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SHORT COMMUNICATION

Human granulocytic anaplasmosis in Austria: Epidemiological, clinical, and laboratory findings in five consecutive patients from Tyrol, Austria

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Abstract

We report five consecutive cases of *Anaplasma* (*A.*) *phagocytophilum* infection (the causative agent of human granulocytic anaplasmosis (HGA)) from western Austria. All infections were acquired between June and August in 2003 and 2004 in the Inn valley (Tyrol, Austria). Four patients required hospitalisation, one patient was treated as an outpatient. During the acute stage of illness, laboratory findings included thrombocytopenia (5/5), elevated C-reactive protein (5/5), elevated neopterin (5/5), elevated lactate dehydrogenase (4/5), and elevation of liver enzymes (4/5). Leukopenia (3/5) and elevated procalcitonin (2/5) were less frequently observed. All patients were treated with tetracyclines, which led to prompt improvement of the clinical conditions. Anti-platelet antibodies were observed in one of four patients, but remained unchanged after complete covalescence.

Keywords: *Anaplasma phagocytophilum*; HGA; HGE; Austria; Neopterin; Anti-platelet antibodies

Introduction

Human granulocytic anaplasmosis (HGA), formerly known as human granulocytic ehrlichiosis (HGE), is a tick-borne zoonosis caused by the intracellular bacterium *Anaplasma (A.) phagocytophilum* (formerly *Ehrlichia* (*E.*) *phagocytophila, Ehrlichia equi*, or HGE-agent) (Dumler et al., 2001). The clinical presentation is uncharacteristic. According to the Centers of Disease Control and Prevention (CDC), HGA is considered possible in patients with a history of tick exposure who present with a non-specific febrile illness, a temperature above 37.6 °C, and an otherwise unrevealing physical examination. It is considered probable, when such patients yield a single titre of at least 1:80 in the immunofluorescence assay (IFA) or PCR is positive or when typical morulae can be observed in neutrophils. The diagnosis is confirmed by an at least four-fold titre movement or when *A. phagocytophilum* can be grown in cell culture (Bakken and Dumler, 2000).

In Europe, the number of clinical cases is still comparably low: Less than 70 clinical cases have hitherto been reported from 11 European countries (Morais et al., 1991; Pierard et al., 1995; Baumann et al.,

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2003; Strle, 2004; Juceviciene and Marciuskiene, personal communication), although seroepidemiological studies imply that the causative agent is widespread in many European countries with seroprevalence rates ranging from 2 to almost 30% depending on the applied method and the study group (Strle, 2004).

To learn more about this emerging infectious disease in Europe, we here describe clinical symptoms and laboratory findings in five consecutive patients with HGA.

Materials and methods

Patients

Between 1 May and 31 October, 2003 and 2004, 191 patients were tested for antibodies against *A. phagocytophilum*, either because this was requested by the sender (142 cases, among them patients 1, 2, and 5) or because we suggested doing so after we had been contacted by the doctor in clinical charge (49 cases, including patients 3 and 4). We recommended testing for HGA when at least two of the following three criteria were fulfilled: (1) Tick bite within four weeks prior to onset of symptoms; (2) $>38\,^{\circ}C$ fever; (3) platelet count $<100{,}000/\mu l$ and C-reactive protein (CRP) >0.7 mg/dl and leukocyte count $<12{,}000/\mu l$ (Strle, 2004).

Serologic investigation

Sera were tested for IgG and IgM antibodies to *A. phagocytophilum* beginning at a dilution of 1:128 (IgG) or 1:160 (IgM) using a commercially available IFA (Focus Technologies, Cypress, California, USA) according to the manufacturer's protocol. For detection of HGA-specific IgM antibodies, samples were preincubated with goat monospecific antiserum to human IgG. All sera were serially diluted to the endpoint titre.

Sera were further tested for IgG antibodies with a commercially available Western blot (MarDx, Trinity Biotech, Dublin, Ireland) coated with purified proteins at 42 and 44 kDa of *A. phagocytophilum* according to the manufacturer's protocol. All titre movements were assessed by contemporary testing of paired samples. Plasma samples and aspirate from bone marrow were investigated by PCR as previously described (Walder et al., 2003a). Neopterin and procalcitonin concentrations were determined using commercially available immunoassays (BRAHMS Diagnostica, Hennigsdorf-Berlin, Germany).

Antibodies against platelets were assessed by an in-house validated ELISA. In brief, platelets were isolated from blood group 0/Rh-positive donors or from convalescent serum of the respective patient, lysed in lysis buffer, and the supernatant of the lysed platelets was used for the coating of flat bottom microtitre plates. Remaining binding sites were blocked with 250 µl/well of 1% low-fat milk powder (Merck 15363). Incubation was 60 min at room temperature followed by five washes with ELISA washing buffer, incubation with a horseradish peroxidase-labelled anti-human rabbit IgG conjugate (P212, Dako) for 30 min at room temperature, five washes with ELISA washing buffer, and finally incubation with tetramethylbenzidine in acetate puffer for 5 min in the dark. The reaction was stopped by adding 50 µl 1 M H_2SO_4, and the test was evaluated in a micro-ELISA reader at 450/620 nm.

Results

Five patients fulfilled the WHO-criteria for confirmed infection with *A. phagocytophilum* (HGA), i.e., the PCR yielded a positive result with subsequent seroconversion (patient 2) or they seroconverted against *A. phagocytophilum* with an at least fourfold titre rise within 2–4 weeks after onset of symptoms (four patients) (Bakken and Dumler, 2000). All infections were acquired in the Inn valley between June and August. Four patients were diagnosed in 2003 and one patient in 2004. Epidemiologic data, clinical findings, and laboratory parameters are summarised in the Tables 1–3.

One patient (#4) was treated as an outpatient, four patients required hospitalisation. Symptoms persisted for 5 (#4) to 22 (#1) days before HGA was diagnosed and never subsided before specific treatment was initiated. Patient 1 observed a particularly prolonged course of illness with four episodes of fever separated by several days of fatigue and general malaise.

Establishing the correct diagnosis turned out to be a particular challenge: Only in one patient, HGA was considered at the first visit, all other patients attended 2–3 different practitioners or hospitals. Three patients received antibiotic treatment at the first or second visit under suspicion of infection with atypical bacteria or

Table 1. Epidemiologic findings in human granulocytic anaplasmosis (HGA) patients in Tyrol, Austria

Patient #	1	2	3	4	5
Sex	Male	Male	Male	Female	Male
Age	33	40	63	46	32
Initial diagnosis	Viral infection	Suspected HGE	TBE	Suspected myelodysplastic syndrome	Unclear infection
Number of tick bites	1	Exposure	27	4	Exposure
Area of exposure	Rum	Innsbruck	Kundl	Innsbruck	Vomperbach or Absam
Work or leisure	Leisure	Leisure	Work	Leisure	Work

TBE: Tick-borne encephalitis.