JCM
Journals.ASM.org

First Reported Case of *Ehrlichia ewingii* Involving Human Bone Marrow

M. Brandon Allen,[a] Bobbi S. Pritt,[b] Lynne M. Sloan,[b] Christopher D. Paddock,[c] Chaitanya K. Musham,[d] Jeanette M. Ramos,[a] Neslihan Cetin,[a] Eric R. Rosenbaum[a]

Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA[a]; Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, Minnesota, USA[b]; Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[c]; Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA[d]

A 65-year-old female with a history of multiple tick bites presented with fever and pancytopenia. Intracytoplasmic rickettsial morulae were detected on peripheral smear and bone marrow biopsy specimens, and PCR amplified *Ehrlichia ewingii* DNA from both specimens. To our knowledge, this is the first report of *E. ewingii* infection of human bone marrow.

CASE REPORT

A 65-year-old female from rural north-central Arkansas presented in July to an emergency department with weakness and fatigue. She had a 5-day history of subjective fever, productive cough, generalized myalgia, and progressive fatigue. She denied nausea, diarrhea, bleeding, and rash but stated that she had had a urinary tract infection (UTI) 1 month prior that had been treated with sulfamethoxazole-trimethoprim without resolution. She reported multiple recent tick bites while working in her yard and was in close contact with several pet dogs.

Past medical history was significant for type 2 diabetes mellitus, hypertension, hypothyroidism, and laryngeal squamous carcinoma that had been in remission since treatment with chemotherapy and radiation 2 years prior to presentation. The physical examination was significant for fever of 101.3°F and bilateral upper extremity petechiae and bruising.

Initial laboratory studies revealed a white blood count (WBC) of 2,000/μl (reference range, 3,000 to 12,000/μl), a hemoglobin level of 8.9 g/dl (reference range, 11.5 to 16 g/dl), and a platelet count of 32,000/μl (reference range, 150,000 to 500,000/μl). Each of these values had been within normal limits during an evaluation for her UTI that had been performed 3 weeks earlier. The total bilirubin level was 1.3 mg/dl (reference range, 0.2 to 1.2 mg/dl), and the lactate dehydrogenase level was 314 IU/liter (reference range, 100 to 248 IU/liter). Iron studies showed a decreased total iron binding capacity of 234 μg/dl (reference range, 250 to 425 μg/dl) and an increased peripheral blood ferritin level of 732 μg/dl (reference range, 11 to 306 μg/dl) with normal iron and folate levels. The antinuclear antibody (ANA) titer was increased at 1:160. Random-inpatient blood glucose levels ranged from 102 to 111 mg/dl, and her diabetes was adequately controlled with metformin and glipizide by her treatment as an outpatient. HIV serology results were negative.

The patient was admitted to the hospital and was started on vancomycin and cefepime because of fever and neutropenia and was started on doxycycline to address the potential for tick-borne illness. Since recurrent malignancy, myelodysplastic syndrome following chemotherapy, and other marrow processes were in the differential diagnosis for her pancytopenia, peripheral smear and bone marrow aspirate and core biopsy procedures were performed. Review of the peripheral blood smear revealed leukopenia with neutrophilic bands containing intracytoplasmic morulae (Fig. 1), pancytopenia with left-shifted granulopoiesis, reactive lymphocytes, a relative monocytosis, thrombocytopenia, and mild erythrocyte anisopoikilocytosis. The marrow aspirate showed occasional intracytoplasmic morulae within cells of the myeloid lineage and plasma cells (Fig. 1 and 2, respectively). Histology revealed hypercellular marrow with mild erythroid dyspoiesis. The core biopsy was performed 2 days after antibiotic treatment was initiated, and a few morulae seen demonstrated morphological features consistent with treatment response (Fig. 2) (1).

Given the microscopic findings and the extent of tick exposure, peripheral blood specimens were referred to outside laboratories for further characterization of the tick-borne infection using serologic and molecular methods. Results of serologic studies (ARUP Laboratories, Salt Lake City, UT) were incongruous. A positive *Anaplasma phagocytophilum* IgG titer of 1:640 (reference range, <1:80) suggested recent or past infection. This result was supported by the finding of granulocytotropic morulae on a peripheral smear; however, *A. phagocytophilum* is not endemic to Arkansas and the IgM titer was negative at <1:16 (reference range, <1:16). "*Rickettsia rickettsii* (Rocky Mountain spotted fever)" serology was reactive for IgM at a low titer (1:64; reference range, <1:64) but negative for IgG (<1:64; reference range, <1:64), suggesting possible infection with a spotted fever group *Rickettsia* species. *Francisella tularensis* serology was negative (IgM = 2 U/ml and IgG = 9 U/ml; reference range, ≤9 U/ml). *Ehrlichia* serology was not pursued due to an initial negative PCR result for *E. chaffeensis* (Arkansas Children's Hospital Clinical Laboratory, Little Rock, AR).

