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Capnocytophaga canimorsus meningitis diagnosed using next- generation sequencing of microbial cell-free DNA

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ABSTRACT

Capnocytophaga canimorsus meningitis is frequently caused by exposure to dog or cat bites and occurs more commonly in immunocompromised individuals. CSF analysis is the first step in diagnosis; however, in situations where CSF cultures turn negative, molecular techniques such as 16S rRNA gene amplification followed by polymerase chain reaction (PCR) product sequencing have shown promise. Next generation sequencing of cell free DNA (NGS cfDNA) can assist in identifying the causative agent in a quick and accurate manner. We present a rare case of *C. canimorsus* meningitis in an immunocompetent host that highlights the utility of NGS cfDNA in timely diagnosis after exhausting all other available diagnostic techniques.

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Introduction

Capnocytophaga species have been implicated as a rare but serious cause of infection in humans and are found in the oral flora of humans, cats, and dogs [1]. Risk factors for infection with *Capnocytophaga canimorsus* include a history of animal or human bite, immunocompromised condition, asplenia, cirrhosis or a history of heavy alcohol use. Due to the fastidious nature of *C. canimorsus*, traditional culture of the organism delays diagnosis in such cases [2]. We present a case of bacterial meningitis in an immunocompetent host that highlights both the importance of *C. canimorsus* as a possible pathogen in appropriate clinical settings and the utility of next generation sequencing in timely diagnosis of meningitis with unilluminating cerebrospinal fluid (CSF) results.

CASE PRESENTATION

A 55-year-old female presented to an outlying emergency department (ED) with complaints of chills, shortness of breath and headache, 2 days after being bitten by a family dog. She had a past medical history of hypertension, coronary artery disease, stage 2 chronic kidney disease, seasonal allergies, and a remote history of ovarian teratoma. She had no history of chemotherapy exposure, asplenia, alcohol abuse or cirrhosis. At that time, she did not have any erythema, edema, or pain at the site of the bite, and she was discharged home. Her chills and shortness of breath improved without treatment, but her headache continued to worsen, prompting her presentation to the ED 3 weeks later. She was again afebrile, with no noted erythema, edema or pain at the site of the previous bite. She did not exhibit any neurologic abnormalities or meningeal signs on physical exam and her workup revealed leukocytosis, thrombocytosis, and elevated C reactive protein (CRP). She was discharged home to follow up with her primary care provider (PCP). Due to persistent symptoms, her PCP ordered an MRI brain scan which showed leptomeningeal enhancement with a cyst appearing foci in the temporal lobe (Fig. 1), after which she was admitted to the hospital. She underwent a lumbar puncture and was empirically started on vancomycin 1250 mg intravenously daily, ceftriaxone 2 g intravenously twice daily, ampicillin 2 g intravenously every 4 h and acyclovir 10 mg/kg intravenously every 8 h.

Investigations

Her complete blood count (CBC) on initial ED presentation showed leukocytosis, anemia, and thrombocytosis, and she was

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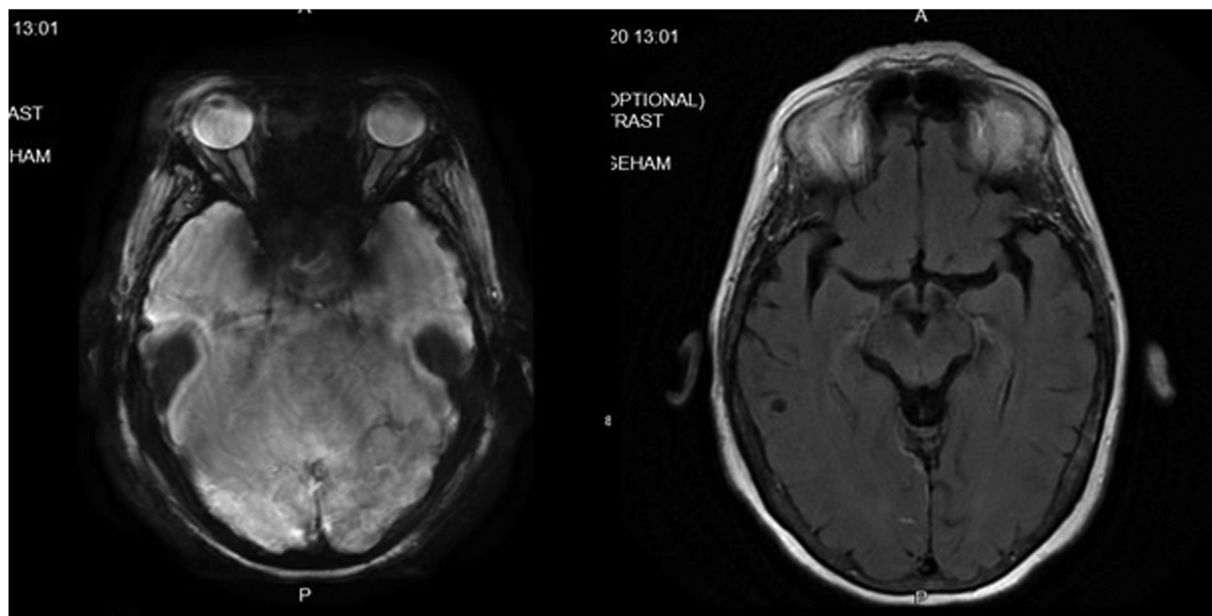

