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BRIEF REPORT

Characteristics of *Rickettsia typhi* Infections Detected with Next-Generation Sequencing of Microbial Cell-Free Deoxyribonucleic Acid in a Tertiary Care Hospital

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We present 10 patients with *Rickettsia typhi* infection in whom next-generation sequencing of microbial cell-free deoxyribonucleic acid (mcfDNA) was used as a diagnostic tool. *Rickettsia typhi* mcfDNA was detected in all cases and was more rapid and specific than rickettsial serology. *Rickettsia typhi* mcfDNA impacted antibiotic management in 50% of patients.

Keywords. clinical characteristics; murine typhus; next-generation sequencing; *Rickettsia typhi*.

Murine or flea-borne typhus—a bacterial disease caused by *Rickettsia typhi* most prevalent in tropical and subtropical environments—is carried by mammals and transferred to humans primarily by abrasion of feces from rat fleas (*Xenopsylla cheopis*) and cat fleas (*Ctenocephalides felis*) into bite wounds [1]. Although murine typhus peaked in the United States in the 1940s, subtropical regions of the United States, especially California and Texas, remain endemic regions with a growing number of annual cases. In Texas, cases quadrupled between 2008 and 2018 [2]; the incidence is highest on the coast and in the south but expanding inland and northward with metropolitan areas becoming foci of increasing transmission [1–3]. The diagnosis of murine typhus represents a challenge to clinicians given a diverse range of presentations from mild constitutional and gastrointestinal symptoms to severe sepsis-like physiology with multiorgan involvement [4, 5]. The diagnostic work-up of *R typhi* often includes imaging studies and extensive laboratory evaluation, which can lead to increased patient care costs [6] and delays in timely management. Given the prolonged time to diagnosis by serological testing, doxycycline is generally started empirically while awaiting confirmatory testing [7]. In recent years, next generation sequencing (NGS) of microbial cell-free deoxyribonucleic acid (mcfDNA) has been utilized as a send-out test at Baylor St. Luke's Medical Center (BSLMC), where this retrospective case series was conducted. The Karius test (Redwood City, CA) used in this study is a validated, College of American Pathologists-accredited, Clinical Laboratory Improvement Amendments-certified open-ended tool that detects and quantifies the plasma concentration of mcfDNA of >1000 pathogens enabling the rapid, noninvasive diagnosis of infectious diseases [8]. The aim of the following case series is to correlate the mcfDNA NGS signal with the clinical features of murine typhus and to characterize its role in the diagnosis of *R typhi* infection and in tailoring antibiotic management.

METHODS

Our study was performed at BSLMC, a quaternary academic medical center, between May 2017 and May 2020. Patients with *R typhi* detected by mcfDNA NGS were reviewed. All mcfDNA NGS tests were ordered by infectious diseases attendings. Patients were included in the study if the *R typhi* mcfDNA concentration met the commercial threshold or if the serology and clinical manifestations were consistent with murine typhus based on discussion with an infectious disease physician (M.A.). A previously described cohort of 684 healthy adults was interrogated for *R typhi* mcfDNA as a means of negative control [8]. A chart review of BSLMC patient records was conducted. All mcfDNA NGS tests were sent to Karius; serology samples were sent to Quest Diagnostics (San Juan Capistrano, CA). This study was approved by Baylor College of Medicine Institutional Review Board.

Patient Consent Statement

This work was a retrospective chart review approved by the Baylor College of Medicine Institutional Review Board. Because of the nature of the study, consent was not required. No identifiable patient information is included in this manuscript.

