Clinicopathological Conference

Into the Unknown: Diagnosing Mysterious Brain Lesions

Authors

Ihab Kassab[1], Carlos Isada[2], Marwan M. Azar[3], Nadine Sarsam[1], Min Jiang[1], Sandra Camelo-Piragua[4], Daniel Kaul[1], Maricar Malinis[3]

Affiliations:

[1] Department of Internal Medicine, University of Michigan, Ann Arbor, MI

[2] Department of Infectious Disease, Respiratory Institute, Cleveland Clinic Foundation, Cleveland, OH

[3] Section of Infectious Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT

[4] Department of Pathology, University of Michigan, Ann Arbor, MI

Keywords

Brain Abscess, Organ Transplant, *Bartonella henselae*

Running Title

Brain Lesion in Organ Transplant

Corresponding Author

Maricar Malinis, MD

Section of Infectious Diseases, Yale School of Medicine

PO Box 208022, New Haven, CT. 06520-8022

E-mail: maricar.malinis@yale.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tid.13829.

Contributions

All the authors equally contributed to the draft of the manuscript.

Twitter

Ihab Kassab @KassabIhab

Daniel Kaul @dan_dkauld

Maricar Malinis @maricar_malinis

Financial Disclosures

No financial support was received for this work.

Daniel Kaul: Research support from AstraZeneca, Janssen, Gilead, Merck, NIAID, Nobelpharma; Data Safety Monitoring Board Member for Noveome

Conflict of interest

The authors have no conflict of interest to disclose as described by the Transplant Infectious Disease Journal.

Permission for Figures

Permission was obtained for Figures 3 and 4 from Elsevier (License 52477310213) and for figure 5 from Wolters Kluwer Health, Inc (License 5247761192322)

Acknowledgments

We thank Drs Steve Miller and Charles Chiu for providing Figure 8 of this manuscript.

List of abbreviations

Abbreviation	*Meaning*
ADEM	acute disseminated encephalomyelitis
ADC	apparent diffusion coefficient

AIDS	acquired immunodeficiency syndrome
CNS	central nervous system
CMV	cytomegalovirus
COVID-19	Coronavirus Disease 2019
CSF	cerebrospinal fluid
CT	computed tomography
DNA	deoxyribonucleic acid
EEG	electroencephalogram
EBV	Epstein-Barr virus
GRE	gradient recalled echo
H&E	hematoxylin and eosin
HACEK	*Haemophilus* species, *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*
HIV	human immunodeficiency virus
HSV	herpes simplex virus
HHV-6	human herpesvirus 6
JCV	John Cunnigham virus
IRIS	immune reconstitution inflammatory syndrome

mNGS	metagenomic next generation sequencing
MRI	magnetic resonant imaging
NMDAR	N-methyl-D-aspartate receptor
PAS	periodic acid-Schiff
PCR	polymerase chain reaction
PTLD	post-transplant lymphoproliferative disease
PML	progressive multifocal leukoencephalopathy
PRES	posterior reversible encephalopathy syndrome
RMSF	Rocky Mountain spotted fever
SOT	solid organ transplant
TB	tuberculosis
TMP-SMX	trimethoprim-sulfamethoxazole
VZV	varicella zoster virus
WBC	white blood cell

Abstract

The case discussed involves a 49-year-old male with simultaneous pancreas-kidney transplant who presented with fever, headache and was found to have multifocal brain lesion on brain imaging.

Initial Case Presentation (Ihab Kassab)

A 49-year-old male with a history of type 1 diabetes mellitus, end stage renal disease, who presented 8 months after a simultaneous pancreas-kidney transplant with a three-day history of fever, tension-like headache, nausea, and non-bloody emesis. He had no cough, vomiting, diarrhea, or urinary symptoms. He denied any exposure to cats, but he owned two dogs. He lives in central Michigan with his wife, unemployed and did not have any recent travel or unusual dietary habits. Induction immunosuppression received was unknown. His maintenance immunosuppression regimen at the time of presentation was tacrolimus and mycophenolate mofetil. He was receiving trimethoprim sulfamethoxazole (TMP-SMX) 400-80 mg once daily for infectious prophylaxis.

Physical examination revealed a fever of 39.5°C, tachycardia of 135 beats per minute, blood pressure of 122/74 mmHg, respiratory rate 16 breaths/minute, and oxygen saturation of 97% on ambient air. He was alert and oriented, without any focal neurological signs, and neck was supple. Breath and heart sounds were normal on auscultation. The rest of the exam was unremarkable. Laboratory values were significant for elevated white blood cell count (WBC) of 13.5 K/ul. Laboratory test results are shown in **Table 1**.

Chest radiograph showed no focal opacities. Urine analysis was unremarkable. Transthoracic echocardiography was performed showing a normal heart with no vegetations. Computed tomography (CT) of the brain showed multifocal areas of hypodensity predominantly involving the subcortical white matter of both cerebral hemispheres, cerebellar hemispheres, and right thalamus (**Figure 1**).

Differential Diagnosis (Carlos Isada)

Neurologic syndromes are common in solid organ transplantation (SOT), with about one-third (10-85%) experiencing a neurologic complication.[1] Etiologies can be both infectious and non-infectious, often with many overlapping features. In most instances, neurologic complications should be considered urgent in the SOT population, given a high associated mortality.

When approaching a patient with suspected central nervous system (CNS) infection it is useful to classify the patient into the predominant clinical and imaging syndrome. Common neurologic syndromes in the immunocompromised include meningitis, space occupying lesion(s), limbic encephalitis, brainstem lesions, leukoencephalopathy (white matter disease) and stroke. Examples of infectious and non-infectious etiologies[1-3] associated with these neurologic syndromes are outlined in **Table 2**. I will discuss the first two syndromes, meningitis, and space occupying lesion(s) in more detail later. These syndromes are not mutually exclusive, and some immunocompromised patients may present with more than one syndrome.

Anatomic localization of neurologic deficits is an important early step and is based on serial bedside examinations and imaging findings. Some SOT patients present with a diffuse encephalopathy without discrete lesions on neuroimaging. For the remainder, important anatomic considerations include the presence of cortical or subcortical deficits, deficits that follow a vascular distribution, cranial nerve palsies, posterior fossa involvement (e.g., cerebellar lesions), and spinal cord and/or peripheral nerve involvement. Another important diagnostic clue is involvement of extra-neural sites, particularly the paranasal sinuses, ocular structures, cardiac valves, and the lung.

The timeline for CNS infections in SOT follows the timeline for most infections in this population.[1] In the postoperative period, weeks one to four, CNS infection is uncommon. One exception are those who have had intense exposure pre-operatively such as exposure to *Aspergillus spp*. from hospital construction. Another cause of postoperative phase infection is donor derived infection such as West Nile virus, albeit rare. The peak time for CNS syndromes is the early phase (1-6 months post-transplant). A variety of pathogens have been described in both early and late (> 6 months) phase, outlined in **Table 3**. Routine post-transplant antibiotic prophylaxis likely impacts the incidence and spectrum of CNS infections, such as TMP-SMX decreasing the risk of infection with *Listeria monocytogenes*, *Nocardia* spp., and *Toxoplasma gondii*.

Based on the available information our case would be characterized as a late posttransplant syndrome with multiple space occupying lesions involving subcortical white matter, with no significant extra-neural involvement. The CT scan findings of hypodensities is nonspecific and could represent abscesses with edema, cerebritis, areas of ischemia, demyelination,

metabolic insult, and others. The lesions are in multiple vascular distributions. The lesions seem to be clinically silent despite the relatively large size on CT scan. For example, there are no cerebellar signs on examination despite at least one lesion in the left cerebellar hemisphere.

The patient also has features of a concurrent meningitis, with fever, leukocytosis, and neck stiffness. One hypothesis is that the hypodensities on CT scan represent abscesses and the patient has acute meningitis. However, it is uncommon to have brain abscesses and acute meningitis occur simultaneously, which I will discuss in more detail later.

At this point the differential diagnosis remains wide. This includes all the "late CNS syndrome" pathogens as well as other rare opportunistic organisms. To assess the latter, additional history would be helpful including social history, habits, exposures, and epidemiologic risk factors Empiric antibiotics are indicated and would treat immediately for bacterial meningitis with vancomycin, ampicillin, and ceftriaxone. Doxycycline is also a consideration depending on geographic location and exposure history. Diagnostic studies would include magnetic resonance imaging (MRI) of the brain followed by lumbar puncture with measurement of opening pressure. If the MRI confirms the presence of mass lesions, I will consider adding an antifungal agent.

Regarding the possibility of tuberculous meningitis or abscesses, the incidence of tuberculous meningitis in a non-endemic country such as the United States is predictably low, with only 69 cases reported to the U.S. Centers for Disease Control and Prevention in 2018.[4] Tuberculous brain abscesses are even less common than TB meningitis, including

countries in which TB is endemic.[5-8] Given these considerations it would be reasonable to defer starting antituberculosis therapy unless more suggestive epidemiologic or exposure history comes to light.

Clinical Course (Ihab Kassab)

The patient was given empiric intravenous antibiotic regimen of ceftriaxone 2 gm every 12 hours, vancomycin 13 mg/kg every 8 hours, and ampicillin 2 grams every 4 hours. A lumbar puncture was performed with cerebrospinal fluid (CSF) analysis showing leukocytes of 1005 cells/cm^3 (80% neutrophils), protein of 186 mg/dL and glucose of 63 mg/dL. CSF cytology, bacterial and fungal cultures, and serologies were all negative. MRI of the brain showed innumerable bilateral scattered supratentorial, infratentorial, and brainstem ring-enhancing lesions compatible with a multifocal infection (**Figure 2**).

Interpretation of Findings and Differential Diagnosis (Carols Isada)

The MRI scan shows small rim-enhancing lesions consistent with parenchymal brain abscesses. The most likely etiologies are bacterial, fungal, parasitic. The appearance virtually excludes viral and rickettsial causes. It should be noted that Epstein Barr virus (EBV) related posttransplant lymphoproliferative disease (PTLD) may have a similar appearance, however the characteristic “partial” ring enhancement on MRI secondary to central necrosis is lacking. The multifocal pattern of lesions is consistent with hematogenous spread of infection or a central embolic source, as opposed to spread from a contiguous focus of infection such as the sinuses.

