Infections With the Tick-Borne Bacterium “Candidatus Neoehrlichia mikurensis” Mimic Noninfectious Conditions in Patients With B Cell Malignancies or Autoimmune Diseases

Anna Grankvist,1 Per-Ola Andersson,2 Mattias Mattsson,3 Monica Sender,2 Krista Vaht,2 Linnea Höper,4 Egidija Sakiniene,4 Estelle Trysberg,5 Martin Stenson,6 Jan Fehr,6 Sona Pekova,7 Christian Bogdan,8 Guido Bloomberg,9 and Christine Wennerås1,2

1Department of Clinical Microbiology/Infectious Diseases, Sahlgrenska Academy, Sahlgrenska University Hospital, Göteborg; 2Department of Hematology and Coagulation, Sahlgrenska University Hospital, Göteborg; 3Department of Internal Medicine, Karlstad Hospital, Karlstad; 4Department of Rheumatology, Sahlgrenska University Hospital, Göteborg and 5Department of Medicine, Kungälv Hospital, Kungälv, Sweden; 6Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; 7Laboratory for Molecular Diagnostics, CHAMBON Laboratories, Prague, Czech Republic; 8Mikrobiologische Institut—Klinische Mikrobiologie, Immunologie und Hygiene, Friedrich Alexander Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany and 9Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Background. Candidatus Neoehrlichia mikurensis is a newly discovered noncultivable bacterium spread among ticks and rodents in Europe and Asia that can infect humans, particularly immunocompromised patients.

Methods. We compiled clinical and laboratory data from 11 patients with hematological malignancies or autoimmune diseases who were diagnosed with Candidatus N. mikurensis infection in Europe 2010–2013. Both published (6) and unpublished cases (5) were included.

Results. The patients had a median age of 67, were mostly male (8/11), and resided in Sweden, Switzerland, Germany, and the Czech Republic. All but one had ongoing or recent immune suppressive treatment and a majority were splenectomized (8/11). Less than half of them recalled tick exposure. The most frequent symptoms were fever (11/11), localized pain afflicting muscles and/or joints (8/11), vascular and thromboembolic events (6/11), that is, deep vein thrombosis (4), transitory ischemic attacks (2), pulmonary embolism (1), and arterial aneurysm (1). Typical laboratory findings were elevated C-reactive protein, leukocytosis with neutrophilia, and anemia. Median time from onset of symptoms to correct diagnosis was 2 months. In at least 4 cases, the condition was interpreted to be due to the underlying disease, and immunosuppressive therapy was scheduled. All patients recovered completely when doxycycline was administered.

Conclusions. Candidatus N. mikurensis is an emerging tick-borne pathogen that may give rise to a systemic inflammatory syndrome in persons with hematologic or autoimmune diseases that could be mistaken for recurrence of the underlying disease and/or unrelated arteriosclerotic vascular events. Awareness of this new pathogen is warranted among rheumatologists, hematologists, oncologists, and infectious disease specialists.

Keywords. B-cell malignancies; human; infection, Neoehrlichia; tick-borne.

“Candidatus Neoehrlichia mikurensis” was first reported to be a human pathogen in 2010 [1–3]. Until now, a total of 15 cases have been reported from Sweden, [2] Germany, [3] Switzerland, [1, 4] the Czech Republic, [5] and China [6]. A little more than half of the published cases concerned apparently healthy persons, [1, 3, 6], whereas the remainder were immunocompromised patients [2–5].
From 2004 but under other names [10]

I. ovatus, I. frontalis

widely distributed among ticks ([12,17, 18], Central [19]. No reports exist from the Amer-

ican or systemic in

As recently as 2018, it has been shown that others have reported

Ca. N. mikurensis

received its name in 2004, after it was dis-

covered in ticks and rodents on the Japanese island of Mikura

by means of polymerase chain reaction (PCR) directed against

conserved genes of the bacterial genome, for example, 16SrRNA

and groESL sequences [7]. Transmission electron microscopy of

infected rats showed small cocci in the cytoplasm of endothelial

cells [7]. Phylogenetic analyses reveal it to be a new species be-

 longing to the family Anaplasmataceae. Its closest relative is

Candidatus Neoehrlichia lotoris, which primarily infects rac-

coons [8]. Other related species are Ehrlichia ruminantium,

Ehrlichia chaffeensis, and Anaplasma phagocytophilum [7,9].

