TRANSPLANT
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CLINICO-PATHOLOGIC CONFERENCE

Into the unknown: Diagnosing mysterious brain lesions

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Abstract

In this inaugural clinicopathological conference, the invited experts discussed the diagnostic approach to central nervous system infections in immunocompromised hosts. The case presented involved a pancreas-kidney transplant recipient with multiple brain abscesses caused by Bartonella henselae. CSF metagenomic next-generation sequencing played a significant role in the diagnosis. Bartonella henselae is a gram-negative zoonotic pathogen that causes cat-scratch disease, which can be transmitted to humans through cat bites or scratches. Symptoms can vary in severity, correlating with the patient's immune status. Visceral organ involvement, ocular involvement, and neurological manifestations have been reported in immunocompromised patients, but brain abscesses are rare.

KEYWORDS
Bartonella henselae, brain abscess, organ transplant

1 | 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 3-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/min, blood pressure of 122/74 mmHg, respiratory rate 16 breaths/min, 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/μl. Laboratory test results are shown in Table 1.

Chest radiograph showed no focal opacities. Urinalyis 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).

2 | 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.

TABLE 1 Initial laboratory data

Test	Result	Reference range
WBC count (K/μl)	13.5	4–11
Differential count (K/μl)		
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/μl)	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/1.73 m^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	

Abbreviations: eGFR, estimated glomerular filtration rate; HPF, high power field; PCR, polymerase chain reaction; SARS-CoV-2, S Acute Respiratory Syndrome Coronavirus 2; WBC, white blood cell.

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

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 1–4, CNS infection is uncommon. One exception includes those who have had intense exposure preoperatively 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 post-transplant 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

TABLE 2 Common neurologic syndromes in solid organ transplant (SOT)[1–3]

Neurologic syndrome[1]	Examples: infectious non-infectious
Leukoencephalopathy[2]	Viral: PML-JCV, HHV, West Nile virus
	IRIS PRES 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 spp, 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

Abbreviations: ADEM, acute disseminated encephalomyelitis; CMV, cytomegalovirus; CNS, central nervous system; HHV, human herpes viruses; HSV, herpes simplex virus; IRIS, immune reconstitution inflammatory syndrome; JCV, John Cunnigham virus; NMDAR, N-methyl-D-aspartate receptor; PML, progressive multifocal leukoencephalopathy; PRES, posterior reversible encephalopathy syndrome; VZV, varicella zoster virus.

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 tuberculosis (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.

3 | CLINICAL COURSE (IHAB KASSAB)

The patient was given empiric intravenous antibiotic regimen of ceftriaxone 2 g every 12 h, vancomycin 13 mg/kg every 8 h, and ampicillin 2 g every 4 h. 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).

## 4 | INTERPRETATION OF FINDINGS AND DIFFERENTIAL DIAGNOSIS (CARLOS ISADA)

The MRI scan shows small rim-enhancing lesions consistent with parenchymal brain abscesses. The most likely etiologies are bacterial, fungal, and parasitic. The appearance virtually excludes viral and rickettsial causes. It should be noted that Epstein–Barr virus (EBV)-related post-transplant 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 five 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 *T. 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

TABLE 3 Timeline for central nervous system (CNS) infection in solid organ transplant (SOT)[1]