RESULTS

Ten patients with *R typhi* detected by mcfDNA NGS were included (Table 1); no *R typhi* mcfDNA NGS reads were detected in the 684-subject healthy cohort (specificity 100%). Median age was 38.5 years (19–75 years); 7 patients were female. Eight patients presented in summer months (May–August). The

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Table 1. Clinical Features of Patients Infected With *Rickettsia typhi*

Case No.	1	2	3	4	5	6	7	8	9	10
Age, gender	23, female	75, male	19, female	35, male	32, female	45, female	42, female	72, female	44, female	29, male
Month of presentation	July	May	June	May	July	May	November	June	January	August
Associated conditions	SLE on methotrexate	None	Hypothyroidism, obesity	None	UC on mesalamine	None	Bell's palsy on Prednisone	Cachexia, back pain, generalized weakness	None	Migraine, asthma
Initial suspected diagnosis	UTI	Gastroenteritis, rickettsial, or viral infection	Viral infection	Fever of unknown origin	Influenza, severe sepsis cholangitis	UTI	Sepsis secondary to EBV vs acute hepatitis	Brucellosis	Fever of unknown origin	Fever of unknown origin, suspected viral sepsis
Fever (°F)	103	101.7	103	102	102	102.8	102.2	104	102.4	101.2
Septic shock	Yes	No	No	No	Yes	No	Yes	No	No	No
Intensive care unit admission	Yes (day of admission)	No	No	No	Yes (day of admission)	No	Yes (day of admission)	No	No	No
Headache	No	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes
Confusion	No	No	No	No	Yes	Yes	No	No	No	No
Rash	Yes	No	Yes	No	No	No	Yes	No	No	No
Myalgia/arthralgia	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Respiratory signs/symptoms	No	Cough	Shortness of breath	No	Respiratory failure/ARDS	Cough	Respiratory failure/ARDS	No	Cough and shortness of breath	Shortness of breath
Gastrointestinal symptoms	No	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes
Hepatomegaly/splenomegaly	No	No	No	No	Yes	No	No	No	No	Yes
Myocarditis	No	No	No	No	Yes	No	No	No	No	No
Acute kidney injury/renal impairment	No	No	No	No	Yes	No	Yes	No	No	No
Hemoglobin gm/dL (RI 12–15)	11.6	14.7	14.1	10.0	11.1	13.2	13.1	16.2	11.6	13.9
White blood cells (RI 4–10 k/μL)	2.9	6.1	4.5	9	9.1	8	2.79	7.3	4	7.8
Neutrophils% (RI 55–70)	56	79	68	67	74	83	79.9	71	76	80
Bands% (RI 0–10)	33	27	21	8	18	6	2	Not reported	2	1
Platelet count k/μL (RI 150–450)	55	95	130	194	16	87	83	339	72	39
Sodium mEq/L (RI 136–145)	127	131	137	134	138	124	138	127	134	130
Blood urea nitrogen mg/dL (RI 7–21)	8	20	11	11	36	11	7	7	5	7
Creatinine mg/dL (RI 0.57–1.25)	0.79	0.95	0.70	0.75	2.07	1.03	0.70	0.64	0.6	0.9

Table 1. Continued

Case No.	1	2	3	4	5	6	7	8	9	10
Aspartate aminotransferase U/L (RI 5–40)	109	229	130	91	199	222	140	26	100	129
Alanine aminotransferase U/L (RI 5–50)	101	95	106	175	117	140	142	28	70	167
Alkaline phosphatase U/L (RI 40–150)	234	120	63	94	133	123	139	117	66	197
Creatine kinase U/L (RI 29–200)	188	Not reported	Not reported	Not reported	193	Not reported	242	71	Not reported	327
Hematuria	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes
Proteinuria mg/dL (RI 0)	10	100	No	No	200	100	30	20	20	No
Lumbar puncture	Not performed	Not performed	3 WBC/μL, Glucose 58 mg/dL, Protein 24 mg/dL	Not performed	Not performed	Not performed	Not performed	Not performed	Not performed	2 WBC/μL Glucose 47 mg/dL, Protein 30 mg/dL
Rickettsia typhi mcfDNA NGS MPM (RI = 0)[#]	5827	1113	84[a]	162	4777	54	Positive[b]	Detected[c,d]	Detected[c]	431
EBV mcfDNA NGS MPM (RI = 1.4)[#]	ND	219	ND	71	ND	418	ND	ND	178	ND
Rickettsia typhi serology	IgM 1:64 IgG > 1:256	IgM 1:128 IgG > 1:256	Not performed	IgM 1:256 IgG > 1:256	IgM > 1:256 IgG 1:64	IgM: >1:256 IgG: Not detected	IgM 1:128 IgG 1:128	IgM: >1:256 IgG: >1:256	IgM 1:64 IgG: Detected reflex Ab assay, no titer	IgM: 1:64 IgG: Not detected
RMSF serology	IgM 1:64 IgG 1:128	IgM not detected IgG 1:64	Not performed	IgM 1:64 IgG not detected	IgM 1:128 IgG Not detected	IgM: Not detected IgG: Not detected	IgM 1:64 IgG 1:64	IgM: Not detected IgG: 1:128	IgM 1:64 IgG: Detected reflex Ab assay, no titer	IgM: Not detected IgG: Not detected
Duration of symptoms onset before collection of mcfDNA (days)	9	7	13	15	10	13	10	18	26	8
Duration of antibiotics before collection of mcfDNA (days)	7	4	9	11	3	8	16	7	18	3