Fig. 1. MRI brain showing leptomeningeal enhancement and cyst appearing foci in the temporal lobe.

Table 1
Laboratory workup.

Pertinent Labs	Units	#1 visit	#2 visit
WBC	10(3)/mcL	12.48	13.07
Neutrophils	47.0–73.0 %	60.5	57.6
Lymphocytes	18.0–42.0 %	30.7	33.1
Monocytes	4.0–12.0 %	8.0	8.1
Eosinophils	0.0–5.0 %	0.4	0.8
Basophils	0.0–1.0 %	0.4	0.4
Hemoglobin	g/dL	9.1	9.4
Hematocrit (%)		29.9	31.2
Platelet count	10(3)/mcL	529	461
CRP	mg/dL	4.53	8.88

Table 2
CSF Studies.

CSF Studies	Value	Reference range & units
Appearance	Cloudy	Clear
WBC	4000	cells/mm(3)
Neutrophil (%)	90	
Leukocytes (%)	2	
Monocytes (%)	8	
Glucose	53	40–70 mg/dL
Protein	79.7	12–60 mg/dL
CSF culture	No growth	No growth
Escherichia Coli K1 PCR	Not detected	
Haemophilus influenza PCR	Not detected	
Listeria monocytogenes PCR	Not detected	
Neisseria meningitidis PCR	Not detected	
Streptococcus agalactiae PCR	Not detected	
Streptococcus pneumoniae PCR	Not detected	
Cytomegalovirus PCR	Not detected	
Enterovirus PCR	Not detected	
Herpes simplex 1 and 2 PCR	Not detected	
Human Herpesvirus 6 PCR	Not detected	
Varicella zoster virus PCR	Not detected	
Cryptococcus neoformans PCR	Not detected	
VDRL	Nonreactive	
Cysticercus antibody	<0.75	<0.75 Antibody Not Detected > or = 0.75 Antibody Detected
Fungal culture	No growth	No growth
Acid fast smear	No fluorescent antibody seen	No fluorescent antibody seen
Fungal/silver stain	Negative	Negative

also found to have an elevated C-reactive protein (CRP) level (Table 1). Her CBC on the second visit to the ED showed increased leukocytosis, and worsening CRP levels (Table 1). Blood cultures showed no growth. Initial CSF studies showed 4000 total white blood cells (WBC) with neutrophilic predominance and elevated protein count, with bacterial, viral, and parasitic testing failing to identify a particular etiology (Table 2). Repeat CSF studies showed 187 total nucleated cells with lymphocytic predominance and elevated glucose, with negative bacterial, and fungal testing. Adjunct testing for mycobacterial and fungal etiologies was unremarkable (Table 3). Screening for immunodeficiency states (Human immunodeficiency virus (HIV) screen), hemoglobin A1C, quantitative immunoglobulins, and T and B cell enumeration) was negative. Blood next-generation sequencing of microbial cell-free DNA (NGS cfDNA) ("Karius Test", Karius, Redwood City, California, USA) detected *C. canimorsus* at 11 molecules/μL.