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

Abbreviations: EBV, Epstein–Barr virus; PTLD, post-transplant lymphoproliferative disorder; TB, tuberculosis; TMP-SMX, trimethoprim-sulfamethoxazole.
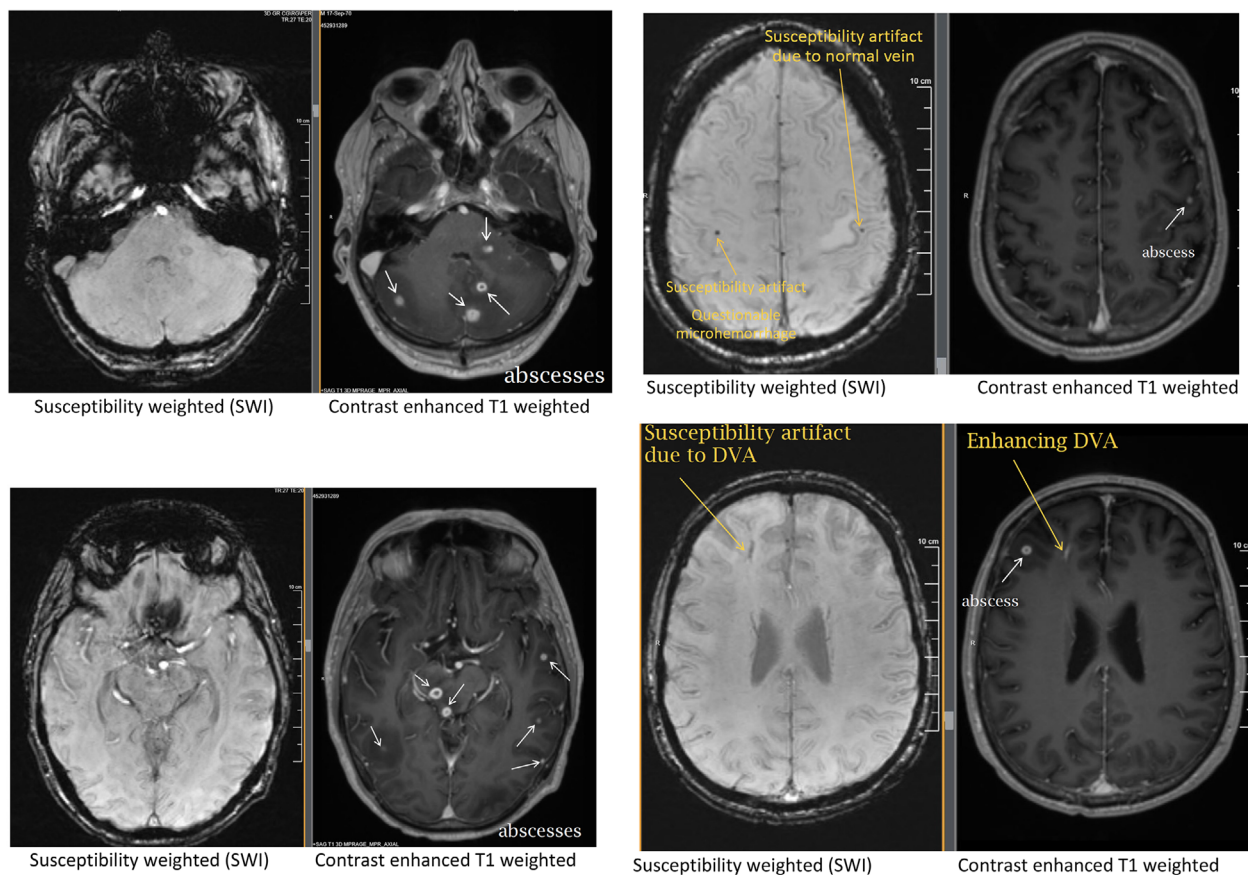
FIGURE 2 Initial magnetic resonance imaging (MRI) of the brain (hospital day 2). The MRI of brain demonstrated innumerable bilateral scattered supratentorial, infratentorial, and brainstem ring-enhancing lesions (arrows on contrast enhanced T1-weighted images)
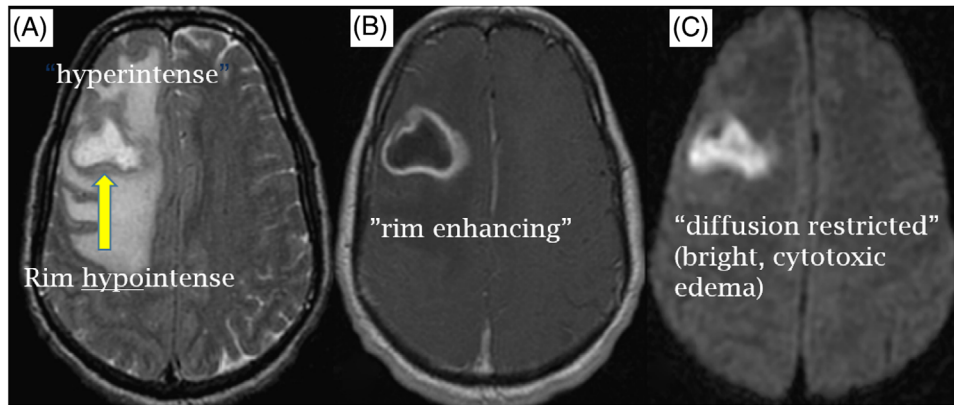