The literature on brain abscess in SOT is limited, consisting of mainly single case reports and small case series of less than 5 patients. One of the largest cohort studies was in 1997, examining SOT recipients on cyclosporine based immunosuppression.[9] This was a 14-year retrospective review performed prior to the azole prophylaxis era. Twenty-eight brain abscesses were identified out of 4628 SOT recipients for an incidence of 0.6%. The most common etiology was fungal (*Aspergillus, Candida* spp.) followed by *Nocardia spp*., and *Toxoplasma gondii*. Common bacterial pathogens were not found in this series. CSF analysis was either normal or with minimal abnormalities; CSF cultures were all negative. Multiple brain lesions were more common than single, and all anatomic areas were involved. In this series most had CNS dissemination from a pulmonary focus. More recent series of brain abscess in SOT recipients generally have been limited to a single opportunistic pathogen (fungal brain abscess[10], *Nocardia spp*. [11,12] *T. gondii* [13,14]), with some series including both SOT and hematologic stem cell recipients. The microbiologic spectrum of brain abscesses in SOT in the azole prophylaxis era with current immunosuppressive regimens is unknown. This is particularly problematic when estimating the risk of pyogenic brain abscesses given the increasing incidence of methicillin-resistant *Staphylococcus aureus* infections in SOT.[15]

Neuroimaging may help differentiate amongst various groups of organisms, particularly different MRI sequences. For example, pyogenic (bacterial) brain abscesses characteristically display central hyperintensity and rim hypointensity on T2 weighted imaging and rim enhancement on T1 weighted imaging with contrast.[16,17] On diffusion weighted image sequences, pyogenic abscesses usually show central diffusion restriction,

indicative of cytotoxic edema. An example of a bacterial brain abscess[17] is shown in **Figure 3** and fungal abscess[17] in **Figure 4**.

Suggestive MRI patterns have been described with other CNS infections including toxoplasmosis, nocardiosis, neurocysticercosis, tuberculosis, and EBV-related PTLD. Other MRI sequences have been reported including apparent diffusion coefficient (ADC), magnetic resonance angiography with vessel wall imaging (for infectious CNS vasculitis), and magnetic resonance spectroscopy.[16,17] MRI blood sequences such as the susceptibility weighted images (SWI) may suggest invasive CNS mold infection given the angioinvasive nature of many pathogenic molds. The presence of acute bleeding can also be seen with herpes simplex encephalitis within areas of cerebritis and occasionally with toxoplasmosis.

It should be noted that there is overlap in these MRI patterns, with some variability. The sensitivity and specificity need further study. In the current case, the lesions are small and spatial resolution is limited. Additional information would be helpful such as the presence of diffusion restriction in the center of the lesions, and whether petechial bleeding is present on susceptibility weighted images.

One MRI pattern that is considered diagnostic is the "starry sky" pattern seen in Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsia.*[18] In severe cases the organism causes an infectious CNS vasculitis, characterized by multiple small acute infarcts. These appear as punctate hyperintensities in the centrum semi-ovale which has been likened to a starry sky (**Figure 5**). RMSF is a consideration in this case given the epidemiology; the state of Michigan reported 48 cases of RMSF in 2020 (Michigan

Surveillance System).[19] However, the clinical presentation, absence of skin rash, and presence of multiple small rim-enhancing lesions rather than infarcts essentially rules this out.

Regarding the CSF findings there was a significant pleocytosis which was predominantly neutrophilic (1005 total nucleated cells/mm^3, 80% polymorphonuclear cells) with elevated CSF protein and normal CSF glucose. This is most consistent with bacterial meningitis, further supported by his fever, headache, and neck stiffness. As mentioned previously, this is an unexpected finding since it is unusual to have brain abscesses co-exist with meningitis; these are usually independent processes. Some notable exceptions include rupture of an abscess directly into a ventricle, leading to ventriculitis and then diffuse meningitis. *Listeria monocytogenes* meningitis can easily cross the blood brain barrier and blood: CSF barrier. It can present with meningitis and brain stem involvement (rhomboencephalitis). *Cryptococcal neoformans* can rarely present with meningitis along with solid tumor-like intracranial masses. None of these seem to be present on his MRI scan. There are reports of Nocardia spp. causing acute meningitis (CSF WBC greater than 500/mm^3) with simultaneous brain abscesses[20], but these are rare and to date have not been reported in SOT.

Some neurotropic viruses can cause an acute aseptic meningitis with a neutrophil predominance and relatively high CSF pleocytosis (greater than 1000 WBC/mm^3). Enteroviruses and West Nile virus can sometimes mimic acute bacterial meningitis in terms of severity of symptoms and CSF profiles. However, the finding of multiple small ring enhancing lesions would be highly unusual for these viruses and are essentially ruled out.

In prior series of brain abscess in SOT, none of the cases reported CSF pleocytosis even approaching 1000 WBC/ mm^3. Values were typically less than 50 WBC/mm^3. This raises the possibility that the brain abscesses in the current patient are more of a secondary process, such as hematogenous spread from a primary bacterial meningitis. In one recent series of bacterial meningitis in SOT[21], a cohort of 6 cases were identified out of approximately 11,000 transplant patients in the Netherlands from 2004-2016. All were renal transplant recipients. Microbial etiologies were *S. pneumoniae* (2 cases), *L. monocytogenes* (2), *E. coli* (1) and *P. aeruginosa* (1). The mean leukocyte counts in CSF were 713 cells/ mm^3 with a range 17-12014 WBC/ mm^3. CT scans of the brain were negative in all cases.

The differential diagnosis thus far includes *Listeria monocytogenes* with brain abscesses, *Nocardia spp.* infection, and other rare bacterial meningitides. Non-bacterial infections include cryptococcal meningitis with abscess formation, CNS toxoplasmosis, CNS aspergillosis, and neurocysticercosis. It should be noted that none of these diagnoses are common even in the immunocompromised, and for several the presentation would be highly atypical.

Approach to Diagnosis in Patients with CNS infection (Marwan Azar)

Despite a battery of conventional testing, including CSF stains, cultures, antigen testing, serology and molecular testing, the etiology of acute meningoencephalitis remains unidentified in more than half of patients presenting with this clinical syndrome.[22]

Delayed and missed diagnoses are associated with increased morbidity and mortality and this is particularly true for immunocompromised patients. Emerging diagnostic tests, including multiplex molecular polymerase chain reaction (PCR) panels and metagenomics of CSF may improve the diagnostic yield for CNS infections. Data on the yield of these tests has not been well defined in immunocompromised and transplant patients.

The Biofire FilmArray Meningoencephalitis (ME) Panel (Salt Lake City, UT) is currently the only commercially available FDA-approved multiplex PCR assay on CSF specimens and includes 7 viral, 6 bacterial and 1 fungal (*Cryptococcus neoformans/gatti*) targets. The clinical utility of the FIlmArray ME assay has not been rigorously evaluated among transplant patients but a study in a heterogenous population concluded that use of this assay did not result in additional pathogen detections compared to conventional testing.[23] Importantly, the sensitivity of the FilmArray ME for *Cryptococcus* appears to be relatively low (71%) compared to CSF *Cryptococcus* antigen testing and has resulted in false negative testing in a kidney transplant recipient with cryptococcosis so the assay should not be used to rule out cryptococcal meningitis in transplant patients.[24,25] Additionally, detection of certain targets including HHV-6 and CMV may not be clinically significant. Overall, this assay could be useful in conjunction with conventional testing, but results must be interpreted in the context of host status and clinical, radiographic, and microbiologic data.

While PCR-based methods can detect known nucleic acid sequences using a specific set of primers, metagenomic next-generation sequencing (mNGS), also known as clinical metagenomics, can detect the nucleic acid of entire microbial communities in a clinical specimen. As a form of hypothesis-independent testing, clinical metagenomics has the potential to detect thousands of potential pathogens simultaneously and to significantly

shorten the turnaround time for testing compared to culture-based methods.[26] Clinical metagenomic assays directed at CNS infection can be performed on CSF, brain or spinal cord tissue or blood/plasma specimens. Though the literature is replete with reports in which clinical metagenomics on CSF, brain tissue or blood detected CNS pathogens missed by conventional testing, a significant proportion of infections (45%) detected by standard testing was missed by mNGS testing on CSF[27-29] in a prospective study of hospitalized patients with meningoencephalitis (44% of whom were immunocompromised). Moreover, clinical metagenomic testing on CSF did not lead to a positive clinical impact in most cases (≥96%). Notably, there are very little data to inform our understanding of the performance of clinical metagenomics in transplant recipients. Due to several drawbacks including lack of standardization across mNGS protocols, increased cost, decreased sensitivity for organisms with thick cell walls (fungi, mycobacteria) and the inability to discern between infection and colonization, targeted PCR-based tests continue to be the preferred test when specific pathogens are suspected. Clinical metagenomics should be considered if conventional testing including targeted PCR assays is negative but suspicion for infection remains high, including in unusual clinical presentations such as this one.

Clinical Course (Ihab Kassab)

Vancomycin and ceftriaxone were stopped, while ampicillin was continued. Meropenem and high dose TMP-SMX were initiated to cover empirically for CNS nocardiosis or toxoplasmosis. CT of chest was performed to evaluate for possible extra-CNS disease involvement. The CT chest was not revealing. Transthoracic echocardiogram did not demonstrate any vegetation. Blood cultures were no growth. When CSF PCR for Listeria was

resulted negative, ampicillin was discontinued. Patient developed leukopenia and worsening renal function which prompted discontinuation of TMP-SMX. Voriconazole was added empirically due to concern for CNS aspergillosis. Donor history was revisited and there was no confirmed significant travel history or findings to suggest of donor-derived disease. Additional diagnostic testing results are outlined in **Table 6**. On hospital day 9, patient had a seizure-like activity and altered sensorium. Voriconazole was discontinued. A 2-day electroencephalogram (EEG) captured a right posterior quadrant onset subclinical seizure. Repeat LP and MRI of brain were performed on hospital day 12 and 13, respectively. CSF revealed 1005 leukocytes (80% neutrophils, 5% lymphocytes), protein 186 g/dl and glucose 63 g/dl. MRI of the brain **(Figure 6)** revealed no interval changes in size or number of the supratentorial and infratentorial ring-enhancing lesions. Ceftriaxone was re-started due to concern for possible nocardiosis and meropenem was discontinued as it may have contributed to the seizure. Brain biopsy was performed, and brain tissue sent for cultures and broad range PCR. Stains and culture for bacteria, fungi and AFB were negative. Brain tissue PCR results were negative for bacterial DNA, fungal DNA, nontuberculous mycobacteria DNA, and mycobacterium tuberculosis complex DNA.

The biopsied brain tissue showed evidence of chronic inflammation embedded in new mesenchymal collagen deposition (**Figure 7**). Purulent neutrophilic content was not evident, indicating that this biopsied tissue was most consistent with an abscess wall only. Hematoxylin and eosin (H&E) staining showing a mixed inflammatory infiltrate in a fibrous background, but acute neutrophilic inflammation is not evident (Figure 7,Panel A). Inflammation is composed of T-cells (CD3) (Panel B) and numerous macrophages (CD68) (

Figure 7, Panel C). Movat staining demonstrated diffuse, acute collagen deposition, often not seen in the brain but consistent with abscess wall (Figure 7,Panel D).

