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Human granulocytic anaplasmosis in eastern France: clinical presentation and laboratory diagnosis☆

Christelle Koebel[a,1], Aurélie Kern[a,1], Sophie Edouard[b], Anh Thu Hoang[a], Noéline Celestin[c], Yves Hansmann[c], Benoît Jaulhac[a,*], Philippe Brouqui[b], Sylvie Josiane De Martino[a]

[a]*Laboratoire de Bactériologie, Hôpitaux Universitaires de Strasbourg, 1, rue Koeberlé, Strasbourg Cedex, France*
[b]*Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), CNRS-IRD UMR 6236, Faculté de Médecine, Marseille, France*
[c]*Service de Maladies Infectieuses et Tropicales, Hôpitaux Universitaires de Strasbourg, 1, place de l'hôpital BP 426, 67091 Strasbourg Cedex, France*

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Abstract

Human granulocytic anaplasmosis (HGA) is a tick-borne infection characterised by an acute, nonspecific febrile illness. To date, few clinical cases have been supported by both a positive polymerase chain reaction (PCR) assay and subsequent seroconversion against *Anaplasma phagocytophilum* antigen all over Europe. We report here 3 consecutive cases of HGA that occurred during the summer of 2009 which fulfilled the epidemiologic, clinical, and biological criteria for HGA. These data highlight PCR assay on ethylenediaminetetraacetic acid blood rather than serology as the diagnostic test of choice during the acute phase of the disease. In endemic areas, HGA should be investigated in patients presenting an undifferentiated febrile illness with cytopenia, elevated rates of liver enzymes, and increased C-reactive protein values.

Keywords: Human granulocytic anaplasmosis; *Anaplasma phagocytophilum*; PCR; Serology; France; *Ixodes ricinus*

1. Introduction

Eastern France is an endemic area for Lyme borreliosis (Hubálek, 2009) and tick-borne encephalitis (Hansmann et al., 2006). The vector of both these diseases is *Ixodes ricinus*. Other illnesses occurring after *I. ricinus* bite—such as anaplasmosis, rickettsiosis, or babesiosis—have been reported in Europe. Human granulocytic anaplasmosis is an acute infectious disease caused by *Anaplasma phagocytophilum.* It has been detected in *I. ricinus* ticks by polymerase chain reaction (PCR) from most European countries including France (Brouqui et al., 2001). The infection rate of ticks in northeastern France is low: approximately 0.4% in nymphs and 1.2% in adult ticks (Ferquel et al., 2006).

HGA was first characterised in northern United States in 1994, and the first European case was reported in 1997 in Slovenia (Petrovec et al., 1997). Since then, although around 70 cases have been reported from several European countries, most of these were only based on the detection of specific antibodies, since PCR in acute phase was not often performed. However, according to European guidelines (Brouqui et al., 2004), laboratory confirmation of HGA must be based either on seroconversion or on 4-fold increase in antibody titer, either on the detection of *A. phagocytophilum* in blood, on culture, or specific PCR. Diagnosis of HGA currently frequently relies on clinical suspicion only due to the limited availability of rapid diagnostic tests such as PCR, and the absence of detectable serum antibodies at the time of clinical presentation. The largest series of confirmed HGA occurred in Central Europe, in particular in Slovenia (Lotric-Furlan et al., 2006) and in Scandinavia (Norway and Sweden) (Bjoërsdorff et al., 1999), but sporadic cases have also been described in the Netherlands (van Dobbenburgh et al., 1999), Austria (Walder et al., 2006), Italy (de la Fuente et al., 2005; Ruscio and Cinco, 2003), and Spain (García et al., 2006; Oteo et al., 2000). In France, the only confirmed laboratory case has been

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* Corresponding author. Tel.: +33-369-55-14-53; fax: +33-369-55-16-98.
E-mail address: jaulhac@unistra.fr (B. Jaulhac).
[1] These authors contributed equally to this work.

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reported in the eastern region of Alsace in 2003 (Remy et al., 2003).

Clinical presentation of HGA is nonspecific and usually consists of fever >38.5 °C, headache, malaise, myalgia, and/or arthralgia, and is often accompanied by laboratory abnormalities such as leukopenia, thrombocytopenia, and increased activity of hepatic enzymes (Brouqui et al., 2004). However, many clinical suspicions of this disease are not investigated, have no proven etiology, and are subsequently not reported.

We report here 3 consecutive HGA cases, confirmed by PCR, which occurred in the same area (Alsace, France) during the summer of 2009 and which fulfilled HGA epidemiologic, clinical, and biological criteria.