All these bacterial species are strict intracellular pathogens

that can only be cultivated in cell lines. N. mikurensis is denom-

inated “Candidatus” because no one to our knowledge has yet

reported its cultivation. The target cells of Neoehrlichia infec-

tion in humans are yet to be defined although polymorphonu-

clear granulocytes [5] and endothelial cells have been implicated

[9]. At present, the only diagnostic option is either pan-bacterial

PCR (targeting the 16S rRNA gene) followed by sequence anal-

ysis, [2] or a specific real-time polymerase chain reaction (RT-

PCR), [4] performed on whole blood, plasma, or bone marrow.

No serological tests are available, and the lack of serological

cross-reactivity with either Anaplasma phagocytophilum or

Ehrlichia chaffeensis [3, 7] precludes the use of Anaplasma-
or Ehrlichia-based indirect fluorescence antibody tests.

In retrospect, it has become clear that others have reported

on the same bacterial species prior to Kawahara’s original report

from 2004 but under other names [10–15]. Ca. N. mikurensis

is widely distributed among ticks (Ixodes ricinus, I. persulcatus,

I. ovatus, I. frontalis), [8,16] rats, field mice, and voles in North-

ern [12,17,18], Central [19–22], and Eastern Europe [16,23,24],

Asia [7,25,26], and Africa [27]. No reports exist from the Am-

ericas or Australia. Rodents appear to be healthy carriers of

Ca. N. mikurensis and may be viewed as the zoonotic reservoir

[17,28]. The only other animal species besides humans that has

been shown to become sick due to Ca. N. mikurensis infection

are dogs [29].

One peculiarity of Neoehrlichia infection in humans is the ac-

cumulation of cases among patients with B-cell malignancies or

rheumatological diseases, many of whom have been splenec-
tomized [2,4,5]. Another distinguishing feature is the high prev-

ealence of thromboembolic complications among these patients

[2–4]. Importantly, diagnosis of infection could be missed or se-

verely delayed as the clinical picture of Neoehrlichia infection

may be misinterpreted as noninfectious conditions, for exam-

ple, arteriosclerotic thromboembolism with secondary fever,
or systemic inflammation due to a new bout or recurrence of

the underlying rheumatologic or hematologic disease.

The objective of this study was to provide a synopsis of new,

unpublished cases of Neoehrlichia infection in patients with

rheumatic/autoimmune diseases or hematologic malignancies

(n = 5) along with already published cases (n = 6) regarding

host factors, clinical picture, and laboratory findings. The goal

is to make rheumatologists, hematol/oncologists, and infec-

tious disease specialists aware of this new infectious disease as

patients may remain untreated, or even worse, be given chem-

otherapy and/or immune suppressive therapy against the un-

derlying disease.

METHODS

Data Collection

All but 1 of the 6 Swedish patients reside on the west coast of

Sweden, within a 100 km-radius from the city of Göteborg.

They attend the rheumatology and/or hematology clinics at

the Sahlgrenska University Hospital in Göteborg and Kungälv’s

Hospital. One patient was treated at Karlstad’s Hospital. The

Swedish patients have given oral or written consent to publish

nonidentifiable data. Some of the patients participate in the

“Neo-VÄST” study, which was approved by the Local Ethics

Committee in Göteborg, Sweden.

The details on the origin, clinical recruitment, and follow-up

of all other patients summarized here have been reported else-

where [3–5].