Differential Diagnosis with Additional Information (Carlos Isada)

This additional information on the clinical course and the brain biopsy makes it clear that we are dealing with a very rare situation, possibly a presentation that has not been previously described. The patient responded to antibiotics in terms of the CSF parameters, with a marked decrease in CSF WBC from 1005 cells/mm^3 to 56 cells/mm^3. He received minimal antifungal or anti-toxoplasma therapy, and no anti-tuberculous therapy. None of these would be expected to improve spontaneously. The most significant antibiotic exposure was to ceftriaxone.

The brain biopsy confirms the presence of an abscess. The histopathology suggests the infectious process is subacute, probably over 14 days corresponding to the late capsule formation stage of brain abscesses[30]. This contrasts with the acute symptom presentation of 3 days in our case, possibly related to his immunocompromised state. No distinctive histopathologic pattern was seen on the brain biopsy such as pyogranulomas (suggestive of bartonellosis, blastomycosis, actinomycosis, and others) or the Splendore-Hoeppli phenomenon (suggestive of nocardiosis, actinomycosis, aspergillosis, blastomycosis, and others). The organism is not visualized on H&E stain, which highlights most yeast, molds, and toxoplasma spp. Numerous macrophages were seen on the biopsy, but not reported as foamy macrophages, which would have suggested CNS Whipple's disease. However, results

of periodic acid-Schiff (PAS) stain are not reported, which is the preferred stain for identifying *Tropheryma whipplei*. CNS Whipple's cannot be ruled out.

Numerous microbial cultures from several sites are negative including blood, CSF, and brain biopsy making common etiologies of meningitis unlikely such as *S. pneumoniae*, *L. monocytogenes*, and *N. meningitidis*. The multiplex PCR panel from CSF is also negative which includes these three organisms. Additional molecular diagnostic studies on brain tissue are pending. This type of culture-negative scenario is where broad range PCR or mNGS may be particularly useful[31].

The evidence to date suggests an infection with a fastidious bacterial organism. Fastidious organisms have complex or particular nutritional requirements. They fail to grow unless specific nutrients are present in the growth media. Fastidious organisms are best known for their role in causing culture-negative endocarditis. Examples of "classic" fastidious organisms described in older microbiology literature[32] as well as more recently recognized agents are listed in **Table 4**, noting which organisms are unlikely to respond clinically to ceftriaxone.

The pros and cons for fastidious bacteria that typically respond to ceftriaxone are listed in **Table 5**. Parenchymal brain lesions have been described with several organisms including *B. burgdorferi*, *T. whipplei*, *L. interrogans*, *E. rhusiopathiae* but multifocal brain abscesses are exceedingly rare except for *Abiotrophia spp*. and *E. rhusiopathiae*. The clinical presentation of the fastidious organisms in SOT is largely unknown.

Using the framework above the differential diagnosis can be narrowed to the following five organisms: *Abiotrophia spp*., *L. interrogans*, *B. henselae*, *B. burgdorferi*, and *T. whipplei*. As noted in Table 4, no one organism would fit all the major features of our case. This appears to be an unusual manifestation of an unusual infection, possibly a presentation not previously described. Molecular diagnostics such as broad range PCR and mNGS may help establish the diagnosis. The evidence supports that the true diagnosis is one of the five fastidious bacteria listed above. I would favor CNS Whipple's disease as the most likely etiology, but with a moderate degree of diagnostic uncertainty.

Clinical Course (Ihab Kassab)

The patient improved markedly on ceftriaxone and was discharged on 2 grams twice daily for possible *Nocardia* pending further results and given improvement. The repeat CSF fluid that was sent to University of California San Francisco for mNGS resulted with *Bartonella henselae*. There were 22 sequence reads that aligned to *Bartonella henselae* out of a total of 21,539,049 total sequence reads in the sample. This corresponds to RPM ratio (Reads Per Million ratio) of 1.0217, which is below the formal reporting threshold RPM ratio of 10 (**Figure 8**).

However, because *Bartonella* is not a common contaminant organism and potentially clinically significant even if detected at very low levels, this finding was reported so that follow-up test and clinical evaluation could be performed. Repeat discussion with patient

confirmed no exposure to cats. To further confirm the infection, serologies and serum PCR were sent. Antibody test resulted in *Bartonella henselae* IgG titer of 1:1024, consistent with a recent infection (**Table 7**). The diagnosis of *Bartonella henselae* infection with brain abscesses was confirmed.

Ceftriaxone was planned until significant resolution of the brain lesions, and doxycycline was added for a 12-month treatment plan. Patient fully recovered to baseline.

Final Diagnosis : Brain abscess due to *Bartonella henselae*

Bartonella infection is not an uncommon infection, with 12,000 outpatient cases and 500 inpatient cases a year in the United States. *Bartonella henselae* is endemic in warm humid climates and therefore the incidence is highest in the southern United States. The incidence is highest among children. [33,34]

Bartonella henselae is difficult to culture and the best investigations for detecting this pathogen are serology and PCR.[35] Additionally, mNGS is a DNA sequencing technology that has revolutionized genomic research. In microbiology, mNGS has been used for detection of pathogens nucleic acids. In our case, the CSF mNGS played a major role in the diagnosis. [36,37] *Bartonella henselae* commonly presents with a papule persisting for 1-3 weeks. Painful regional lymphadenopathy near the site of the scratch or bite is also commonly seen. More severe atypical manifestations are generally seen in the immunocompromised, such as post-transplant and HIV/AIDS patients. Atypical manifestations include vascular proliferative lesions such as bacillary angiomatosis (red elevated skin lesions) and bacillary peliosis (blood

filled cavities in the liver), neuroretinitis, parinaud oculoglandular syndrome, fever of unknown origin, and blood culture-negative endocarditis. Other manifestations that are more typically seen in children include granulomatous hepatitis, splenitis, and osteomyelitis.[33,38-42]

Neurologic complications are rare and only occur in approximately 0.17% to 2% of *Bartonella henselae* infected patients, with encephalopathy being the most common presentation. Cat scratch encephalopathy most commonly presents with seizures. Other CNS manifestations of *Bartonella henselae* include encephalitis, aseptic meningitis, status epilepticus, transverse myelitis, and radiculitis. [43,44] It can also present with dementia in HIV patients.[45] Although a case of brain granulomatous process involving the right thalamus and surrounding tissues associated with *Bartonella henselae* infection has been reported in the literature[44], to our knowledge, multiple brain abscesses have not been reported before.

Our patient developed severe *Bartonella* infection with meningitis and multiple brain abscesses. His atypical presentation and lack of exposure history made the diagnosis very challenging and *Bartonella* was not suspected, and therefore, serologies were not sent. In our case, the CSF mNGS with its ability to reveal unsuspected pathogens, played a major role in the diagnosis.

From the *Bartonella henselae* cases reported in the literature, some patients have been known to present with neurological symptoms without a history of cat scratch, as is the case in our patient. [46] However, our patient owned two dogs, and *Bartonella henselae* infection in dogs has been described.[47] Antibiotic therapy, including one or a combination of

macrolides, rifampin, doxycycline, TMP-SMX, and ciprofloxacin, is recommended in atypical disease.[34] Our patient was successfully treated with doxycycline, and prior to that he was treated with ceftriaxone which may have variable activity against *Bartonella.*

Bartonella henselae infection is not an uncommon infection, but brain abscesses are very rare.

To our knowledge, apart from our case there has been no previous reports of multiple brain abscesses associated with *Bartonella henselae* infections in the literature. We present this case to heighten physicians' awareness on *Bartonella* infection especially among the immunocompromised and transplant population when presenting with fever of unknown origin, meningitis, and brain abscesses when no common pathogen could be found. This case highlights the importance of using newer genetic techniques such as mNGS as opposed to more direct techniques like directed PCR, as mNGS might reveal a pathogen that is not suggested by the clinical presentation and exposure history.

References

1. Wright AJ, Fishman JA. Central nervous system syndromes in solid organ transplant recipients. Clin Infect Dis 2014;59(7):1001-11. DOI: 10.1093/cid/ciu428.
2. Castro I, Ruiz J, Tasias M, Montero M, Salavert M. Central nervous system infections in immunocompromised patients. Rev Esp Quimioter 2018;31 Suppl 1:56-61. (https://www.ncbi.nlm.nih.gov/pubmed/30209926).
3. Moritani T, Capizzano A, Kirby P, Policeni B. Viral infections and white matter lesions. Radiol Clin North Am 2014;52(2):355-82. DOI: 10.1016/j.rcl.2013.11.001.

4. CDC. Reported tuberculosis in the United States, 2018. (https://www.cdc.gov/tb/statistics/reports/2018/national_data.htm).

5. Bourgi K, Fiske C, Sterling TR. Tuberculosis Meningitis. Curr Infect Dis Rep 2017;19(11):39. DOI: 10.1007/s11908-017-0595-4.

6. Bottieau E, Noe A, Florence E, Colebunders R. Multiple tuberculous brain abscesses in an HIV-infected patient successfully treated with HAART and antituberculous treatment. Infection 2003;31(2):118-20. DOI: 10.1007/s15010-002-2121-2.

7. Qian X, Nguyen DT, Lyu J, Albers AE, Bi X, Graviss EA. Risk factors for extrapulmonary dissemination of tuberculosis and associated mortality during treatment for extrapulmonary tuberculosis. Emerg Microbes Infect 2018;7(1):102. DOI: 10.1038/s41426-018-0106-1.

8. Woldeamanuel YW, Girma B. A 43-year systematic review and meta-analysis: case-fatality and risk of death among adults with tuberculous meningitis in Africa. J Neurol 2014;261(5):851-65. DOI: 10.1007/s00415-013-7060-6.

9. Selby R, Ramirez CB, Singh R, et al. Brain abscess in solid organ transplant recipients receiving cyclosporine-based immunosuppression. Arch Surg 1997;132(3):304-10. DOI: 10.1001/archsurg.1997.01430270090019.

10. Baddley JW, Salzman D, Pappas PG. Fungal brain abscess in transplant recipients: epidemiologic, microbiologic, and clinical features. Clin Transplant 2002;16(6):419-24. DOI: 10.1034/j.1399-0012.2002.02033.x.

11. Coussement J, Lebeaux D, van Delden C, et al. Nocardia Infection in Solid Organ Transplant Recipients: A Multicenter European Case-control Study. Clin Infect Dis 2016;63(3):338-45. DOI: 10.1093/cid/ciw241.

12. Coussement J, Lebeaux D, Rouzaud C, Lortholary O. Nocardia infections in solid organ and hematopoietic stem cell transplant recipients. Curr Opin Infect Dis 2017;30(6):545-551. DOI: 10.1097/QCO.0000000000000404.

13. Robert-Gangneux F, Meroni V, Dupont D, et al. Toxoplasmosis in Transplant Recipients, Europe, 2010-2014. Emerging infectious diseases 2018;24(8):1497-1504. DOI: 10.3201/eid2408.180045.