Peripheral blood sent for molecular testing by PCR (Mayo Medical Laboratories, Rochester, MN) was positive for *E. ewingii* using real-time multiplex PCR (2) and negative for *A. phagocytophilum*, *E. chaffeensis*, and the recently described *E. muris*-like or-

Received 17 June 2014 **Returned for modification** 11 July 2014
Accepted 2 September 2014
Published ahead of print 3 September 2014
Editor: M. J. Loeffelholz
Address correspondence to Eric R. Rosenbaum, errosenbaum@uams.edu.

doi:10.1128/JCM.01670-14

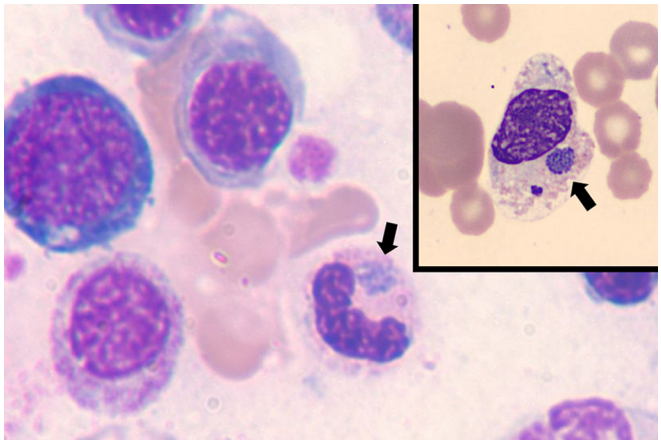

FIG 1 Bone marrow aspirate at a magnification of ×1,000. Wright-Giemsa staining shows a granulocytic band with an intracellular morula (arrow). (Inset) Peripheral blood at a magnification of ×1,000 (CellaVision microscopy, Lund, Sweden). Wright-Giemsa staining shows a band cell in peripheral blood with an intracellular morula (arrow).

ganism. Given the *E. ewingii*-positive PCR result on peripheral blood, PCR was also performed on the decalcified, paraffin-embedded bone marrow core block shavings (Mayo Medical Laboratories) using the same *Ehrlichia/Anaplasma* PCR. *E. ewingii* was successfully amplified, and morulae within myeloid precursor cells were stained by an immunohistochemical method for *Ehrlichia* spp. using an immunoalkaline phosphatase technique and dog hyperimmune anti-*Ehrlichia canis* antiserum at the Centers for Disease Control and Prevention, Atlanta, GA (3, 4) (Fig. 3).

Vancomycin and cefepime were discontinued, and a 10-day course of doxycycline was completed. At discharge, the patient was afebrile and her pancytopenia had resolved.

Ehrlichiosis is a tick-borne rickettsial illness seen during the summer months most commonly in the southeastern and central United States. Species of *Ehrlichia* associated with human illnesses in the United States include *E. chaffeensis* (5), *E. ewingii* (6, 7), and an *E. muris*-like organism (8). Patients with ehrlichiosis may present with fever, headache, myalgia, rash, nausea, vomiting, and laboratory findings of thrombocytopenia, leukopenia, anemia, and elevated liver enzyme numbers (4, 6, 9, 10). In peripheral blood, *E. chaffeensis* generally has tropism for monocytes and *E. ewingii* tropism for granulocytes (1, 4, 6). Anaplasmosis has similar symptoms and was formerly referred to as human granulocytic ehrlichiosis (11, 12). Although such an interpretation is implied by this old nomenclature, leukocyte tropism (i.e., monocytic versus granulocytic) is not specific, as *A. phagocytophilum* has demonstrated in vitro growth in both monocytes and granulocytes (13). Additionally, *E. chaffeensis*, commonly considered specific for monocytes, can be identified *in vivo* within monocytes, granulocytes, lymphocytes (14), and histiocytes (15). On peripheral smears made using a Wright-Giemsa stain, *E. chaffeensis*, *E. ewingii* (Fig. 1), and *A. phagocytophilum* appear as basophilic clusters of intracytoplasmic bacteria referred to as morulae (1, 11, 15). *E. muris*-like bacteria have yet to be morphologically described *in vivo* but infect granulocytic and monocytic cell lineages in cell culture (8).

