**Anaplasmataceae-Specific PCR for Diagnosis and Therapeutic Guidance for Symptomatic Neoehrlichiosis in Immunocompetent Host**

Michael Schwameis, Julia Auer, Dieter Mitteregger, Ingrid Simonitsch-Klupp, Michael Ramharter, Heinz Burgmann, Heimo Lagler

_Candidatus_ Neoehrlichia is increasingly being recognized worldwide as a tickborne pathogen. We report a case of symptomatic neoehrlichiosis in an immunocompetent Austria resident who had recently returned from travel in Tanzania. The use of _Anaplasmataceae_-specific PCR to determine the duration of antimicrobial therapy seems reasonable to avert recrudescence.

**The Study**

In January 2013, a 30-year-old white woman with no relevant medical history was admitted to the Division of Infectious Diseases and Tropical Medicine, General Hospital of Vienna, in Vienna, Austria, because of a 3-week history of high fevers (up to 39.9°C), chills, and night sweats accompanied by headache, muscle pain, and malaise. Four weeks before hospitalization, the woman had returned from a 28-day vacation in Tanzania, and we illustrate the applicability of _Anaplasmataceae_-specific PCR for diagnosis and therapeutic guidance.

**Human Neoehrlichiosis**

Human neoehrlichiosis is an infectious disease that primarily affects immunocompromised persons and persons with severe concurrent medical conditions (1–5). We describe symptomatic _Candidatus_ Neoehrlichia infection in an otherwise healthy woman who had returned from a 28-day vacation in Tanzania, and we illustrate the applicability of _Anaplasmataceae_-specific PCR for diagnosis and therapeutic guidance.

**On hospitalization day 5, a peripheral blood sample was tested by using a 16S rRNA gene–based eubacterial broad range PCR (SepsiTest; Molzym GmbH & Co. KG, Bremen, Germany); results were positive. The amplification products (300 bp) were sequenced (GenBank accession no. EF633744.1; only 1 database entry was available) and 97% (293/301 bp) homology with _Candidatus_ Neoehrlichia lotoris (GenBank accession no. KT895260) and compared, using BLAST (http://blast.ncbi.nlm.nih.gov/), with known sequences in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) database. The sequence showed 98% (294/300 bp) homology with _Candidatus_ Neoehrlichia lotoris (GenBank accession no. EF633744.1; only 1 database entry was available) and 97% (293/301 bp) homology with _Candidatus_ Neoehrlichia microensis (GenBank accession no. KF155504.1; several database entries were available and showed a reproducible single base deletion at position 225). These findings were confirmed by _Anaplasmataceae_-specific 16S ribosomal RNA gene–based PCR. Primer pairs EHR16SD (5′-GTT ACC YAC AGA AGA AGT CC-3′) and EHR16SR (5′-TAG CAC TCA TCG TTT ACA GC-3′) were chosen to amplify a 345-bp fragment (6). The protocol was adjusted to that in the manual for High-Fidelity PCR enzyme mix (Thermo Scientific, Waltham, MA, USA) and to that of Brown et al. (7). Bidirectional sequencing of the 345-bp amplicon showed a sequence of 243 bp corresponding to the cDNA strand (GenBank accession no. KT953340) and yielded similar results: 97% (235/243 bp) sequence homology was shared with _Candidatus_ Neoehrlichia lotoris (GenBank accession no. EF633744.1), and 96% (235/244 bp) sequence homology was shared with _Candidatus_ Neoehrlichia microensis (GenBank accession no. JQ359046.1). Because the percentages of shared homologies were not sufficient to attribute the identified microbial agent to an official species, we tentatively named the agent _Candidatus_ Neoehrlichia Tanzania. In addition, a microscopy review of Giemsa-stained blood smears obtained within the first days of admission...

Author affiliation: Medical University of Vienna, Vienna, Austria

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showed structures possibly equivalent to microbial patho-
gens within leukocytes (Figure 1).