14. Ramanan P, Scherger S, Benamu E, et al. Toxoplasmosis in non-cardiac solid organ transplant recipients: A case series and review of literature. Transplant infectious disease : an official journal of the Transplantation Society 2020;22(1):e13218. DOI: 10.1111/tid.13218.

15. Pereira MR, Rana MM, Practice AICo. Methicillin-resistant Staphylococcus aureus in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant 2019;33(9):e13611. DOI: 10.1111/ctr.13611.

16. Muccio CF, Caranci F, D'Arco F, et al. Magnetic resonance features of pyogenic brain abscesses and differential diagnosis using morphological and functional imaging studies: a pictorial essay. J Neuroradiol 2014;41(3):153-67. DOI: 10.1016/j.neurad.2014.05.004.

17. Saini J, Gupta RK, Jain KK. Intracranial infections: key neuroimaging findings. Semin Roentgenol 2014;49(1):86-98. DOI: 10.1053/j.ro.2013.09.001.

18. Kontzialis M, Zamora CA. Teaching NeuroImages: starry-sky appearance in Rocky Mountain spotted fever. Neurology 2015;85(12):e93. DOI: 10.1212/WNL.0000000000001959.

19. Weekly disease report for the week ending August 7th 2021. Michigan Disease Surveillance System. (https://www.michigan.gov/documents/mdch/Current_WSR_272689_7.pdf.).

20. van Veen KE, Brouwer MC, van der Ende A, van de Beek D. Bacterial meningitis in solid organ transplant recipients: a population-based prospective study. Transplant infectious disease : an official journal of the Transplantation Society 2016;18(5):674-680. DOI: 10.1111/tid.12570.

21. Bross JE, Gordon G. Nocardial meningitis: case reports and review. Rev Infect Dis 1991;13(1):160-5. DOI: 10.1093/clinids/12.5.160.

22. Gelfand JM, Genrich G, Green AJ, Tihan T, Cree BA. Encephalitis of unclear origin diagnosed by brain biopsy: a diagnostic challenge. JAMA Neurol 2015;72(1):66-72. DOI: 10.1001/jamaneurol.2014.2376.

23. Cailleaux M, Pilmis B, Mizrahi A, et al. Impact of a multiplex PCR assay (FilmArray(R)) on the management of patients with suspected central nervous system infections. Eur J Clin Microbiol Infect Dis 2020;39(2):293-297. DOI: 10.1007/s10096-019-03724-7.

24. O'Halloran JA, Franklin A, Lainhart W, Burnham CA, Powderly W, Dubberke E. Pitfalls Associated With the Use of Molecular Diagnostic Panels in the Diagnosis of Cryptococcal Meningitis. Open Forum Infect Dis 2017;4(4):ofx242. DOI: 10.1093/ofid/ofx242.

25. Walker M, Sheets J, Hamer D, O'Neal C, . Performance of the Biofire Filmarray Meningitis/Encephalitis Panel in Cryptococcal Meningitis Diagnosis. Open Forum Infect Dis 2018;5:1.

26. Simner PJ, Miller S, Carroll KC. Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases. Clin Infect Dis 2018;66(5):778-788. DOI: 10.1093/cid/cix881.

27. Wilson MR, Sample HA, Zorn KC, et al. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. N Engl J Med 2019;380(24):2327-2340. DOI: 10.1056/NEJMoa1803396.

28. Salzberg SL, Breitwieser FP, Kumar A, et al. Next-generation sequencing in neuropathologic diagnosis of infections of the nervous system. Neurol Neuroimmunol Neuroinflamm 2016;3(4):e251. DOI: 10.1212/NXI.0000000000000251.

29. Niles DT, Wijetunge DSS, Palazzi DL, Singh IR, Revell PA. Plasma Metagenomic Next-Generation Sequencing Assay for Identifying Pathogens: a Retrospective Review of Test Utilization in a Large Children's Hospital. Journal of clinical microbiology 2020;58(11). DOI: 10.1128/JCM.00794-20.

30. Britt RH, Enzmann DR, Yeager AS. Neuropathological and computerized tomographic findings in experimental brain abscess. J Neurosurg 1981;55(4):590-603. DOI: 10.3171/jns.1981.55.4.0590.

31. Ramachandran PS, Wilson MR. Metagenomics for neurological infections - expanding our imagination. Nat Rev Neurol 2020;16(10):547-556. DOI: 10.1038/s41582-020-0374-y.

32. Doern GV. Detection of selected fastidious bacteria. Clin Infect Dis 2000;30(1):166-73. DOI: 10.1086/313586.

33. Nelson CA, Saha S, Mead PS. Cat-Scratch Disease in the United States, 2005-2013. Emerging infectious diseases 2016;22(10):1741-6. DOI: 10.3201/eid2210.160115.

34. Batts S, Demers DM. Spectrum and treatment of cat-scratch disease. The Pediatric infectious disease journal 2004;23(12):1161-2. (https://www.ncbi.nlm.nih.gov/pubmed/15626957).

35. Windsor JJ. Cat-scratch disease: epidemiology, aetiology and treatment. Br J Biomed Sci 2001;58(2):101-10. (https://www.ncbi.nlm.nih.gov/pubmed/11440202).

36. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed 2013;98(6):236-8. DOI: 10.1136/archdischild-2013-304340.

37. Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol 2017;243:16-24. DOI: 10.1016/j.jbiotec.2016.12.022.

38. Dura-Trave T, Yoldi-Petri ME, Gallinas-Victoriano F, Lavilla-Oiz A, Bove-Guri M. Neuroretinitis Caused by Bartonella henselae (Cat-Scratch Disease) in a 13-Year-Old Girl. Int J Pediatr 2010;2010:763105. DOI: 10.1155/2010/763105.

39. Pappas G, Cascio A, Rodriguez-Morales AJ. The immunology of zoonotic infections. Clin Dev Immunol 2012;2012:208508. DOI: 10.1155/2012/208508.

40. Galindo-Bocero J, Sanchez-Garcia S, Alvarez-Coronado M, Rozas-Reyes P. Parinaud's oculoglandular syndrome: A case report. Arch Soc Esp Oftalmol 2017;92(1):37-39. DOI: 10.1016/j.oftal.2016.02.003.

41. Okaro U, Addisu A, Casanas B, Anderson B. Bartonella Species, an Emerging Cause of Blood-Culture-Negative Endocarditis. Clin Microbiol Rev 2017;30(3):709-746. DOI: 10.1128/CMR.00013-17.

42. Al-Matar MJ, Petty RE, Cabral DA, et al. Rheumatic manifestations of Bartonella infection in 2 children. J Rheumatol 2002;29(1):184-6. (https://www.ncbi.nlm.nih.gov/pubmed/11824958).

43. Fan J, Ali H. Cat scratch disease causing encephalitis. Proc (Bayl Univ Med Cent) 2020;33(3):440-441. DOI: 10.1080/08998280.2020.1756141.

44. Breitschwerdt E, Sontakke S, Hopkins S. Neurological Manifestations of Bartonellosis in Immunocompetent Patients: A Composite of Reports from 2005–2012. NOvember 4, 2012 (https://www.omicsonline.org/open-access/neurological-manifestations-of-bartonellosis-in-immunocompetent-patients-a-composite-of-reports-from-2314-7326-3-124.php?aid=15914).

45. Marra CM. Neurologic complications of Bartonella henselae infection. Curr Opin Neurol 1995;8(3):164-9. DOI: 10.1097/00019052-199506000-00002.

46. Wheeler SW, Wolf SM, Steinberg EA. Cat-scratch encephalopathy. Neurology 1997;49(3):876-8. DOI: 10.1212/wnl.49.3.876.

47. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. Bartonella spp. in pets and effect on human health. Emerging infectious diseases 2006;12(3):389-94. DOI: 10.3201/eid1203.050931.

TABLES

Table 1. Initial Laboratory Data

Test	Result	Reference Range
White blood cell count (K/uL)	13.5	4 - 11
Differential count (K/uL)		
Neutrophils	11.5	1.5 - 7.2
Lymphocytes	0.9	1.2 - 4.0
Monocytes	0.6	0.1 - 1.1
Eosinophils	0.3	0.0 - 0.5
Basophils	0.1	0.0 - 0.2

Platelet count (K/uL)	414	150 - 400
Hemoglobin (g/dL)	13.7	13.5 - 17
Hematocrit (%)	41.4	40 - 50
Sodium (mmol/L)	133	135-145
Potassium (mmol/L)	4.5	
Chloride (mmol/L)	102	
Bicarbonate (mmol/L)	24	
Blood Urea Nitrogen (mg/dL)	10	
Serum creatinine (mg/dL)	0.93	0.7 – 1.3
eGFR (mL/min/1.73m^2)	>90	>59
Aspartate aminotransferase (U/L)	29	8 - 30
Alanine aminotransferase (U/L)	26	=<35
Total bilirubin (mg/dL)	0.7	0.2 – 1.2
Urinalysis	Color: yellow Glucose: 300 Ketone: 10 No protein WBC: 2/HPF Nitrate: negative Leukocyte esterase: negative	
SARS-CoV-2 PCR	Negative	
Respiratory viral panel	Negative	
Blood cultures	Drawn and pending	

Table 2. Common neurologic syndromes in SOT[1-3]

Neurologic Syndrome[1]	**Examples- Infectious** **Non-infectious**
Leuko-encephalopathy[2]	Viral : PML- JC virus, Human herpes viruses, West Nile virus
	Immune reconstitution inflammatory syndrome (IRIS) Posterior reversible encephalopathy syndrome (PRES) Acute disseminated encephalomyelitis (ADEM) Due to medications such as cyclosporine, tacrolimus, rituximab, amphotericin B Pontine/extrapontine osmotic demyelination Radiation injury, many cancer chemotherapies
Stroke(s)	Viral : VZV CMV endocarditis with emboli vasculitis post-meningitis Fungal: *Aspergillus, Mucor spp*.
	Radiation-related arteriopathy Non-bacterial thrombotic endocarditis CNS vasculitis
Limbic encephalitis	Viral: HSV 1 and 2, HHV-6
	Hashimoto encephalopathy Autoimmune (paraneoplastic) syndromes (NMDAR) Repetitive seizures
Brainstem	Bacterial: *Listeria monocytogenes* Fungal: *Cryptococcus neoformans* Viral: VZV, PML-JCV

	Wernicke encephalopathy Osmotic demyelination PRES radiation necrosis

Table 3. Timeline for CNS infection in SOT[1]

Phase	**CNS Infection**	**Comment**
Postoperative (1-4 weeks)	Opportunistic CNS infection unusual	Exceptions: Intense exposure peri-op (e.g., Aspergillosis from hospital construction) Donor derived viral infections
Early CNS Syndromes (1-6 months)	*Listeria monocytogenes* *Toxoplasma gondii* *Nocardia* species Herpesviruses *Aspergillus* species (median 6 months) Other molds *Cryptococcus neoformans* Endemic fungi *Mycobacterium tuberculosis*	Peak time for opportunistic CNS infections Routine prophylaxis likely impacts CNS infections. e.g., TMP-SMX has activity against *Listeria, Nocardia spp , T. gondii*