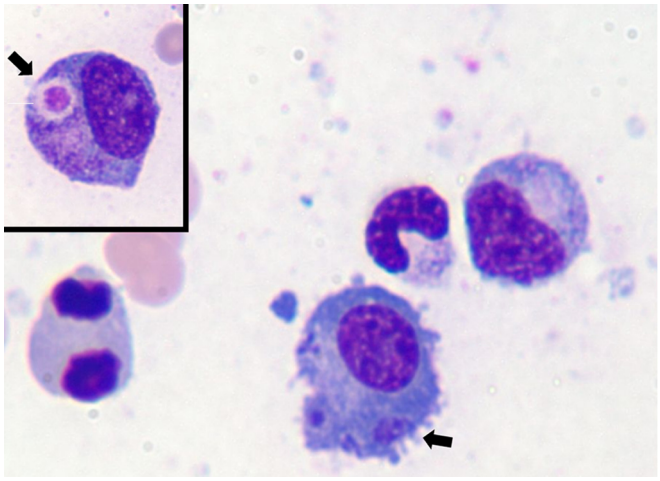

FIG 2 Bone marrow aspirate at a magnification of ×1,000. Wright-Giemsa staining shows a plasma cell with an intracellular morula (arrow). (Inset) Myeloid precursor with an intracellular morula (arrow) demonstrating treatment effect "halo."

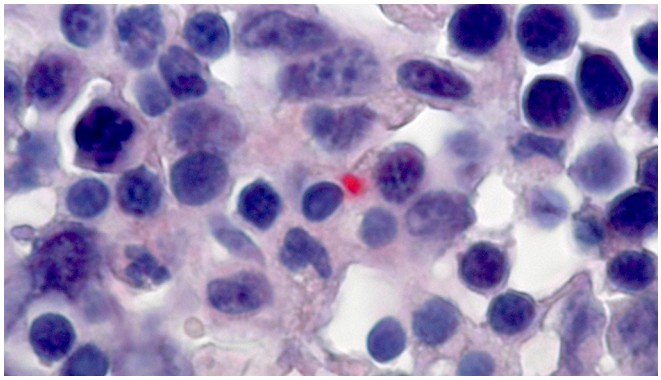

FIG 3 Bone marrow core biopsy specimen at a magnification of ×1,000. An *Ehrlichia* morula (pink) within a myeloid precursor was identified using an immunohistochemical stain.

E. chaffeensis invasion of myeloid cells in human bone marrow and other organs has been previously described (3, 4, 15), and *A. phagocytophilum* in association with bone marrow infection has also been previously described (16). Prior to this case report, *E. ewingii* infection of human marrow cells had yet to be documented. However, we observed *E. ewingii* within myeloid precursors and plasma cells in the bone marrow aspirate (Fig. 2) and in myeloid precursors of the immunohistochemically stained bone marrow core biopsy specimen (Fig. 3). In animal studies, *A. phagocytophilum* and *E. muris* have been linked to cytopenias and dyshematopoiesis (17, 18). We postulate that a similar bone marrow process in humans may have been responsible for the pancytopenia observed in this case involving *E. ewingii*.

As demonstrated in the description of this case, identification of the specific organism causing an ehrlichiosis-like illness can pose a challenge for the clinician. A history of fever and tick bite during summer months in areas of endemicity is commonly diagnosed as "tick fever," empirical treatment with doxycycline is administered, the patient generally recovers, and no further testing is performed. Thus, these tick-borne diseases go under- or misreported to public health departments. Obstacles to identification to