Antimicrobial treatment with oral doxycycline (300
mg per day) was subsequently initiated, resulting in im-
provement in the patient’s overall condition within 2 days
and in a continuous decrease of all inflammation markers,
normalization of platelet counts, and abatement of fever
(Figure 2). However, serum Neoehrlichia DNA remained
detectable at high levels. To provide the optimal duration
of antibiotic treatment, we performed daily Anaplasmata-
ceae-specific 16S PCR measurements of blood samples.
Over the next 10 days of therapy, the DNA signal intensity
continuously diminished. Doxycycline was stopped 1 day
after disappearance of Neoehrlichia serum DNA.

In contrast with patients in previously published re-
ports of human neoehrlichiosis, the patient described in
our report was a healthy young woman without concurrent
medical conditions. She had signs and symptoms of disease

Table. Clinical data at admission and primary diagnostic test results for a patient with Candidatus Neoehrlichia infection,
Austria, 2013*

| Clinical variable                          | Finding/value                                      |
|------------------------------------------|----------------------------------------------------|
| Subjective symptoms                      | Malaise, diffuse muscle pain, dull headache (without signs of meningism), and tenderness in the left upper abdominal quadrant |
| Tympnic temperature                      | 37.8°C, while taking acetaminophen                 |
| Heart auscultation                        | Systolic murmur (right sternal border), tachycardia (125 beats per minute) |
| Condition of skin                         | No rash or signs of cutaneous exposure to arthropods |
| Laboratory testing†                       |                                                    |
| C-reactive protein                        | 5 mg/dL (<0.5)                                    |
| Procalcitonin                             | 0.14 ng/mL (<0.5)                                 |
| Leukocyte count                           | 3.9 x 10^9/L (4–10)                               |
| Neutrophils                               | 53% (50–75)                                       |
| Lymphocytes                               | 27% (25–40)                                       |
| Monocytes                                 | 16% (0–12)                                        |
| Fibrinogen                                | 480 mg/dL (180–390)                               |
| Serum amyloid A                           | 164 mg/dL (<5)                                    |
| γ-globulins                               | 26.2% (11.1–18.8)                                 |
| Erythrocyte sedimentation rate            | 70 mm/h (<15)                                     |
| Platelet count                            | 121 x 10^9/L (150–350)                            |
| Hemoglobin                                | 9 g/dL (12–16)                                    |
| Chest radiography                         | No consolidations, no opacities                   |
| Abdominal ultrasonography                 | Splenomegaly of 15.5 × 6.7 cm                     |
| Transesophageal echocardiography          | Normal cardiac function and valves, no evidence of vegetations |
| Cranial computed tomography               | Parasagittal meningioma, otherwise normal         |
| Ophthalmologic examination                | Bilateral papilloedema                             |
| Cerebrospinal fluid                       | Clear and colorless; absolute cell count 4/μL protein, glucose, and lactate levels within reference range |
| Abdominal ultrasonography                 | Splenomegaly, 15.5 × 6.7 cm                       |
| Urinary dip stick and urinary cultures    | No growth                                          |
| Blood cultures                            | No growth                                          |
| Serologic testing                         |                                                    |
| HIV                                       | Negative                                           |
| Hepatitis B and C viruses                 | Negative                                           |
| Epstein-Barr virus                        | Negative                                           |
| Cytomegalovirus                           | Negative                                           |
| Mycoplasma spp.                           | Negative                                           |
| Adenovirus                                | Negative                                           |
| Enterovirus                               | Negative                                           |
| Coxsackievirus                            | Negative                                           |
| Influenza A, B, and C viruses             | Negative                                           |
| Parainfluenza virus                       | Negative                                           |
| Anaplasma spp.                            | Negative                                           |
| Rickettsia spp.                           | Negative                                           |
| Tuberculous mycobacteria                  | Negative                                           |
| Plasmodium spp.                           | Negative                                           |
| Syphilis (VDRL, TPPA)                     | Negative                                           |
| PCR testing                               |                                                    |
| Leishmania spp.                           | Negative                                           |
| Trypanosoma spp.                          | Negative                                           |
| Plasmodium spp.                           | Negative                                           |
| Giemsa-stained thin and thick blood smears|                                                    |

*TPPA, Treponema pallidum particle agglutination assay; VDRL, Venereal Disease Research Laboratory test.
†Laboratory data are given as absolute number or percentage (reference range).
for 4 weeks without any symptomatic improvement before therapy was initiated. Treatment led to a rapid clinical response and rapid clearance of serum Neoehrlichia DNA, which may be attributable to her otherwise good medical condition but may also reflect high antimicrobial efficiency of the high-dose therapeutic regimen applied.

