture [54].

An investigation of cannabis samples from the patients' dispensary demonstrated contamination with several varieties of *Cryptococcus*, including *C. neoformans*, and other opportunistic fungi [55]. These findings raise concern regarding the safety of cannabis, even in immunocompetent users. Smoking marijuana could be a direct source of inoculation in the lungs and subsequent hematogenous dissemination. Both reported patients were non-alcohol consumers and had no evidence of cirrhosis or liver decompensation such as portal hypertension (ascites, esophageal varices), hyponatremia, hepatorenal or hepatopulmonary syndrome. They did not have any type of cancer, were not undergoing chemo- or corticosteroid therapy, and had no evidence of autoimmune diseases. However, they smoke marijuana frequently.

Amphotericin B (AmB) has a strong fungicidal activity against *Cryptococcus*, but it has poor penetration into the CSF [56,57]. To achieve higher concentration in CSF, intrathecal administration of AmB deoxycholate (AmBd) has been used for the treatment of CM, and observational studies suggest that it could be associated with improved survival [58]. However, AmBd has a direct irritant effect and intrathecal administration is poorly tolerated [59,60]. Lipid formulations of AmB (lAmB) are effective and less irritating than AmBd [61-64]. The safety and tolerability of the intrathecal administration of lAmB for the treatment of CM in HIV-infected

patients have been demonstrated [65]. External Ventricular Drain (EVD) and intrathecal administration of lAmB could help reduce complications and mortality. Furthermore, this strategy may decrease the duration of the intravenous AmB to 7 to 14 days without observing an increase in mortality compared to standard of care. Adverse events in patients who received intrathecal lAmB were mild and transient [66]. Intrathecal lAmB through lumbar punctures was safe and well-tolerated by HIV-patients, and it was not associated with an increased mortality compared with the standard of care. Large clinical multicentre trials are needed to confirm the survival benefits of EVD and the intrathecal administration of lAmB in HCV infected patients with CM.

Conclusion

Our cases illustrate the importance of considering CNS cryptococcosis in CHC patients regardless of the fibrosis score, as this fungal organism may be a more common culprit of infections in these patients than previously considered. An extremely high index of suspicion will help aid in prompt diagnosis of cryptococcal infections in this population and aggressive interventions may improve outcomes in HCV-infected patients. Patients with CHC should be advised to avoid smoking marijuana, a potential source of opportunistic *C. neoformans* infection.

Important epidemiologic evidence suggests that HCV could be a risk factor for mental illness and neurological diseases however, the association between antiviral therapy and resolution of BBB damage and neuroinflammation has remained unclear. Understanding this association may help develop strategies to reduce neuropsychiatric occurrences and Opioid Use Disorders (OUD) in CHC patients. Future research should examine whether any additional improvements in neurocognition and white matter integrity among SVRs occur with longer follow-up periods. To solve this conundrum, we may need to study the neuroinflammatory mechanisms in patients with CHC before and after receiving antiviral therapy. Cofounding factors that may cause neuroinvasion or affect the mental status should be excluded such as advanced liver disease and cirrhosis (fibrosis stage 4), HIV, infectious meningoencephalitis, metabolic encephalopathy, and CNS diseases. The study should be prospective, controlled (DAA vs. placebo), double blinded, enrolling non-cirrhotic patients with CHC (fibrosis score F0-F3). Patients should have imaging studies of the brain with MRI and/or PET scan, and lumbar puncture before enrolment and 12 weeks after discontinuation of therapy. Patients should be evaluated for mental illness throughout the process to follow on improvement of their neuropsychiatric illness. Furthermore, we need to evaluate the impact of virological cure (virological cure vs. chronic infection) on addiction in terms of addiction therapy retention.

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