the species level include the turnaround time for PCR and serology and the fact that morulae are seen on peripheral blood smears in only 22% to 38% of ehrlichiosis cases (1, 19). Additionally, serologic studies can be misleading, as cross-reactivity is seen among *Ehrlichia* spp., *A. phagocytophilum*, and *Rickettsia* spp. (6, 9, 20). Further, serologic tests for *E. ewingii* and the *E. muris*-like agent are presently not available and these species may be reported as *E. chaffeensis* due to cross-reactivity (6, 7, 21). Specific to *E. ewingii* reporting, this cross-reactivity may partially explain statistics from the Centers for Disease Control and Prevention where only 28 cases of *E. ewingii* infection were reported between 2008 and 2010 compared to 2,645 cases of *E. chaffeensis* infection (22). Unfortunately, identification of *E. ewingii* is readily available only to clinicians using PCR. Finally, there is accumulating evidence of an association between the severity of ehrlichiosis disease and treatment with sulfa-containing antimicrobials (23–25). It is noteworthy that the patient reported here was treated prior to hospital admission with sulfamethoxazole-trimethoprim for UTI.

In summary, we report the first recognized case of *E. ewingii* infection in human bone marrow and demonstrate some diagnostic challenges that may arise when identifying human-infecting *Rickettsiales* isolates to the species level. As other methodologies may yield confounding results, PCR is currently the only modality available for definitive identification of *E. ewingii*.

ACKNOWLEDGMENTS

We declare that we have no conflicts of interest.

The findings and conclusions are ours and do not necessarily represent the official position of the U.S. Department of Health and Human Services.