Late CNS syndromes (> 6 mos.)	Herpesviruses Progressive multifocal leukoencephalopathy (median 12 months) Bacteria: *S. pneumoniae*, brain abscess *Aspergillus* Mucorales (12 months) and other molds Endemic fungi (median 12 months) *Cryptococcus* spp.(median 19 months)	Pathogens similar to early CNS syndromes, less common e.g.,TB, toxoplasmosis may be delayed CNS posttransplant lymphoproliferative disorder (PTLD): EBV (+) median 11.5 months EBV (-) median 69 months

Table 4. Fastidious bacteria

"Classic" Fastidious Bacteria[1]	Newer/Other
**Legionella spp*	*Abiotrophia/Granulicatella* (Formerly nutritionally variant streptococci)
**Brucella*	**Coxiella burnetii* (Q fever)
**Francisella tularensis* (Tularemia) Susceptible to B-lactams *in vitro* but clinical failures	*Tropheryma whipplei* (Whipple's disease)
Borrelia burgdorferi (Lyme)	HACEK organisms– previously considered fastidious. Modern blood culture instruments detect organisms < 5 days. Grow in routine media.
Leptospira	*Nocardia spp*. – difficult to visualize and requires modified acid-fast stain. Grows readily on several routine laboratory media
Bartonella (Cat scratch disease)	*Erysipelothrix rhusiopathiae*– some do not consider this fastidious
Bordetella pertussis (pertussis)	

*** = unlikely to respond to ceftriaxone**

Table 5. Fastidious bacteria with *in vitro* susceptibility to ceftriaxone, with selected pros and cons related to the case

Fastidious Bacteria	Comments
Abiotrophia, Granulicatella (Formerly nutritionally variant *Streptococci)*	**PRO**: known cause of acute bacterial meningitis, including one report in immunocompromised pt. One case with CSF WBC > 6000/mm^3. Recognized as a cause of culture negative endocarditis, a potential source of multifocal brain abscesses. **CON**: most meningitis cases are post-neurosurgical. No brain abscess reported.
Borrelia burgdorferi (Lyme disease)	**PRO**: neuroborreliosis well recognized, aseptic meningitis during disseminated phase. Numerous CNS manifestations. One report of rim enhancing lesion with surrounding edema. Ceftriaxone is drug of choice for CNS Lyme. Increasing cases reported in Michigan; 565 in 2020 (previous years 300-400, Michigan Disease Surveillance System) **CON**: No history of tick bite, erythema chronicum migrans rash, arthritis. No reports of multifocal brain abscesses. Presentation in SOT unknown
Tropheryma whipplei (Whipple's disease)	**PRO:** CNS manifestations common. Isolated CNS infection well described. MRI with a variety of abnormalities including enhancing focal lesions, mass-like lesions, multiple diffuse cerebral lesions. CSF pleocytosis > 300 cells/mm^3 reported. Long periods of asymptomatic latency reported. Ceftriaxone drug of choice **CON**: CSF WBCs usually low, lymphocytic. Isolated CNS disease uncommon. Usually chronic infection. Brain abscesses not reported. Pt did not have oculomasticatory myorhythmias, gaze palsy or other characteristic findings. Several case reports in SOT
Bartonella (Cat scratch disease)	**PRO:** variety of neurologic manifestations of *B. henselae* described. Bartonella encephalitis well recognized; case reports of aspetic meningitis. *B. henselae* known cause of culture negative endocarditis and CNS lesions may be related. **CONS:** no exposure to cats (not always necessary). Reported cases are encephalitis rather than acute meningitis. No reports of brain abscess.

Leptospira	**PRO**: can occur as an isolated CNS infection, neuroleptospirosis. Aseptic meningitis, but usually lymphocytic. Seizures. Parenchymal brain lesions reported. Spirochetes may be difficult to visualize on brain biopsy. Case report of brain biopsy of meninges with chronic T cell infiltrates. In 2020, four cases reported in Michigan **CONS**: isolated neurologic disease rare. No conjunctival suffusion, myalgias, jaundice, renal injury, bleeding. SOTS cases- 5 in total, all with Weil's syndrome (no CNS disease). Organism can be seen on biopsy with silver stain. No cases of brain abscess reported.
Erysipelothrix rhusiopathiae	**PRO**: pus former. Rare cause acute meningitis, with or without endocarditis. Immunocompromised state associated with severe disease and endocarditis. One case report of multiple brain infarcts. **CON:** no occupational exposure (but in several series, some do not have occupational or animal exposure). No cases of brain abscess. Organism can potentially grow in routine culture
Bordetella pertussis	***CON:*** *B. pertussis* not a cause of meningitis or brain abscess *B. hinzii* reported: meningitis in renal transplant– not fastidious

Table 6. Additional Diagnostic Tests

Test	**Result**	**Reference Range**
Blood cultures	Negative	
COVID-19 by PCR	Negative	
Respiratory viral panel	Negative	
Serum *Histoplasma* antigen (ng/mL)	Not detected	0.4 - 19
Serum *Toxoplasma* PCR (copies/mL)	Not detected	376 - 1.00 x10^8
Serum *Aspergillus* antigen [galactomannan] (index)	<0.500	<0.5
Serum *Cryptococcus neoformans* antigen	Not detected	
Plasma CMV PCR	Not detected	
Plasma EBV PCR	Not detected	
Serum beta-d-glucan (pg/mL)	<31	<80
Serum cysticercosis antibody IgG	Negative	
Urine *Legionella pneumophila* serogroup 1 antigen	Negative	
Urine *Streptococcus pneumoniae* antigen	Negative	
Urine *Histoplasma* antigen	Negative	
CSF analysis Leukocytes Protein Glucose	 1005 (80% Neutrophils, 5% Lymphocytes) 186 63	
CSF culture and gram stain	Negative	

CSF fungal stain and culture	Negative	
CSF viral panel	Not detected	
CSF *Cryptococcus neoformans* antigen	Not detected	
CSF acid fast bacilli stain and culture	Negative	
CSF beta-d-glucan (pg/mL)	<60	<60
CSF *Aspergillus* antigen [galactomannan] (index)	0.040	<0.500
CSF EBV PCR (IU/ml)	Not detected	52 - 1.69 x 10^8
CSF *Toxoplasma* PCR (copies/mL)	Not detected	183 - 1.00 x10^8
CSF Arbovirus IgM	Not detected	

Table 7. Bartonella PCR and Serology

Test	Results	Reference Range
Bartonella PCR	Negative	
Bartonella henselae IgG	**1:1024**	<1:128 titer
Bartonella henselae IgM	< 1:20	< 1:20 titer
Bartonella quintana IgG	<1:128	<1:128 titer
Bartonella quintana IgM	<1:20	<1:20 titer

PCR: Polymerase Chain Reaction; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

FIGURES

Figure 1. Computed Tomography of the Brain

Computed tomography of the brain showed multifocal areas of hypodensity predominantly involving the subcortical white matter of both cerebral hemispheres, cerebellar hemispheres, and right thalamus

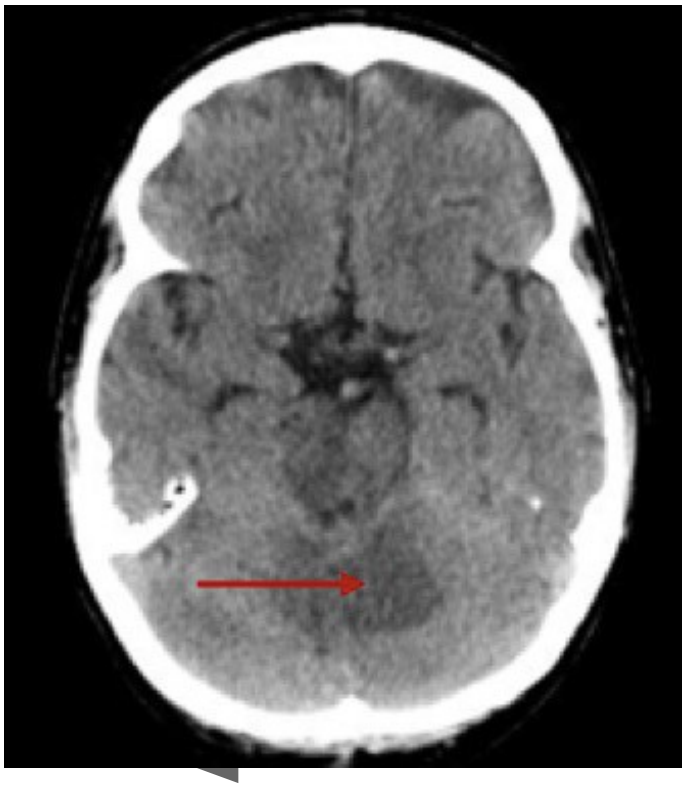
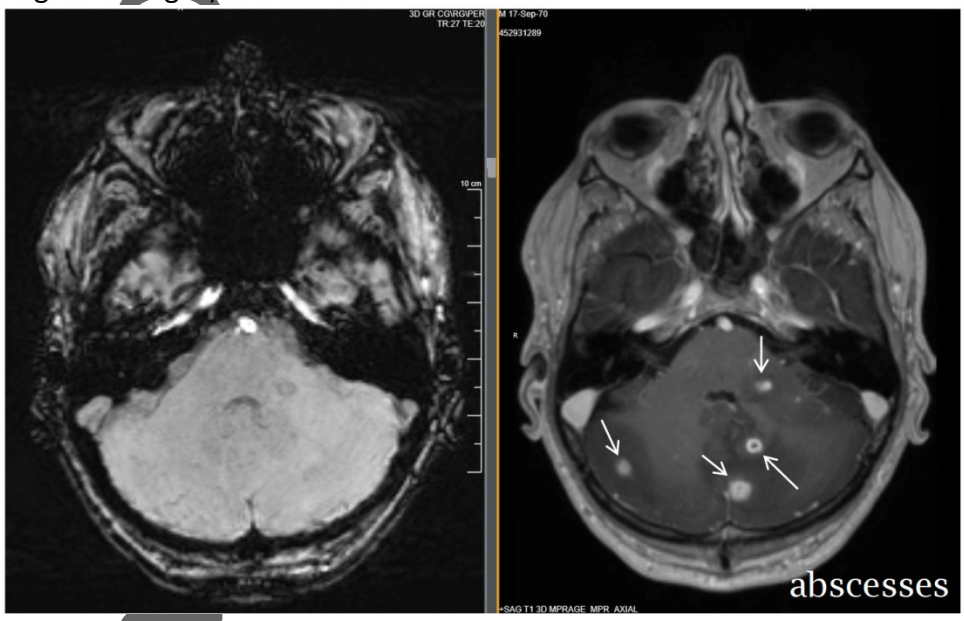
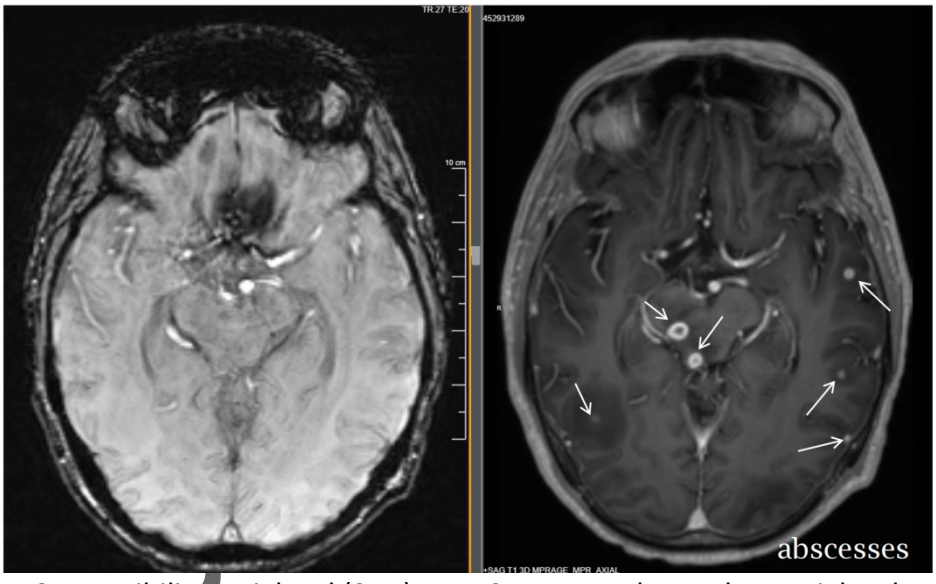