FIGURE 3 Magnetic resonance imaging (MRI) appearance of typical bacterial brain abscess.[17] (A) T2-weighted image showing space occupying lesion with hyperintense (bright) central signal, with hypointense (dark) surrounding rim. (B) T1-weighted image with contrast. Lesion with contrast "rim" enhancement. (C) Diffusion-weighted image. Diffusion restriction in center and rim of lesion. Reproduced from Ref. 17 and published by Elsevier (License 5247731021328). All rights reserved
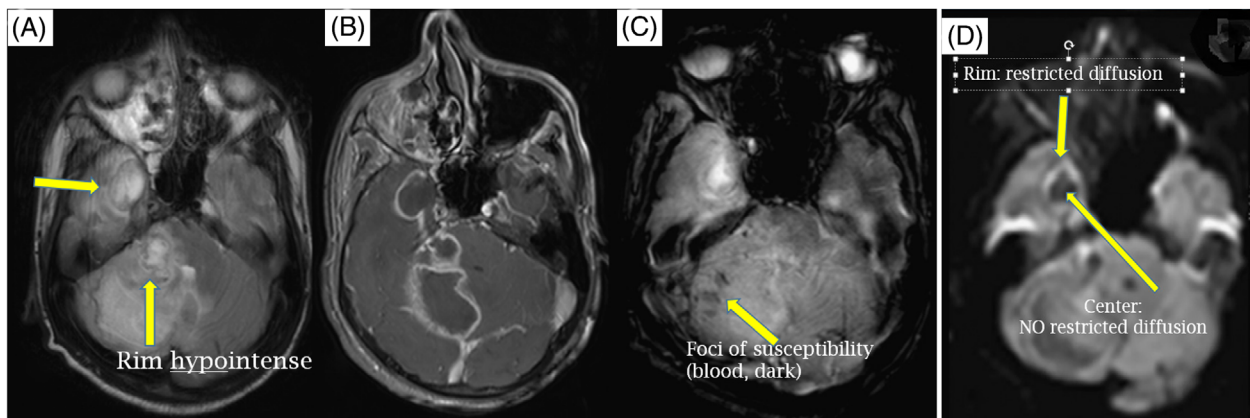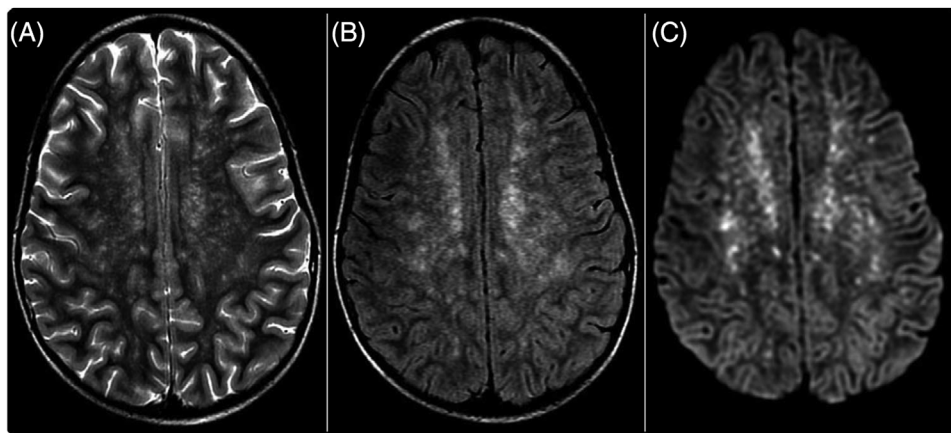