Abbreviations: Ab, antibody; ARDS, acute respiratory distress syndrome; EBV, Epstein-Barr virus; Ig, immunoglobulin; mcfDNA, microbial cell-free deoxyribonucleic acid; MPM, molecules per microliter; ND, not detected; NGS, next-generation sequencing; RI, reference interval; RMSF, Rocky Mountain spotted fever; SLE, systematic lupus erythematous; UC, ulcerative colitis; UTI, urinary tract infection; WBC, white blood cells.

[#]The reference interval for a specific pathogen's mcfDNA is the 97.5 percentile of that pathogen's mcfDNA MPM in a cohort of healthy adult subjects; the cohort was initially 167 subjects [8] and has been extended to 684 healthy adults.

[a]The mcfDNA NGS sample failed quality control measures and was quantity insufficient for repeat analysis; the *R typhi* mcfDNA MPM is included as a research use only data point for clinical correlation.

[b]The sample met the commercial threshold but could not be accurately quantified due to lack of sequencing depth.

[c]*Rickettsia typhi* mcfDNA was present in the raw data but under the commercial threshold of the assay.

[d]Microbial cell-free DNA NGS was positive for *Micrococcus luteus* that was considered clinically insignificant by the treating team.

median clinical turnaround time (the time between ordering and result reporting) for mcfDNA NGS was 3 days (interquartile range [IQR], 2.5–3.5). The median laboratory turnaround time for mcfDNA NGS (the time between sample receipt by the laboratory and result reporting) was 1 day (IQR, 1–2).

Rickettsia typhi serology results were available in 9 patients and were positive with variable titers. Rocky Mountain spotted fever serologies were positive in 5 of 9 patients (specificity 44%). *Rickettsia typhi* mcfDNA NGS and serology was 100% concordant. The median clinical turnaround time for serology was 3 days (IQR, 3–5.5), and the median laboratory turnaround time was 2 days (IQR, 2–3). The median time between symptom onset and collection of mcfDNA was 11.5 days (IQR, 8.75–15.75), whereas the median time between hospitalization and collection of mcfDNA was 4 days (IQR, 2.5–6.25 days). The median duration of antibiotic treatment before the collection of mcfDNA was 7.5 days (IQR, 3.75–12.25).

The range of clinical presentation was broad with wide differential diagnoses; initial presumptive etiological considerations included nonrickettsial diseases in every case (Table 1). Three patients required admission to the intensive care unit due to septic shock, and 2 others experienced acute respiratory distress syndrome and acute kidney injury. One had cardiac arrest and combined respiratory/metabolic acidosis requiring extracorporeal membrane oxygenation. Hematology was consulted due to concern for hemophagocytic lymphohistiocytosis in both patients; one received bone marrow biopsy demonstrating macrophage activation syndrome.

Viral infection was suspected in 7 of 10 patients at presentation; urinary tract infection was diagnosed in 2 patients. All patients received viral testing including respiratory panels, hepatitis panels, and Epstein-Barr virus testing. Nine of ten patients received other antibiotics before or alongside doxycycline, including vancomycin, meropenem, rifampin, gentamicin, cefepime, amoxicillin, nitrofurantoin, and azithromycin. Doxycycline was initiated due to suspicion of rickettsial infection in patients with elevated liver enzymes, thrombocytopenia, lack of improvement with broad-spectrum antibiotics, and unrevealing viral panels. In 4 patients, non-tetracycline antibiotics were discontinued before confirmatory results due to clinical improvement. *Rickettsia typhi* mcfDNA affected antibiotic management in 5 patients (50%). In 4 patients, antibiotics were narrowed to doxycycline after *R typhi* mcfDNA detection. One patient's doxycycline was discontinued after clinical improvement but restarted when mcfDNA NGS confirmed *R typhi* infection.