Treatment

The patient was empirically started on vancomycin 1250 mg intravenously daily, ceftriaxone 2 g intravenously twice daily, ampicillin 2 g intravenously every 4 h, and acyclovir 10 mg/kg intravenously every 8 h. Her acyclovir and ampicillin were discontinued following negative herpes simplex and Listeria species on polymerase chain reaction (PCR) studies. After CSF cultures failed to grow, the decision was made to continue empirical treatment with 2 weeks of Vancomycin 1250 mg intravenously and Ceftriaxone (2 g intravenously twice daily).

Outcome and follow up

After next-generation sequencing of microbial cell-free DNA (NGS cfDNA) detected *C. canimorsus*, the patient's regimen was

Table 3
Serologic Studies.

QuantiFERON-TB Gold assay	Negative
Histoplasma capsulatum antibody H band	Negative
Histoplasma capsulatum antibody M band	Negative
Coccidioides immitis antibody	Negative
Blastomyces dermatitidis antibody	Negative
Aspergillus fumigatus antibody	Negative
Histoplasma capsulatum antigen	Negative
Blastomyces dermatitidis antigen	Negative
Aspergillus fumigatus antigen	Negative
Cryptococcus neoformans antigen	Negative
(1, 3) Beta-D-Glucan	Negative

simplified to Ceftriaxone 2 g intravenously twice daily with plans for 3 weeks of total therapy. At follow up, the patient endorsed resolution of her symptoms with no recurrence following the completion of therapy.

Discussion

Meningitis due to *C. canimorsus* was first reported in 1976 by Bobo and Newton, wherein they described a case of a 42-year-old male who developed fever, seizures and headache one week after a dog bite. The organism was identified on blood and CSF cultures, and the patient recovered satisfactorily with antimicrobial therapy [3].

C. canimorsus meningitis is frequently caused by exposure to dog or cat bites and occurs more commonly in immunocompromised males around the age of 60. It classically presents with fever, altered mentation, headache, photophobia and neck stiffness, but other symptoms such as seizures, fatigue and hearing loss have also been reported [4]. Our patient did not have any immunocompromising risk factors and likely acquired the infection after the dog bite.

CSF analysis results are consistent with bacterial meningitis, and blood and CSF cultures are traditional methods for diagnosis. The fastidious nature of C. canimorsus and the requirement for prolonged incubation can, however, make a timely diagnosis a challenge in clinical practice [5]. CSF culture can take a median time of 5 days (range of 1–19 days) to grow this microorganism. In situations where CSF culture turns negative, molecular techniques such as 16S rRNA gene amplification followed by polymerase chain reaction (PCR) product sequencing have been regarded as promising diagnostic modalities [1,2].

In our patient, diagnosis was made by blood next-generation gene sequencing. In this review, we discuss the use of the molecular technique to make the diagnosis. In order to gather more information on the extent of use of these molecular techniques, we performed a literature review by searching keywords '(capnocytophaga canimorsus) and (meningitis)' on PubMed, which revealed a total of 52 articles. All the articles published in the English language were thoroughly reviewed. Our literature focuses on immunocompetent patients with *C. canimorsus* meningitis in whom molecular diagnostic techniques (e.g., 16S ribosomal RNA or DNA sequencing) were used to make the diagnosis (Table 4). Immunocompromised patients with histories of splenectomy, cirrhosis, autoimmune diseases, steroid and other immunosuppressant use were excluded.

In our patient, after the blood culture, CSF culture and PCR testing failed to identify an organism, we resorted to blood NGS

Table 4
Literature review.