FIGURE 4 Magnetic resonance imaging (MRI) appearance of typical fungal brain abscesses.[16] (A) T2-weighted image with hypointense (dark) rim around two fungal abscesses. (B) T1-weighted image with contrast, showing rim enhancement. (C) Gradient recalled echo (GRE) sequence, demonstrating foci of susceptibility consistent with blood. (D) Diffusion-weighted image, showing restricted diffusion involving the rim, and no restriction in the center of the lesion. Reproduced from Ref. 17 and published by Elsevier (License 5247731021328). All rights reserved

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 among 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, TB, and EBV-related PTLD. Other MRI sequences have been reported including apparent diffusion coefficient, 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 (SWIs) 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 SWIs.

FIGURE 5 Magnetic resonance imaging (MRI) scan in Rocky Mountain spotted fever, with the "starry sky" pattern.[18] Reproduced from Ref. 18 and published by Wolters Kluwer Health, Inc. (License 5247761192322). All rights reserved

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). *Cryptococcus neoformans* can rarely present with meningitis along with solid tumor-like intracranial masses. None of these seem to be present in 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 six cases were identified out of approximately 11 000 transplant patients in the Netherlands from 2004 to 2016. All were renal transplant recipients. Microbial etiologies were *Streptococcus pneumoniae* (two cases), *Listeria monocytogenes* (two), *Escherichia coli* (one), and *Pseudomonas aeruginosa* (one). The mean leukocyte counts in CSF were 713 cells/mm^3 with a range 17–12 014 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 is common even in the immunocompromised, and for several the presentation would be highly atypical.

5 | 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 have not been well defined in immunocompromised and transplant patients.

The Biofire FilmArray Meningoencephalitis (ME) Panel (Salt Lake City, UT, USA) is currently the only commercially available FDA-approved multiplex PCR assay on CSF specimens and includes seven viral, six bacterial, and one 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 human herpesvirus 6 and cytomegalovirus 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.

6 | 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 had no growth. When CSF PCR for *Listeria* was resulted negative, ampicillin was discontinued. Additionally, CSF studies including EBV PCR, Toxomplasma PCR, Cryptococcus neoformans antigen, Arborvirus IgM, were not detected (Table 4). 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 4. On hospital day 9, patient had a seizure-like activity and altered sensorium. Voriconazole was discontinued. A 2-day electroencephalogram captured a right posterior quadrant onset subclinical seizure. Repeat lumbar puncture and MRI of brain were performed on hospital days 12 and 13, respectively. CSF revealed 56 leukocytes (0% neutrophils, 85% lymphocytes), protein 82 g/dl, and glucose 50 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 acid fast bacilli were negative. Brain tissue PCR results were negative for bacterial DNA, fungal DNA, nontuberculous mycobacteria DNA, and mycobacterium TB 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 7A). Inflammation is composed of T cells (CD3) (Figure 7B) and numerous macrophages (CD68) (Figure 7C). Movat staining demonstrated diffuse, acute collagen deposition, often not seen in the brain but consistent with abscess wall (Figure 7D).

7 | 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 to 56 cells/mm^{3}. He received minimal antifungal or anti-toxoplasma therapy, and no antituberculous 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

TABLE 4 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	$376–1.00 \times 10^8$
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	1005 (80% neutrophils, 5% lymphocytes)	
Protein	186	
Glucose	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	$52–1.69 \times 10^8$
CSF *Toxoplasma* PCR (copies/ml)	Not detected	$183–1.00 \times 10^8$
CSF Arbovirus IgM	Not detected	

Abbreviations: CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; CSF; cerebrospinal fluid; EBV, Epstein–Barr virus; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction.