Procedures

Clinical data have mainly been derived from the patients’ jour-

nals and attending physicians in charge of the respective pa-

tients; anamnestic data were derived from the patients

themselves. Some data were obtained from published patient

cases [2–5]. Laboratory data were also obtained from patient

charts and published case reports. GraphPad prism 5.0 software

was used to calculate medians and 25/75 percentiles.

The diagnosis of Ca. N. mikurensis infection was in all cases

based on PCR analysis of peripheral blood samples followed by

sequence analysis. Specimens of bone marrow, cerebrospinal

fluid, and blood culture contents were also tested for the

presence of Neoehrlichia DNA. Pan-bacterial PCR directed

against either the V1–V4 region (Sweden, Germany, Switzer-

land) [2–4] or V4–V8 region (Czech Republic) [5] of the 16S

rRNA-gene was used to amplify bacterial DNA. Taqman-

based real-time PCR assays targeting either parts of the groEL

gene or the 16 SrRNA gene [2–4] of Ca. N. mikurensis, incor-

porating internal control plasmids containing the same respec-

tive gene sequences, were used to estimate the concentrations

of bacterial gene copies in patients’ samples.

Statistical Analyses

Statistical analyses were not performed because there was no

control group for comparative analyses.

Neoehrlichia in Immunocompromised Patients • CID 2014:58 (15 June) • 1717
The funding sources (Västra Götaland Regional Research and Development Fund, ALF Research Fund, and Cancer and Allergy Foundation) are noncommercial organizations that have had no impact on the design of the present study.

RESULTS

Host Factors

The 11 patients were all middle-aged or elderly (median age 67 years, range 54–77) and had an underlying disease involving the adaptive immune system (Table 1). More specifically, the patients had either malignant clonal expansion of lymphocytes, in most cases malignant lymphoma or chronic lymphocytic leukemia engaging B cells, or clonal expansion of autoreactive lymphocytes, giving rise to autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or psoriasis (Table 1). Three patients first had an autoimmune disease and subsequently also developed a malignant hematological disease (patients 3, 4, and 6). A surprisingly high fraction of the patients (73%) had no spleen; in most instances it had been removed to diagnose the suspected hematologic disease (Table 1). Also, a majority of the patients had ongoing or recent (preceding 3 months) chemotherapy or immunosuppressive treatment with systemic corticosteroids and/or rituximab (anti-CD20 monoclonal antibody).

Symptoms and Clinical Signs

All patients had systemic inflammation, manifested as high fever, often spiking up to 40°C, with chills and nightly sweats (clinical signs are shown in Table 2). Another prominent finding was different types of localized pain, such as migrating muscular pain, stiff neck, tender subcutaneous veins, and joint pain engaging knees, mandibular/temporal joints, and elbows—2 of the patients with myalgia and arthralgia required opiates because the pain was not relieved by corticosteroid treatment. All but one of the patients were hospitalized, and 2 were admitted more than once until the correct diagnosis was established.

The most striking finding was the high rate of vascular and thromboembolic events associated with this infectious disease. More than half of the patients were affected (6/11), and some of them severely: 2 patients had deep vein thrombosis above the knee, a third patient developed deep vein thrombosis twice (upper arm and leg), and another patient developed deep vein thrombosis, pulmonary embolism, and transitory ischemic attacks. The 2 patients with transitory ischemic attacks had repeated episodes that engaged both sides of the body. One patient had severe arterial inflammation with aneurysms.
Possibly, the ankle swelling observed among several patients reflected local blood vessel inflammation. Two patients who did not develop any vascular or thromboembolic events were on prophylactic anticoagulation treatment at the time of diagnosis (patient 4, low-dose aspirin because of atrial fibrillation; patient 6, chronic low-molecular heparin because of repeated DVT and pulmonary embolism). Hence, multiple thromboembolic events seem to characterize Ca. N. mikurensis infection.