Figure 2. Initial MRI of the Brain (Hospital Day 2)

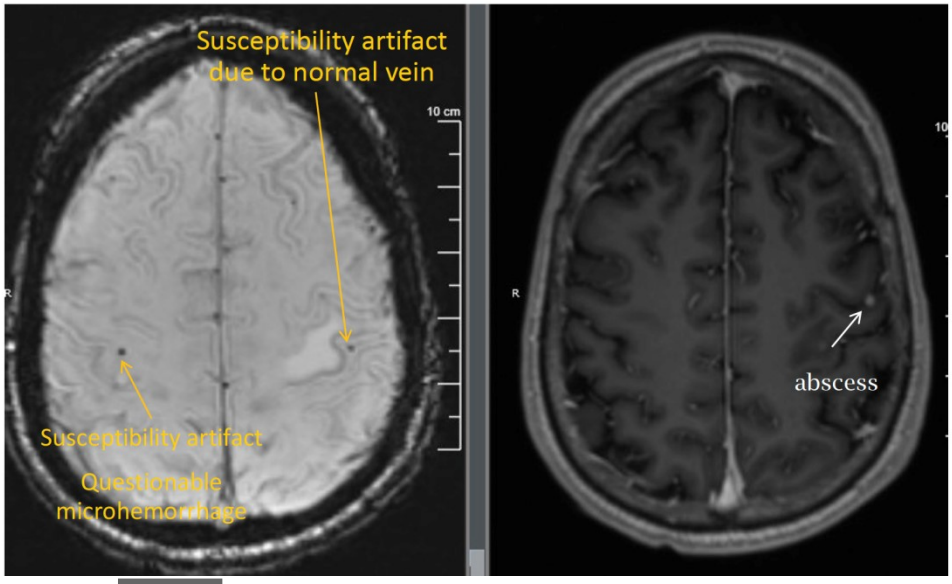
The MRI of brain demonstrated Innumerable bilateral scattered supratentorial, infratentorial, and brainstem ring-enhancing lesions (arrows on contrast enhanced T1 weighted images)



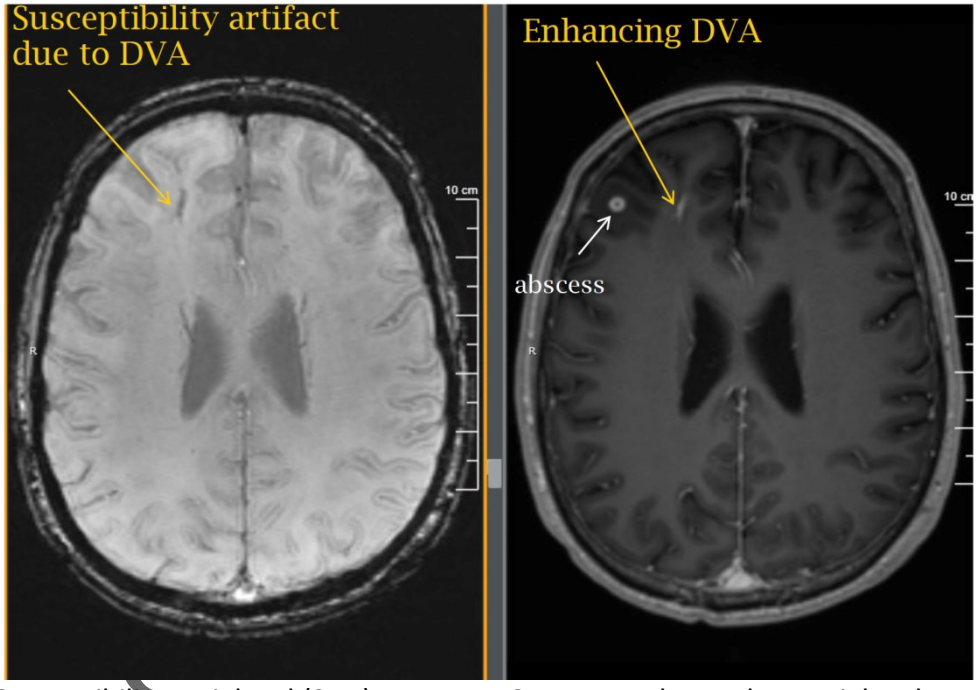
Susceptibility weighted (SWI) Contrast enhanced T1 weighted



Susceptibility weighted (SWI) Contrast enhanced T1 weighted



Susceptibility weighted (SWI) Contrast enhanced T1 weighted



Susceptibility weighted (SWI) Contrast enhanced T1 weighted

Figure 3. MRI appearance of typical bacterial brain abscess[17]
Panel A. T2 weighted image showing space occupying lesion with hyperintense (bright) central signal, with hypointense (dark) surrounding rim.

Panel B. T1 weighted image with contrast. Lesion with contrast "rim" enhancement.
Panel C. Diffusion weighted image. Diffusion restriction in center and rim of lesion.
Reproduced from: *Saini J, Gupta RK, Jain KK. Intracranial infections: key neuroimaging findings. Semin Roentgenol 2014;49(1):86-98 published by Elsevier (License 5247731021328). All rights reserved*

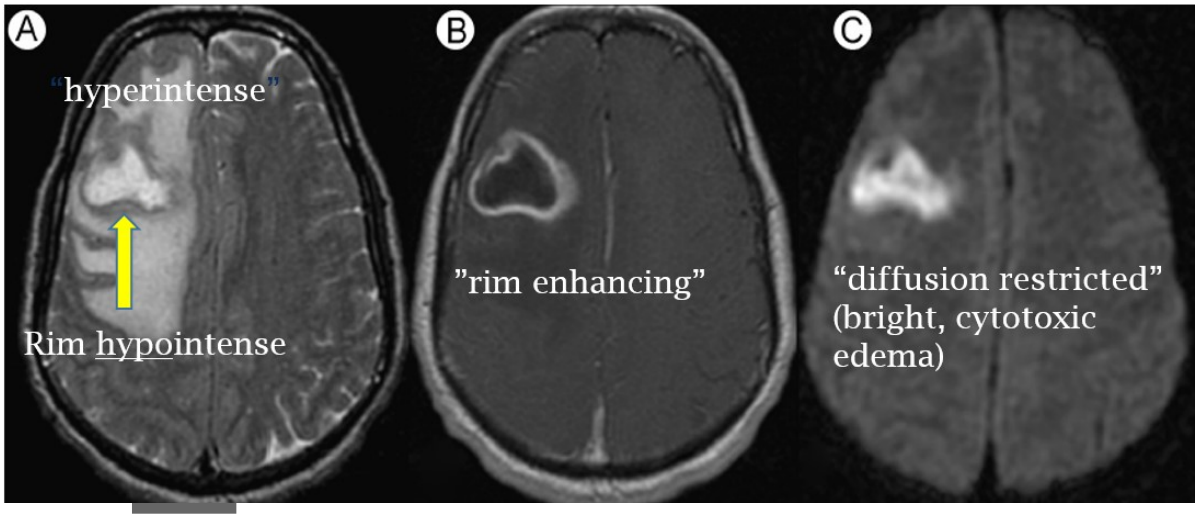
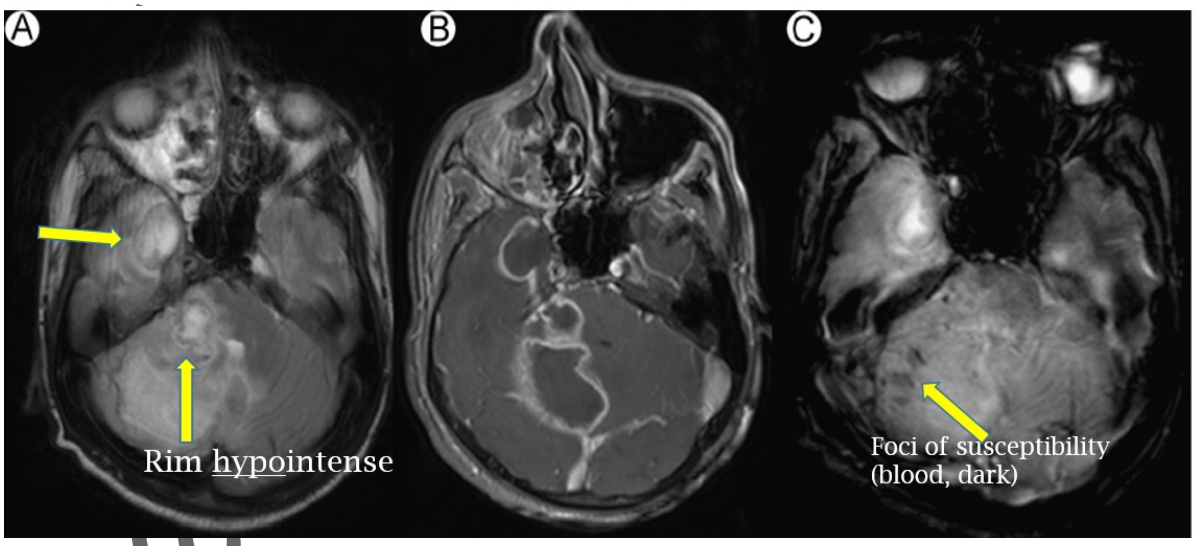


Figure 4. MRI appearance of typical fungal brain abscesses[16]
Panel A. T2 weighted image with hypointense (dark) rim around two fungal abscesses.
Panel B. T1 weighted image with contrast, showing rim enhancement.