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 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 *Streptococcus pneumoniae*, *Listeria monocytogenes*, and *Neisseria 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 5, 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 6. Parenchymal brain lesions have been
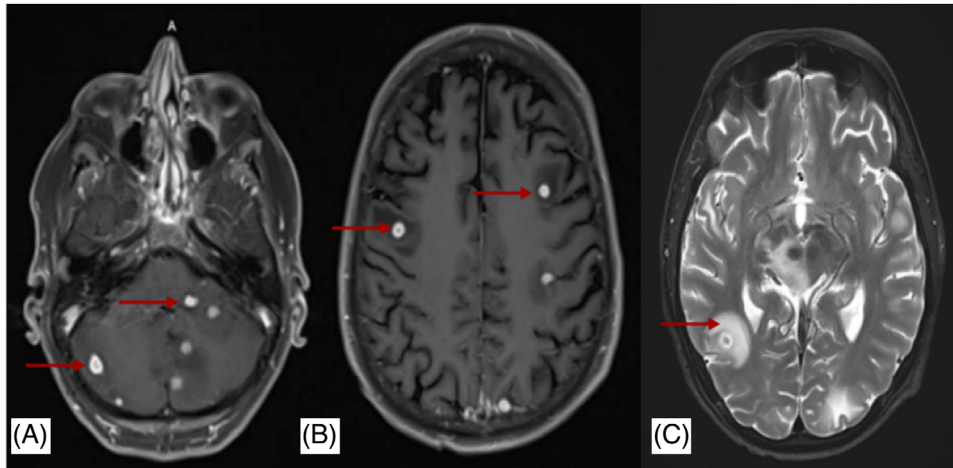
FIGURE 6 Subsequent magnetic resonance imaging (MRI) brain (hospital day 13). (A and B) T1-weighted MR brain showing innumerable bilateral scattered supratentorial, infratentorial lesions (red arrows). (C) T2-weighted MR brain showing hyperintense signal surrounding the lesions that is consistent with surrounding edema (red arrow)
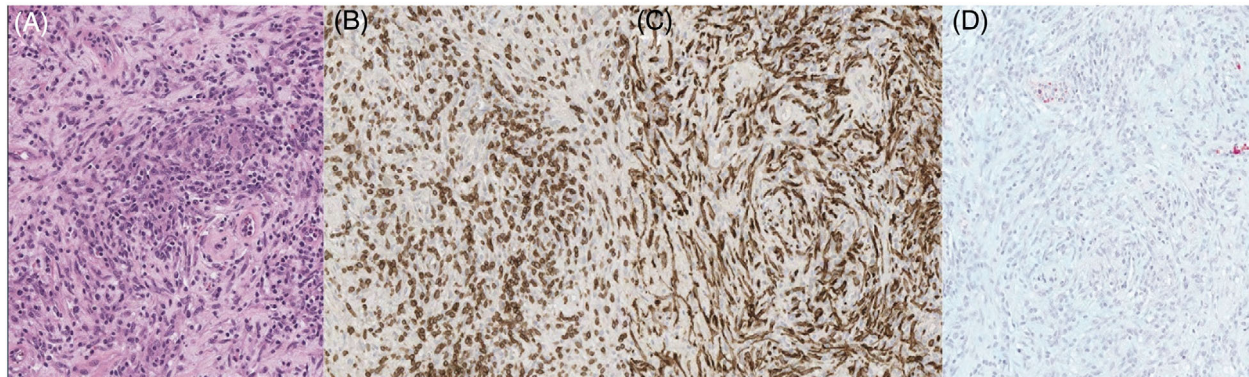
FIGURE 7 Brain tissue histopathology. (A) Hematoxylin and eosin staining showing a mixed inflammatory infiltrate in a fibrous background; acute neutrophilic inflammation is not evident (A). (B and C) Inflammation is composed of T cells: CD3 (B) and numerous macrophages: CD68 (C). (D) Movat staining demonstrated diffuse, acute collagen deposition, often not seen in the brain but consistent with abscess wall

TABLE 5 Fastidious bacteria

"Classic" fastidious bacteria[1]	Newer/other
Legionella spp.[a]	Abiotrophia/Granulicatella (formerly nutritionally variant streptococci)
Brucella[a]	Coxiella burnetii[a] (Q fever)
Francisella tularensis[a] (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)	

Abbreviation: HACEK, Haemophilus species, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and Kingella kingae.
[a]Unlikely to respond to ceftriaxone.