Less specific findings displayed by some patients were skin rashes of the lower extremities, that is, erythema nodosum and erysipelas, and diarrhea. Significant weight loss developed in a few patients, particularly those with systemic inflammation of long duration. Pulmonary symptoms such as cough were discrete and infrequent, and the majority of patients lacked pulmonary infiltrates on chest radiograms.

### Laboratory Findings

The most abnormal laboratory findings among the routine parameters that are usually analyzed in patients with systemic inflammation are listed in Table 3. The only consistent finding among all patients was elevation of the acute phase reactant C-reactive protein in serum, with levels ranging from 4- to 74-fold higher than the cutoff level. Leukocytosis with neutrophilia and anemia were also typical findings. About half of the patients had at some point raised levels of lactate dehydrogenase in serum and hyponatremia, respectively. Lowered platelet counts and elevated transaminase concentrations were rare findings.

### Diagnostics and Bacterial Load

The diagnosis of Ca. Neoehrlichia infection was in all cases based on pan-bacterial PCR analysis of segments of the 16S rRNA gene followed by sequence and homology analyses. EDTA- or citrate-anticoagulated peripheral blood samples were uniformly used for diagnosis, but Neoehrlichia DNA was also detected in plasma specimens (most patients), blood culture flask contents (patient 1) and in bone marrow samples (patients 7, 8, and 11). However, Neoehrlichia was not detected in cerebrospinal fluid of patient 3 despite neurologic symptoms; these symptoms were interpreted to result from infection-related transitory ischemic attacks.

Indirect estimates of the concentrations of bacteria in patient samples were performed using real-time PCR and internal control plasmids. If one assumes that Neoehrlichia contains only 1 copy of the 16S rRNA gene or heat shock protein gene groEL, bacterial concentrations were on the order of 10^9/mL blood among these patients (median value of 2 × 10^8 bacterial gene copies/mL in 5 assessed patients, 25/75 percentile = 0.05–7.8 × 10^6/mL). The levels were even higher in bone marrow: up to 35 × 10^7/mL (patient 7).

### Diagnostic Delay

Diagnostic delay was considerable, the median number of days from the onset of symptoms to diagnosis was 60 (25/75 percentile = 26–135). All patients were subjected to extensive microbiological investigations and given empiric antibiotics. Some patients received corticosteroids because of suspected “disease fever”, for example, “tumor fever” or “autoimmune fever.” At least 4 of the patients had been scheduled to receive chemotherapy but narrowly escaped such treatment once Ca. N. mikurensis infection was diagnosed. Five of the 11 patients recalled tick bites. Three patients fell ill during winter/early spring (January–March in Europe), one of whom (patient 4) received blood transfusions prior to development of infectious symptoms (Table 1).

### Response to Antibiotics

All patients had been given empiric antibiotics treatment other than doxycycline before the diagnosis of Ca. N. mikurensis infection was set. No response was seen to benzyl-penicillin or piperacillin/tazobactam, amoxicillin ± clavulanate, clindamycin, third-generation cephalosporines (cefotaxime, ceftazidime), aminoglycosides (gentamicin), or quinolones (ciprofloxacin and levofloxacin). A transient response to meropenem was seen in a few cases. A complete response was achieved in all patients once they were treated with doxycycline (100 mg PO BID) for 3–6 weeks. Many patients experienced dramatic and rapid improvement within 1 day of initiation of doxycycline therapy. The median time to resolution of symptoms was 5 days (25/75 percentile = 2–8).
DISCUSSION

The purpose of this case review study was to highlight one reason for why human cases of *Neoehrlichia* infection may go unrecognized: the aberrant clinical picture seen among 2 particular groups of patients—middle-aged persons with autoimmune diseases or hematologic malignancies. First, myalgia, arthralgia, and/or fever are typical findings among patients with some of the autoimmune conditions described in this study, for example, psoriatic arthropathy, rheumatoid arthritis, or systemic lupus erythematosus. Second, if no infectious agents are discovered despite extensive microbiological investigations in patients with fever and systemic inflammation with a serious underlying condition, such as malignant lymphoma, chronic lymphatic leukemia, or autoimmune systemic rheumatic disease, the most common interpretation is that the condition reflects recurrence/deterioration of the underlying morbidity. This may ultimately result in erroneous, and potentially dangerous, administration of immune suppressive therapy such as chemotherapy, rituximab, and corticosteroids, which almost occurred in at least 4 of the cases documented in this study.