Panel C. Gradient recalled echo (GRE) sequence, demonstrating foci of susceptibility consistent with blood.

Panel D. Diffusion weighted image, showing restricted diffusion involving the rim, and no restriction in the center of the lesion.

Reproduced from: *Saini J, Gupta RK, Jain KK. Intracranial infections: key neuroimaging findings. Semin Roentgenol 2014;49(1):86-98 published by Elsevier (License 5247731021328). All rights reserved*



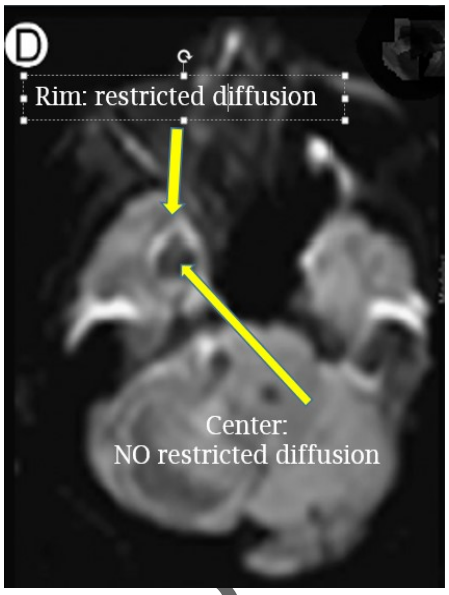
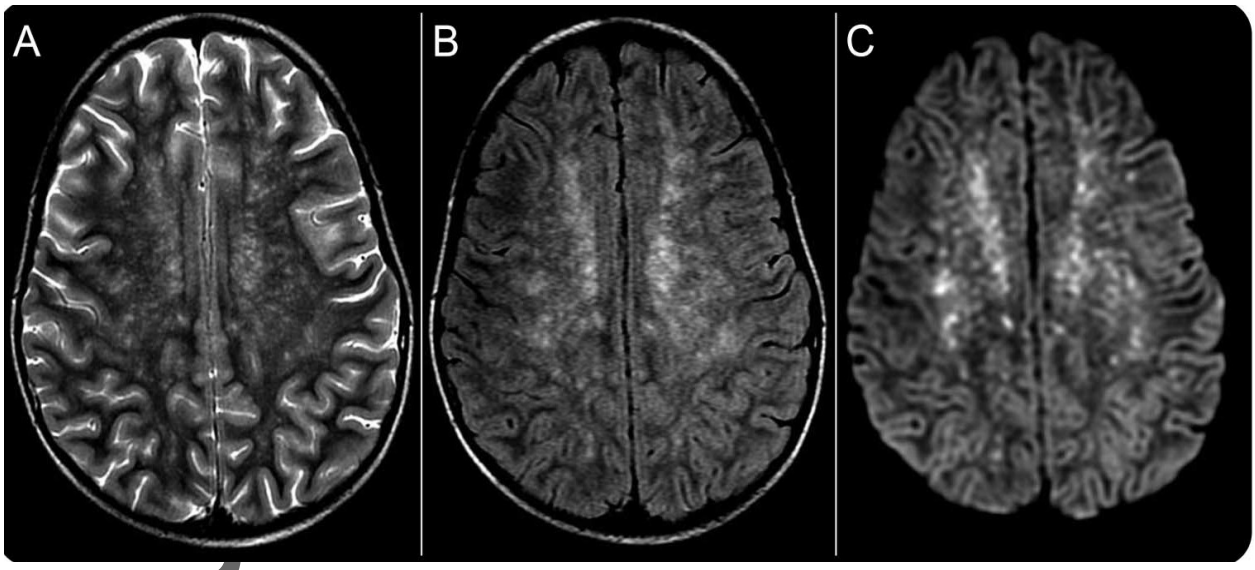


Figure 5. MRI scan in Rocky Mountain spotted fever, with the "starry sky" pattern[18]



Reproduced from *Kontzialis M, Zamora CA. Teaching NeuroImages: starry-sky appearance in Rocky Mountain spotted fever. Neurology 2015;85(12):e93 published by Wolters Kluwer Health, Inc (License 5247761192322). All rights reserved*

Figure 6. Subsequent MRI Brain (Hospital Day 13)
Panel A and B. T1-weighted MR brain showing innumerable bilateral scattered supratentorial, infratentorial lesions (red arrows).
Panel C. T2-weighted MR brain showing hyperintense signal surrounding the lesions that is consistent with surrounding edema (red arrow).

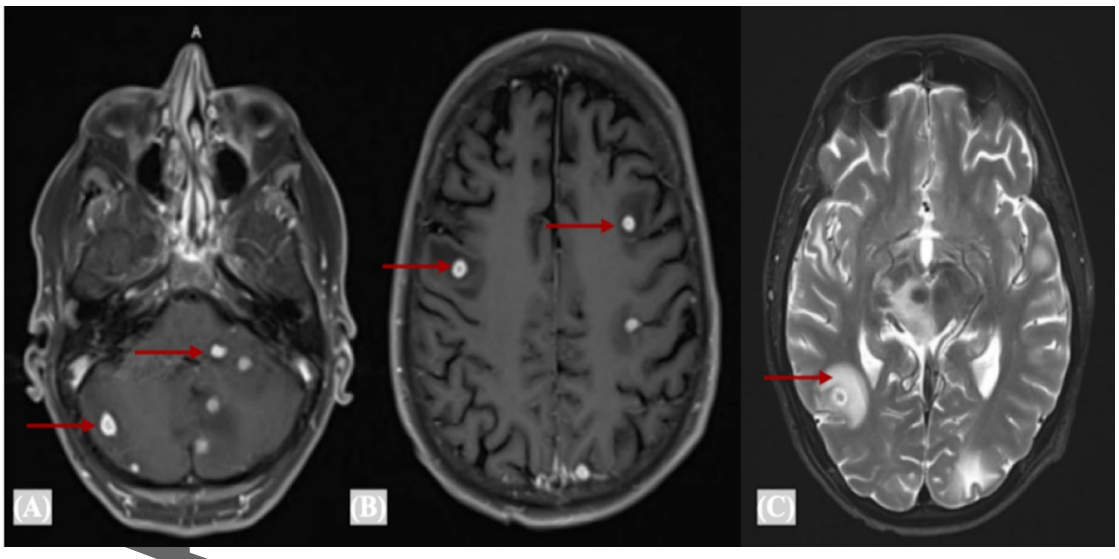


Figure 7. Brain tissue Histopathology

Panel A. Hematoxylin and eosin staining showing a mixed inflammatory infiltrate in a fibrous background; acute neutrophilic inflammation is not evident (Panel A).

Panel B and C. Inflammation is composed of T-cells: CD3 (Panel B) and numerous macrophages: CD68 (Panel C).

Panel D. Movat staining demonstrated diffuse, acute collagen deposition, often not seen in the brain but consistent with abscess wall

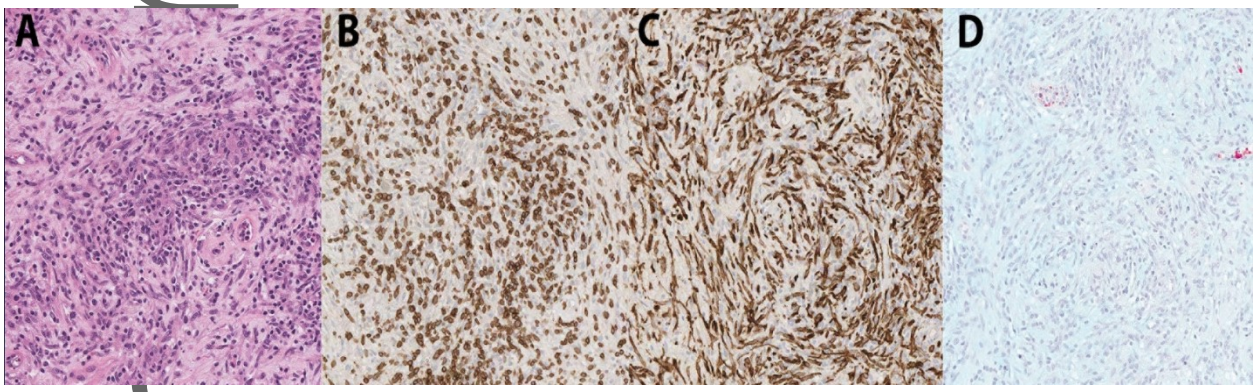
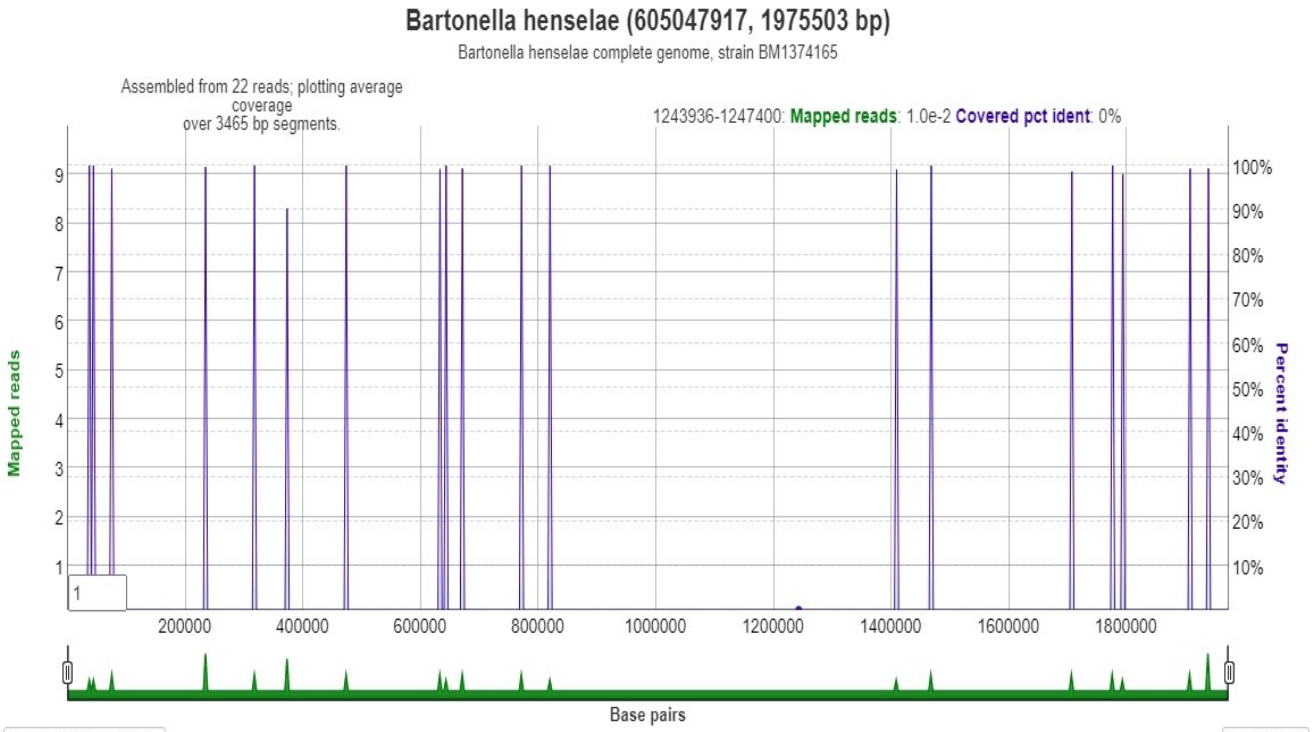