TABLE 6 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 *Bartonella henselae* described. Bartonella encephalitis well recognized; case reports of aseptic meningitis. *B. henselae* known cause of culture-negative endocarditis and CNS lesions may be related
	CON: 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
	CON: isolated neurologic disease rare. No conjunctival suffusion, myalgias, jaundice, renal injury, bleeding. SOTS cases: five 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

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; MRI, magnetic resonant imaging; SOT, solid organ transplant; WBC, white blood cell.

described with several organisms including *Borrelia burgdorferi*, *T. whipplei*, *Leptospira interrogans*, *Erysipelothrix 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*, *Bartonella henselae*, *B. burgdorferi*, and *T. whipplei*. As noted in Table 5, 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.

8 | CLINICAL COURSE (IHAB KASSAB)

The patient improved markedly on ceftriaxone and was discharged on 2 g 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 *B. henselae*. There were 22 sequence reads that aligned to *B. henselae* out of a total of 21 539 049 total sequence reads in the sample. This corresponds to reads per million (RPM) 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 *B. henselae* immunoglobulin G titer
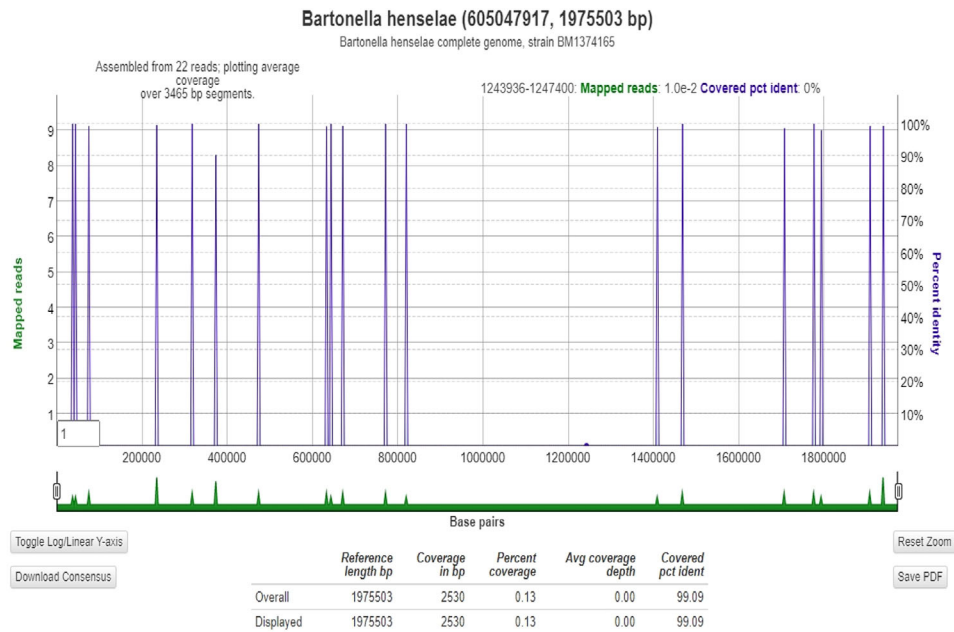
FIGURE 8 Metagenomic next-generation sequencing coverage map

TABLE 7 Bartonella polymerase chain reaction (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

Abbreviations: IgG: Immunoglobulin G; IgM: Immunoglobulin M.

of 1:1024, consistent with a recent infection (Table 7). The diagnosis of *B. 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.

9 | FINAL DIAGNOSIS: BRAIN ABSCESS DUE TO *B. HENSELAE*

Bartonella infection is not an uncommon infection, with 12 000 outpatient cases and 500 inpatient cases per year in the United States. *B. 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]

B. 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]

B. 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 human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (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%–2% of *B. henselae*-infected patients, with encephalopathy being the most common presentation. Cat scratch encephalopathy most commonly presents with seizures. Other CNS manifestations of *B. 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 *B. 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 *B. 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 *B. 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*.

B. 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 *B. 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.

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

CONFLICT OF INTEREST
Daniel Kaul received research support from AstraZeneca, Janssen, Gilead, Merck, NIAID, Nobelpharma and is a member of the Data Safety Monitoring Board Member for Noveome.

The rest of the authors declare no conflict of interest.

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