Another common finding among the *Neoehrlichia*-infected patients was the high incidence of thromboembolic/vascular complications; these were not interpreted to be the consequence of an infection but coincidental to a systemic inflammatory condition of unknown cause. In one case, fever was suspected to be secondary to widespread venous embolism [2]. As transitory ischemic attacks are quite common among older persons, it is understandable that this complication was initially seen as a separate phenomenon, not connected to the febrile episode. Finally, we report a case of arterial aneurysm in a *Neoehrlichia*-infected patient with underlying autoimmune disease. Although it is impossible to attribute causality between the development of the aneurysm and *Neoehrlichia* infection, there has been a previous report of cerebral arterial aneurysm in a purported previously healthy person with suspected *Neoehrlichia* infection, who actually died from intracerebral hemorrhage [3].

The pathogenic mechanisms behind the thromboembolic complications associated with *Neoehrlichia* infection are unknown. Is it a direct effect of infection? Does *Neoehrlichia* infect endothelial cells or exert toxic effects on the endothelium? Or is it an indirect effect of long-standing systemic inflammation with concomitant activation of coagulation [30]? Blood clot formation is a primitive infectious defense mechanism used by horse shoe crabs and other invertebrates to limit infectious processes [30]; the high rate of thromboembolic complications in our patients may have been the next best alternative for those with depressed B-cell immune responses to curb the infectious process.

The importance of the spleen in the infectious defense against *Neoehrlichia* is reflected by the high prevalence of splenectomized patients in this study. The well-known function of the spleen in the defense against encapsulated bacteria is not likely to be of relevance for *Ca. Neoehrlichia mikurensis* with its frail cell wall [7]. Instead, the spleen’s importance for the generation of “natural antibodies” [31] and maintenance of immunoglobulin M (IgM) memory B cells [32] is probably more pertinent. The very high loads of bacteria in the blood of patients most likely reflect their impaired immune state. However, no patient died despite the fact that diagnostic delay was significant. Moreover, the patients recovered within a week after doxycycline therapy was initiated. It appears as if 100 mg doxycycline twice daily for a 3-week period is sufficient to clear the infection.

There are several more reasons for the paucity of reported human *Neoehrlichia* cases: (1) the bacterium does not grow in cell-free media. In fact, to date no one to our knowledge has yet succeeded in cultivating the bacterium at all, which requires the use of eukaryotic cell lines. Hence, the diagnosis relies on pan-bacterial or specific PCR, which are not used as first-line diagnostic procedures. (2) Awareness of this new microbe is very low among medical practitioners. (3) Some of the symptoms arising from a *Neoehrlichia* infection may be attributed to other