Figure 8. Metagenomic Next Generation Sequencing Coverage map



	Reference length bp	Coverage in bp	Percent coverage	Avg coverage depth	Covered pct ident
Overall	1975503	2530	0.13	0.00	99.09
Displayed	1975503	2530	0.13	0.00	99.09

Test	Result	Reference Range
White blood cell count (K/uL)	13.5	4 - 11
Differential count (K/uL)		
Neutrophils	11.5	1.5 - 7.2
Lymphocytes	0.9	1.2 - 4.0
Monocytes	0.6	0.1 - 1.1
Eosinophils	0.3	0.0 - 0.5
Basophils	0.1	0.0 - 0.2
Platelet count (K/uL)	414	150 - 400
Hemoglobin (g/dL)	13.7	13.5 - 17
Hematocrit (%)	41.4	40 - 50
Sodium (mmol/L)	133	135-145
Potassium (mmol/L)	4.5	
Chloride (mmol/L)	102	
Bicarbonate (mmol/L)	24	
Blood Urea Nitrogen (mg/dL)	10	
Serum creatinine (mg/dL)	0.93	0.7 – 1.3
eGFR (mL/min/1.73m^2)	>90	>59
Aspartate aminotransferase (U/L)	29	8 - 30
Alanine aminotransferase (U/L)	26	=<35
Total bilirubin (mg/dL)	0.7	0.2 – 1.2

Urinalysis	Color: yellow Glucose: 300 Ketone: 10 No protein WBC: 2/HPF Nitrate: negative Leukocyte esterase: negative	
SARS-CoV-2 PCR	Negative	
Respiratory viral panel	Negative	
Blood cultures	Drawn and pending	

Neurologic Syndrome[1]	**Examples- Infectious** **Non-infectious**
Leuko-encephalopathy[2]	Viral : PML- JC virus, Human herpes viruses, West Nile virus
	Immune reconstitution inflammatory syndrome (IRIS) Posterior reversible encephalopathy syndrome (PRES) Acute disseminated encephalomyelitis (ADEM) Due to medications such as cyclosporine, tacrolimus, rituximab, amphotericin B Pontine/extrapontine osmotic demyelination Radiation injury, many cancer chemotherapies
Stroke(s)	Viral : VZV CMV endocarditis with emboli vasculitis post-meningitis Fungal: *Aspergillus, Mucor spp*.
	Radiation-related arteriopathy Non-bacterial thrombotic endocarditis CNS vasculitis
Limbic encephalitis	Viral: HSV 1 and 2, HHV-6
	Hashimoto encephalopathy Autoimmune (paraneoplastic) syndromes (NMDAR) Repetitive seizures
Brainstem	Bacterial: *Listeria monocytogenes* Fungal: *Cryptococcus neoformans* Viral: VZV, PML-JCV
	Wernicke encephalopathy Osmotic demyelination PRES radiation necrosis

Phase	CNS Infection	Comment
Postoperative (1-4 weeks)	Opportunistic CNS infection unusual	Exceptions: Intense exposure peri-op (e.g., Aspergillosis from hospital construction) Donor derived viral infections
Early CNS Syndromes (1-6 months)	*Listeria monocytogenes* *Toxoplasma gondii* *Nocardia* species Herpesviruses *Aspergillus* species (median 6 months) Other molds *Cryptococcus neoformans* Endemic fungi *Mycobacterium tuberculosis*	Peak time for opportunistic CNS infections Routine prophylaxis likely impacts CNS infections. e.g., TMP-SMX has activity against *Listeria, Nocardia spp , T. gondii*
Late CNS syndromes (> 6 mos.)	Herpesviruses Progressive multifocal leukoencephalopathy (median 12 months) Bacteria: *S. pneumoniae*, brain abscess *Aspergillus* Mucorales (12 months) and other molds Endemic fungi (median 12 months) *Cryptococcus* spp.(median 19 months)	Pathogens similar to early CNS syndromes, less common e.g.,TB, toxoplasmosis may be delayed CNS posttransplant lymphoproliferative disorder (PTLD): EBV (+) median 11.5 months EBV (-) median 69 months

"Classic" Fastidious Bacteria[1]	Newer/Other
**Legionella spp*	*Abiotrophia/Granulicatella* (Formerly nutritionally variant streptococci)
**Brucella*	**Coxiella burnetii* (Q fever)
**Francisella tularensis* (Tularemia) Susceptible to B-lactams *in vitro* but clinical failures	*Tropheryma whipplei* (Whipple's disease)

Borrelia burgdorferi (Lyme)	HACEK organisms– previously considered fastidious. Modern blood culture instruments detect organisms < 5 days. Grow in routine media.
Leptospira	*Nocardia spp*. – difficult to visualize and requires modified acid-fast stain. Grows readily on several routine laboratory media
Bartonella (Cat scratch disease)	*Erysipelothrix rhusiopathiae*– some do not consider this fastidious
Bordetella pertussis (pertussis)	

Fastidious Bacteria	**Comments**
Abiotrophia, *Granulicatella* (Formerly nutritionally variant *Streptococci)*	**PRO**: known cause of acute bacterial meningitis, including one report in immunocompromised pt. One case with CSF WBC > 6000/mm^3. Recognized as a cause of culture negative endocarditis, a potential source of multifocal brain abscesses. **CON**: most meningitis cases are post-neurosurgical. No brain abscess reported.
Borrelia burgdorferi (Lyme disease)	**PRO**: neuroborreliosis well recognized, aseptic meningitis during disseminated phase. Numerous CNS manifestations. One report of rim enhancing lesion with surrounding edema. Ceftriaxone is drug of choice for CNS Lyme. Increasing cases reported in Michigan; 565 in 2020 (previous years 300-400, Michigan Disease Surveillance System) **CON**: No history of tick bite, erythema chronicum migrans rash, arthritis. No reports of multifocal brain abscesses. Presentation in SOT unknown
Tropheryma whipplei (Whipple's disease)	**PRO:** CNS manifestations common. Isolated CNS infection well described. MRI with a variety of abnormalities including enhancing focal lesions, mass-like lesions, multiple diffuse cerebral lesions. CSF pleocytosis > 300 cells/mm^3 reported. Long periods of asymptomatic latency reported. Ceftriaxone drug of choice **CON**: CSF WBCs usually low, lymphocytic. Isolated CNS disease uncommon. Usually chronic infection. Brain abscesses not reported. Pt did not have oculomasticatory myorhythmias, gaze palsy or other characteristic findings. Several case reports in SOT

Bartonella (Cat scratch disease)	**PRO:** variety of neurologic manifestations of *B. henselae* described. Bartonella encephalitis well recognized; case reports of aspetic meningitis. *B. henselae* known cause of culture negative endocarditis and CNS lesions may be related. **CONS:** no exposure to cats (not always necessary). Reported cases are encephalitis rather than acute meningitis. No reports of brain abscess.
Leptospira	**PRO**: can occur as an isolated CNS infection, neuroleptospirosis. Aseptic meningitis, but usually lymphocytic. Seizures. Parenchymal brain lesions reported. Spirochetes may be difficult to visualize on brain biopsy. Case report of brain biopsy of meninges with chronic T cell infiltrates. In 2020, four cases reported in Michigan **CONS**: isolated neurologic disease rare. No conjunctival suffusion, myalgias, jaundice, renal injury, bleeding. SOTS cases- 5 in total, all with Weil's syndrome (no CNS disease). Organism can be seen on biopsy with silver stain. No cases of brain abscess reported.
Erysipelothrix rhusiopathiae	**PRO**: pus former. Rare cause acute meningitis, with or without endocarditis. Immunocompromised state associated with severe disease and endocarditis. One case report of multiple brain infarcts. **CON:** no occupational exposure (but in several series, some do not have occupational or animal exposure). No cases of brain abscess. Organism can potentially grow in routine culture
Bordetella pertussis	***CON:*** *B. pertussis* not a cause of meningitis or brain abscess *B. hinzii* reported: meningitis in renal transplant– not fastidious

Test	**Result**	**Reference Range**
Blood cultures	Negative	
COVID-19 by PCR	Negative	
Respiratory viral panel	Negative	
Serum *Histoplasma* antigen (ng/mL)	Not detected	0.4 - 19
Serum *Toxoplasma* PCR (copies/mL)	Not detected	376 - 1.00 x10^8
Serum *Aspergillus* antigen [galactomannan] (index)	<0.500	<0.5
Serum *Cryptococcus neoformans* antigen	Not detected	
Plasma CMV PCR	Not detected	
Plasma EBV PCR	Not detected	
Serum beta-d-glucan (pg/mL)	<31	<80
Serum cysticercosis antibody IgG	Negative	
Urine *Legionella pneumophila* serogroup 1 antigen	Negative	
Urine *Streptococcus pneumoniae* antigen	Negative	

Urine *Histoplasma* antigen	Negative	
CSF analysis Leukocytes Protein Glucose	 1005 (80% Neutrophils, 5% Lymphocytes) 186 63	
CSF culture and gram stain	Negative	
CSF fungal stain and culture	Negative	
CSF viral panel	Not detected	
CSF *Cryptococcus neoformans* antigen	Not detected	
CSF acid fast bacilli stain and culture	Negative	
CSF beta-d-glucan (pg/mL)	<60	<60
CSF *Aspergillus* antigen [galactomannan] (index)	0.040	<0.500
CSF EBV PCR (IU/ml)	Not detected	52 - 1.69 x 10^8
CSF *Toxoplasma* PCR (copies/mL)	Not detected	183 - 1.00 x10^8
CSF Arbovirus IgM	Not detected	

Test	**Results**	**Reference Range**
Bartonella PCR	Negative	
Bartonella henselae IgG	**1:1024**	<1:128 titer
Bartonella henselae IgM	< 1:20	< 1:20 titer
Bartonella quintana IgG	<1:128	<1:128 titer
Bartonella quintana IgM	<1:20	<1:20 titer