DISCUSSION

Previous research has characterized the heterogeneous presentation of murine typhus in large cohorts of patients [9]. Although the frequency of fever, headaches, gastrointestinal symptoms, and hyponatremia is consistent with that seen in prior studies, respiratory complaints were more common in this patient group [9, 10]. Patients experienced complications consistent with previous reports of secondary hemophagocytic lymphohistiocytosis, which has been reported in severe rickettsial disease [4, 5]. The frequency of elevated liver function enzymes (*aspartate aminotransferase* >40, *alanine aminotransferase* >50) was similar to other studies of hospitalized patients (up to 90%) [10]. In contrast, thrombocytopenia (<150 000/μL) was much more common in our patients (80%) than in the literature (16.1%–48% of patients) [9, 10].

This study included 2 patients for whom the mcfDNA NGS result was below the commercial threshold but were positive for *Rickettsia* sp antibody titers and had a compatible clinical presentation. By comparison, no *R typhi* mcfDNA NGS reads are present in a cohort of 684 asymptomatic control subjects. The amount of *R typhi* mcfDNA seemed to correlate with sepsis-like presentation, symptom duration, and pretreatment with antibiotics, suggesting that the molecules per microliter may also be useful to monitor the course of the infection and response to therapy. In 4 patients, mcfDNA NGS also detected Epstein-Barr virus, highlighting the commonality of herpes viral reactivation in the setting of sepsis syndromes and critical illness; in one patient, *Micrococcus luteus* was detected, which was believed to be clinically insignificant by the treating team.

All patients in this series presented with a wide differential diagnosis; doxycycline was started empirically due to suspicion of rickettsial disease along with broad coverage for other possible pathogens. *Rickettsia typhi* mcfDNA NGS results allowed physicians to safely mitigate the burden of unnecessary antibiotics in 4 patients and resume doxycycline in another. Although the best practice remains to treat suspected murine typhus empirically, mcfDNA NGS may prove useful in securing what can be an elusive diagnosis, excluding other competing pathogens in the differential diagnosis that would warrant treatment and de-escalating antibiotic coverage.

Our findings are limited by a small sample size of 10 patients and the selection of patients from a quaternary hospital, a referral center for severely ill patients from the community requiring escalation of care. Patients included in the study also had chronic conditions (systemic lupus erythematosus, ulcerative colitis) that can impact presentation, but their conditions were previously well managed and the trends in laboratory values were consistent with an acute process. In addition, the case series did not include patients for whom rickettsial disease was suspected but excluded from the differential after negative work-up. Cost and limited availability pose challenges of mcfDNA NGS as a diagnostic tool in resource-poor murine typhus-endemic areas within and outside the United States. Future research on this emerging cause of flea-borne illness will be needed to better delineate the role of new diagnostic

techniques such as mcfDNA NGS in accelerating diagnosis and tailoring management.

CONCLUSIONS

Rickettsia typhi is a reemerging cause of febrile illness in the United States with a heterogenous range of presentations and severity. The differential diagnosis for the clinical presentation of *R typhi* is broad and most cases are not identified without exhaustive laboratory work-up; despite these diagnostic efforts the infection is likely underdiagnosed. Empirical treatment with doxycycline is recommended; accurate, rapid diagnosis can help narrow antibiotic coverage. Microbial cell-free DNA NGS offers promise as a rapid, open-ended diagnostic method for the evaluation of the broad array of pathogens implicated in the differential diagnosis of systemic syndromic presentations and for securing the specific diagnosis of *R typhi* among the range of etiological possibilities.

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Potential conflicts of interest. A. A. A. is a senior medical director at Karius. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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