Because symptomatic neoehrlichiosis usually occurs in patients with immunosuppression, we examined the patient for an underlying malignancy or autoimmune disorder. These conditions were largely ruled out by negative test results for HIV and mycobacteria and by a normal finding on 18F-FDG-PET/CT (18F-fluorodeoxyglucose-positron emission tomography/computed tomography) examination (except for enhanced splenic FDG uptake). The patient had moderate disease with nonspecific symptoms partly resembling those of human anaplasmosis. The splenomegaly was attributed to polyclonal B cell activity (indicated by hypergammaglobulinemia), but it could also have resulted from direct infection of splenic sinusoidal cells, as found in Neoehrlichia-infected Wistar rats (8). However, spleen size decreased over the course of antimicrobial treatment and reached a normal diameter by a 3-week follow-up examination.

No evidence exists regarding the exact incubation period of human neoehrlichiosis, but it probably approximates that of human granulocytic anaplasmosis, suggesting that the patient in our study acquired neoehrlichiosis in Tanzania. Nonetheless, several tickborne diseases are highly endemic in Austria. Glatz et al. (9) recently reported a 4.2% prevalence of Candidatus Neoehrlichia in Ixodes ricinus ticks in Austria. However, in the 5-day period between returning home from Tanzania and fever onset, the patient in our study had stayed in the urban area of Vienna; thus, the possibility that she may have been

![Figure 1. Giemsa-stained blood smear from an immunocompetent patient with Candidatus Neoehrlichia infection, Austria, 2013. The blood smear shows possible microbial pathogens within leukocytes. Scale bar indicates 10 µm.](image1)

![Figure 2. Body temperature and markers of inflammation over the course of hospitalization for a patient with Candidatus Neoehrlichia infection, Austria, 2013. Day 0 indicates time of admission. Antimicrobial therapy with doxycycline (300 mg per day) was begun on day 5 and led to a rapid resolution of clinical symptoms and a progressive decrease of all inflammatory parameters. Daily Anaplasmataceae-specific PCR measurements guided therapy, which was safely stopped 1 day after disappearance of serum Candidatus Neoehrlichia DNA. Upper right shows 1.5% agarose gel electrophoresis analysis. The intensity of the 345-bp DNA band amplified from blood samples progressively decreased over the course of treatment. CRP, C-reactive protein; SAA, serum amyloid A; Temp, tympanic temperature.](image2)
exposed to ticks in Austria is limited but not excluded. Furthermore, the patient returned to Vienna at the height of winter, making the possible transmission of Candidatus Neoehrlichia by a domestic tick even less plausible. On the other hand, no epidemiologic data are available on the prevalence of Candidatus Neoehrlichia in ticks in Tanzania, but Candidatus Neoehrlichia mikurensis was recently found in ticks of 2 species collected in Nigeria (10). Thus, the presence of Candidatus Neoehrlichia in ticks in Tanzania and the risk for transmission from ticks to humans seem conceivable. Because a 16S rDNA sequence difference of >2% is arbitrarily considered as indicative for delineation at the species level, it seems possible that a new Candidatus Neoehrlichia agent was detected in the patient in our study.

Conclusions
This case demonstrates that Candidatus Neoehrlichia can affect healthy persons who have no underlying hematologic or autoimmune disorders. Neoehrlichiosis should be considered in the differential diagnosis for patients with appropriate symptoms, independent of concurrent conditions and immune status. As long as no evidence-based recommendations regarding treatment of human neoehrlichiosis exist, it seems reasonable to use Anaplasmataceae-specific PCR to monitor treatment response and determine the duration of antimicrobial therapy to avert recrudescence.

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Dr. Schwameis is an internal medicine resident at the Department of Clinical Pharmacology, Medical University of Vienna. His research interests include staphylococcal bloodstream infections and infection-associated coagulopathy.

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Address for correspondence: Heimo Lagler, Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; email: heimo.lagler@medunivwien.ac.at; Michael Schwameis, Department of Clinical Pharmacology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; email: michael.schwameis@meduniwien.ac.at

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