REFERENCES

1. **Hamilton KS, Standaert SM, Kinney MC.** 2004. Characteristic peripheral blood findings in human ehrlichiosis. Mod. Pathol. **17:**512–517. http://dx.doi.org/10.1038/modpathol.3800075.
2. **Bell CA, Patel R.** 2005. A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii*. Diagn. Microbiol. Infect. Dis. **53:**301–306. http://dx.doi.org/10.1016/j.diagmicrobio.2005.06.019.
3. **Dawson JE, Paddock CD, Warner CK, Greer PW, Bartlett JH, Ewing SA, Munderloh UG, Zaki SR.** 2001. Tissue diagnosis of *Ehrlichia chaffeensis* in patients with fatal ehrlichiosis by use of immunohistochemistry, in situ hybridization, and polymerase chain reaction. Am. J. Trop. Med. Hyg. **65:**603–609.
4. **Paddock CD, Childs JE.** 2003. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. Clin. Microbiol. Rev. **16:**37–64. http://dx.doi.org/10.1128/CMR.16.1.37-64.2003.
5. **Anderson BE, Dawson JE, Jones DC, Wilson KH.** 1991. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. J. Clin. Microbiol. **29:**2838–2842.
6. **Buller RS, Arens M, Hmiel SP, Paddock CD, Sumner JW, Rikhisa Y, Unver A, Gaudreault-Keener M, Manian FA, Liddell AM, Schmulewitz N, Storch GA.** 1999. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. N. Engl. J. Med. **341:**148–155. http://dx.doi.org/10.1056/NEJM199907153410303.
7. **Masters EJ, Storch GA, Sumner JW.** 2009. *Ehrlichia ewingii* in an immunocompetent adult. Mo. Med. **106:**301–303.
8. **Pritt BS, Sloan LM, Johnson DK, Munderloh UG, Paskewitz SM, McElroy KM, McFadden JD, Binnicker MJ, Neitzel DF, Liu G, Nicholson WL, Nelson CM, Franson JJ, Martin SA, Cunningham SA, Steward CR, Bogumill K, Bjorgaard ME, Davis JP, McQuiston JH, Warshauer DM, Wilhelm MP, Patel R, Trivedi VA, Eremeeva ME.** 2011. Emergence of a new pathogenic Ehrlichia species, Wisconsin and Minnesota, 2009. N. Engl. J. Med. **365:**422–429. http://dx.doi.org/10.1056/NEJMoa1010493.
9. **Dumler JS, Madigan JE, Pusterla N, Bakken JS.** 2007. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin. Infect. Dis. **45**(Suppl 1)**:**S45–S51. http://dx.doi.org/10.1086/518146.
10. **Thomas RJ, Dumler JS, Carlyon JA.** 2009. Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis. Expert Rev. Anti Infect. Ther. **7:**709–722. http://dx.doi.org/10.1586/eri.09.44.
11. **Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS.** 1996. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. JAMA **275:**199–205.
12. **Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR.** 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. **51:**2145–2165. http://dx.doi.org/10.1099/00207713-51-6-2145.
13. **Klein MB, Miller JS, Nelson CM, Goodman JL.** 1997. Primary bone marrow progenitors of both granulocytic and monocytic lineages are susceptible to infection with the agent of human granulocytic ehrlichiosis. J. Infect. Dis. **176:**1405–1409. http://dx.doi.org/10.1086/517332.
14. **Paddock CD, Sumner JW, Shore GM, Bartley DC, Elie RC, McQuade JG, Martin CR, Goldsmith CS, Childs JE.** 1997. Isolation and characterization of *Ehrlichia chaffeensis* strains from patients with fatal ehrlichiosis. J. Clin. Microbiol. **35:**2496–2502.
15. **Dumler JS, Dawson JE, Walker DH.** 1993. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. Hum. Pathol. **24:**391–396. http://dx.doi.org/10.1016/0046-8177(93)90087-W.
16. **Bayard-Mc Neeley M, Bansal A, Chowdhury I, Girao G, Small CB, Seiter K, Nelson J, Liveris D, Schwartz I, Mc Neeley DF, Wormser GP, Aguero-Rosenfeld ME.** 2004. In vivo and in vitro studies on *Anaplasma phagocytophilum* infection of the myeloid cells of a patient with chronic myelogenous leukaemia and human granulocytic ehrlichiosis. J. Clin. Pathol. **57:**499–503. http://dx.doi.org/10.1136/jcp.2003.011775.
17. **Borjesson D, Macnamara K, Johns J, Winslow G.** 2009. *Anaplasma phagocytophilum* and *Ehrlichia muris* induce cytopenias and global defects in hematopoiesis. Clin. Microbiol. Infect. **15**(Suppl 2)**:**66–67. http://dx.doi.org/10.1111/j.1469-0691.2008.02182.x.
18. **MacNamara KC, Racine R, Chatterjee M, Borjesson D, Winslow GM.** 2009. Diminished hematopoietic activity associated with alterations in innate and adaptive immunity in a mouse model of human monocytic ehrlichiosis. Infect. Immun. **77:**4061–4069. http://dx.doi.org/10.1128/IAI.01550-08.
19. **Childs JE, Sumner JW, Nicholson WL, Massung RF, Standaert SM, Paddock CD.** 1999. Outcome of diagnostic tests using samples from patients with culture-proven human monocytic ehrlichiosis: implications for surveillance. J. Clin. Microbiol. **37:**2997–3000.
20. **Walls JJ, Aguero-Rosenfeld M, Bakken JS, Goodman JL, Hossain D, Johnson RC, Dumler JS.** 1999. Inter- and intralaboratory comparison of *Ehrlichia equi* and human granulocytic ehrlichiosis (HGE) agent strains for serodiagnosis of HGE by the immunofluorescent-antibody test. J. Clin. Microbiol. **37:**2968–2973.
21. **Paddock CD, Folk SM, Shore GM, Machado LJ, Huycke MM, Slater LN, Liddell AM, Buller RS, Storch GA, Monson TP, Rimland D, Sumner JW, Singleton J, Bloch KC, Tang YW, Standaert SM, Childs JE.** 2001. Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in persons coinfected with human immunodeficiency virus. Clin. Infect. Dis. **33:**1586–1594. http://dx.doi.org/10.1086/323981.
22. **Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD).** Last updated 5 September 2013. Statistics and epidemiology: annual cases of ehrlichiosis in the United States. http://www.cdc.gov/ehrlichiosis/stats/.
23. **Peters TR, Edwards KM, Standaert SM.** 2000. Severe ehrlichiosis in an adolescent taking trimethoprim-sulfamethoxazole. Pediatr. Infect. Dis. J. **19:**170–172. http://dx.doi.org/10.1097/00006454-200002000-00021.
24. **Schutze GE, Buckingham SC, Marshall GS, Woods CR, Jackson MA, Patterson LE, Jacobs RF.** 2007. Human monocytic ehrlichiosis in children. Pediatr. Infect. Dis. J. **26:**475–479. http://dx.doi.org/10.1097/INF.0b013e318042b66c.
25. **Nayak SU, Simon GL.** 2013. Myocarditis after trimethoprim/sulfamethoxazole treatment for ehrlichiosis. Emerg. Infect. Dis. **19:**1975–1977. http://dx.doi.org/10.3201/eid1912.121459.