Table 3. Laboratory Parameters of 11 Patients Diagnosed With Neoehrlichiosis

<table>
<thead>
<tr>
<th>Laboratory Parameter</th>
<th>Level</th>
<th>Range of Altered Parameter</th>
<th>Reference Level</th>
<th>Unit</th>
<th>No. of Patients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td>↑</td>
<td>19–370</td>
<td>&lt;5</td>
<td>g/L</td>
<td>11/11</td>
<td>100</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>↑</td>
<td>11–26</td>
<td>3.5–8.8</td>
<td>×10⁸/L</td>
<td>9/11</td>
<td>82</td>
</tr>
<tr>
<td>B-Neutrophils</td>
<td>↑</td>
<td>NR</td>
<td>1.8–7.5</td>
<td>×10⁹/L</td>
<td>9/11</td>
<td>82</td>
</tr>
<tr>
<td>B-Hemoglobin</td>
<td>↓</td>
<td>85–119</td>
<td>117–170</td>
<td>g/L</td>
<td>9/11</td>
<td>82</td>
</tr>
<tr>
<td>S-Sodium</td>
<td>↓</td>
<td>127–136</td>
<td>137–145</td>
<td>mmol/L</td>
<td>6/11</td>
<td>54</td>
</tr>
<tr>
<td>B-Lactate dehydrogenase</td>
<td>↑</td>
<td>3.9–15</td>
<td>1.8–3.4</td>
<td>μmol/L</td>
<td>5/11</td>
<td>45</td>
</tr>
<tr>
<td>B-platelet counts</td>
<td>↓</td>
<td>Slight decrease</td>
<td>145–348</td>
<td>×10⁹/L</td>
<td>2/11</td>
<td>18</td>
</tr>
<tr>
<td>S-Aspartate aminotransferase</td>
<td>↑</td>
<td>Slight increase (&lt;2-fold)</td>
<td>(0.25–0.75)</td>
<td>μmol/L</td>
<td>2/11</td>
<td>18</td>
</tr>
<tr>
<td>S-Alanine aminotransferase</td>
<td>↑</td>
<td>Slight increase (&lt;2-fold)</td>
<td>(0.15–1.1)</td>
<td>μmol/L</td>
<td>2/11</td>
<td>18</td>
</tr>
</tbody>
</table>

Abbreviations: B, blood; NR, not reported; S, serum.
infectious agents such as Borrelia, Anaplasma or a random “summer virus”. Doxycycline, the treatment of choice for Neoehrlichia infection, is equally efficient to treat infections perceived to be caused by Borrelia, Anaplasma, and Ehrlichia respectively. We propose the use of the term “neoehrlichiosis” as a complement to the longer “Candidatus Neoehrlichia mikurensis” infection, both in the interest of brevity, but also to focus on this new clinical infectious disease. Moreover, this nomenclature has already been used elsewhere [4].

The most likely route of transmission of the infection to humans is direct inoculation of the bacteria through the skin via the bite of infected ticks. The incubation period is unknown but has been estimated to be 5–21 days for the other human pathogenic ehrlichiae [33]. The fact that more than half of the patients were unaware of tick bite is in agreement with studies of Borrelia, where it is well-known that tick bites may pass unnoticed by patients [34]. Three patients apparently fell ill during winter/early spring, which was surprising as ticks are less active during this period of the year. Because one of the patients had received blood transfusions prior to the development of fever, we investigated if this might have been the route of transmission, as has been shown for Anaplasma [35] and Ehrlichia [36]. Unfortunately, no aliquot from the erythrocyte concentrate had been stored. One of the 2 blood donors claimed to have had a flulike episode prior to donation of blood; this donor tested negative for Neoehrlichia DNA but had recovered completely and was asymptomatic at the time of testing. Thus, we were unable to determine if blood transfusions are a possible route of Neoehrlichia infection.

To conclude, we hope to raise the awareness of this newly discovered microbe that may give rise to an infectious disease in elderly persons with autoimmune conditions and hematological malignancies. Physicians within the fields of rheumatology, hematology, oncology, and infectious diseases should be aware of this new infectious agent and their attention drawn to patients with suspected recurrence of the underlying autoimmune or hematologic disease where the clinical picture is atypical.

Notes

Financial support. This work was supported by Västra Götaland Regional Research and Development Fund, ALF Foundation, Strategic ALF Grant for Transplantation, the Bavarian Ministry for Environment, Health and Consumer Protection and the Swedish Cancer and Allergy Foundation.

Potential conflicts of interest. All authors: No reported conflicts. 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.

References