Year	Author	Age/ Sex	Risk factors	Blood culture	CSF culture	Treatment	Reference
2020	Hannon DM et al	77/F	–	positive	CSF - 16S rRNA gene sequence positive	Ceftriaxone and ampicillin x 14 days	[6]
2020	Prasil et al	74/M	–	negative	CSF bacterial DNA sequencing positive	Ceftriaxone x 14 days, ampicillin x 1 day	[7]
2020	Bering et al	67/M	CLL on ibrutinib therapy	Positive Identified on DNA sequencing (14d after discharge)	Negative	Vancomycin, ceftriaxone, ampicillin → merrem x 4 weeks	[5]
2019	Hansen M et al	71/F	History of lung cancer status post lobectomy	Positive	CSF culture negative; PCR positive	Vancomycin, ceftriaxone, ampicillin x 4 days → Meropenem x 17 days; (21 days total)	[4]
2018	Bertin et al	69/F	–	Positive PCR positive	–	Vancomycin, zosyn x 28 days	[8]
2018	Bertin et al	65/M	–	Positive	Positive 16S rRNA gene sequence positive	Ceftriaxone, ampicillin x 14 days → ampicillin-sulbactam, moxifloxacin x 28 days	[8]
2017	Beltramone et al	49/M	–	Positive	Negative, 16S rRNA gene sequencing negative	Ceftriaxone x 28 days	[9]
2016	Beernink et al	52/M	–	Not taken	Gram stain positive Culture Negative, 16 s rRNA PCR positive	Ceftriaxone, amoxicillin x 4 days → iv penicillin x 12 days	[10]
2012	Monrad et al	79/M	–	–	Positive/16S rDNA PCR positive	Ampicillin, ceftriaxone, acyclovir → merrem 3 days → ceftriaxone x 21 days	[11]
2012	Monrad et al	66/M	alcoholism	Negative	Positive/ 16S rDNA PCR positive	Penicillin 2 days → ampicillin 6d, ceftriaxone x 20 days	[11]
2006	Risi GF et al	65/F	Status post CT myelogram	Not reported	Positive/ identified by 16S rRNA sequencing	Cefepime, ampicillin, flagyl → ceftriaxone x 14 days	[12]
2006	Meybeck et al	65/M	–	Positive	Positive/ identified by 16S rRNA gene sequencing	Gentamicin, cefotaxime 1day → flagyl, amoxicillin, cefotaxime (total 15 days)	[13]
2006	Gottwein et al	54/M	alcoholism	Positive	Positive/ identified by 16S rDNA gene sequencing	Ceftriaxone x 13 days	[14]

cfDNA testing, which identified *C. canimorsus* as the causative agent. It was reported within 24 h of receiving the blood sample.

Metagenomic next generation sequencing (NGS) involves extraction of cell free (cf) DNA, cfRNA, or both from a sample of body fluid which is then amplified via PCR. This is followed by generation of libraries and shotgun sequencing nucleic acids at very high depth [15]. When compared with other traditional culture methods, metagenomic next generation sequencing showed higher sensitivity especially in the blood, sputum and broncho-alveolar lavage (BAL) samples (23.6 % vs 67.4 %) [16]. Other advantages of using this test include rapid and more accurate results, shortened diagnosis time, ability to analyze a broad spectrum of microorganisms at once and small impact of prior antibiotic exposure on results [17]. This is supported by multiple large multicenter prospective studies that were conducted to investigate the utility of metagenomic NGS [18,19]. WIlson et al. reported that of 58 total CNS infections, 13 were diagnosed by metagenomic NGS that were not identified by clinical testing [18]. Another prospective study on metagenomic NGS reported a 57 % positive detection rate of definite CNS infections and 73.3 % sensitivity of definite bacterial meningitis [19].

The causes of meningitis are not identified in up to 50 % of the cases [20]; therefore, in culture negative cases, NGS, due to its high specificity, can prove beneficial to tailor down to narrow spectrum antibiotics. The disadvantages of the test to be considered are its cost, its inability to be performed at all institutions and its inability to report susceptibility to antimicrobials.

Due to the gradually increasing prevalence of beta-lactamase strains of Capnocytophaga species, the higher generation cephalosporins, such as cefepime or ceftriaxone (parenterally), penicillin/beta-lactamase inhibitors and carbapenems are more commonly being used for a total duration of 3 weeks [5,7]. In our case, after *C. canimorsus* was identified on the NGS cfDNA test, we treated the patient with a 3-week course of parenteral ceftriaxone therapy.

Conclusions

C. canimorsus meningitis is a rare disease that has an affinity for immunocompromised individuals. However, its presentation in immunocompetent patients should not be overlooked, as timely diagnosis and treatment can be lifesaving in such cases. NGS cfDNA assists in identifying the causative agent in a quick and accurate manner while not allowing prior antibiotic exposure to impact its results. These benefits provide it an edge over other traditional diagnostic modalities, and therefore, we recommend NGS cfDNA be considered at an early stage in the disease course.

Author statement

Abuzar A. Asif: Conceptualization, Writing- original draft preparation **Moni Roy**: Writing- Review & Editing, Investigation. **Benjamin Tellier**: Resources, Writing- Review & Editing **Sharjeel Ahmad**: Supervision, Visualization.

Consent

A written consent was obtained from the patient.

Declaration of Competing Interest

The authors report no declarations of interest.

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