2. Materials and methods

2.1. Patients and samples

In Alsace, during the period June to September 2009, 15 patients presenting a febrile syndrome with a recent history of tick bites or exposure to ticks were tested for HGA at the Laboratory of Bacteriology, Strasbourg University Hospital. Ethylenediaminetetraacetic acid (EDTA) whole blood samples were systematically collected for blood smear and specific PCR assay during the febrile phase of the disease. For serologic testing, 2 sera were also collected: the first during the acute phase and the second 2 to 3 weeks afterwards, during the convalescent phase.

2.2. Microscopic examination

Blood smears were obtained from whole blood samples, stained with May–Grünwald–Giemsa and examined for the presence of morulae within the cytoplasm of neutrophils.

2.3. Molecular assay

DNA was extracted from whole-blood samples with the QIAamp® Mini kit (Qiagen®, Hilden, Germany). A Taqman®-based real-time PCR was applied to amplify a 73-bp fragment from the *A. phagocytophilum msp2/p44* gene. We used Primer Express® software version 2.0 (Applied Biosystems) to design specific oligonucleotides: forward primer, 5′-TGT AGC TAT GGA AGG CAG TGT TG-3′; reverse primer, 5′-GCG CTC GTA ACC AAT CTC AAG-3′; and probe, 6-VIC-CGG TAT TGG TGG TGC CAG GGT TGA-TAMRA. Real-time PCR was performed on ABI Prism® 7000 SDS (Applied Biosystems) under standard PCR conditions. All runs included DNA isolation controls and no-template controls to monitor the presence of contaminants during DNA extraction and/or in PCR reagents. The specificity of the primer set was confirmed by testing genomic DNA from a large diversity of bacteria including Gram-negative and Gram-positive bacteria, *Borrelia* sp., *Treponema* sp., *Leptospira* sp., *Mycoplasma* sp., and *Chlamydia* sp. (data not shown). Genomic DNA from *Rickettsia* sp., *Wolbachia* sp., *A. marginale*, and *A. platys* was not amplified by the PCR assay either (data not shown). Veterinary blood samples from cattle, dogs, and sheep infected by *A. phagocytophilum* were used as positive control (data not shown).

Serologic diagnosis of infection with *A. phagocytophilum* was made by IFA (Focus Diagnostics, Cypress, CA, USA). Specimens with IgM titers ≥1/40 and IgG titers ≥1/64 were considered positive. Demonstration of seroconversion or at least 4-fold increase in antibody titre between acute and convalescent serum was used to confirm HGA (Comer et al., 1999).

3. Results

Molecular testing using the *msp2/p44* gene yielded positive results in the acute-phase blood of 3 of the 15 patients tested. For the 12 patients with negative PCR assay, microscopic examination was negative and no specific antibodies were detected in acute-phase serum. Convalescent sera were available for 5 of these patients and were all negative. Laboratory findings were available for 6 patients among the 12 with a negative PCR assay; all these 6 presented leukopenia, thrombocytopenia, and increased levels of liver enzymes.

For the 3 patients with positive PCR results, examination of peripheral blood smears revealed cytoplasmic inclusions suggestive of *A. phagocytophilum* morulae (Fig. 1). All 3 patients presented undifferentiated illness characterised by high-degree fever, headache, myalgia and/or arthralgia, and lymphadenopathy; associated with bicytopenia; and characterised by elevated serum levels of liver enzymes (Table 1). Although the serologic test result of acute-phase serum was negative for these 3 patients, specific antibodies were detected in convalescent serum for each of them (Table 2).

Patients 1 and 2 were living in rural areas: both had domestic animals at home and were also in contact with wild animals. Patient 2 was a hunter and a fisherman. Patient 3 was a forest worker: she reported a tick bite 10 days before the onset of symptoms and cutaneous examination at the time of the disease revealed tick bite scars on her limbs.

Patient 1 presented a biphasic course of the illness starting with febrile syndrome. Three days after the onset of symptoms, he was admitted to hospital. Initial investigations showed neutropenia, thrombopenia, and elevated C-reactive protein (CRP) values, but etiologic investigation results remained negative. As the symptoms spontaneously decreased, the patient returned home with empiric antibiotic therapy (cefixime per os). The patient underwent a new onset of symptoms and came back to hospital where the treatment was stopped. New investigations showed a positive PCR result for *A. phagocytophilum*, but no haematologic and biochemical abnormalities. Doxycycline was started on day 24 following the first onset of symptoms. Patients 2 and 3