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

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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 ethylenediaminetraacetic 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.

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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, ricketttsiosis, 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, or 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...
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 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
received doxycycline 3 days after onset of fever, which led to prompt improvement of the clinical manifestations: the fever dropped within 24 h, the other symptoms resolved afterwards, and biological markers gradually returned to normal values. Only a marked fatigue persisted in both patients through to the third week after hospitalisation. Further clinical evaluation showed that all patients completely recovered. Overall, the course of illness was mild for these 3 patients.

4. Discussion

Eastern France, especially Alsace, is an area where *I. ricinus* is highly prevalent and Lyme borreliosis is very common (Hubálek, 2009). In the meantime, many febrile illnesses that occur after tick bites have no proven etiology and other pathogens such as *A. phagocytophilum*, the agent of HGA, share the same epidemiologic features as *B. burgdorferi*, since they are transmitted by the same vector.
During the summer of 2009, we investigated patients with febrile illness together with a recent history of tick bites or exposure to ticks. Three patients fulfilled the microbiological criteria for confirmed HGA (Brouqui et al., 2004): presence of morulae within granulocytes, positive PCR assay for *A. phagocytophilum*, and subsequent seroconversion against *A. phagocytophilum* antigen. All 3 patients had been exposed to ticks as they lived in rural areas or practiced leisure activities in wooden areas. Clinical characteristics and laboratory findings were similar to those reported in the United States (Dumler et al., 2007) and other European countries (Blanco and Oteo, 2002), including illness characterised by high-degree fever, headache, myalgia and/or arthralgia, and lymphadenopathy; associated with bicytopenia; and characterised by elevated levels of liver enzymes. The rapid clinical improvement observed in 2 patients shortly after initiation of doxycycline was also typical of this disease. This early antibiotic treatment may explain the weak antibody titers in convalescent sera, especially as these samples were obtained 50 to 90 days after the onset of symptoms. Similar to previous observations (Lotric-Furlan et al., 2006), the disease appeared to be mild and to resolve quickly, even in the absence of specific antibiotics: in patient 1, fever abated before the use of doxycycline.

To date, only a few clinical cases have been supported by both a positive PCR assay and subsequent seroconversion against *A. phagocytophilum* antigen all over Europe, with the largest series of patients being from Slovenia (Lotric-Furlan et al., 2006) (15 proven cases) and Scandinavia (Bjoërsdorff et al., 1999) (10 patients). There were also individual reports from other European countries. Nevertheless, seroepidemiologic surveys of HGA in the two mentioned countries have found prevalence of specific antibodies of 0–2.9% in blood donors and 1.5–21% in tick-exposed persons (Blanco and Oteo, 2002), which may suggest that HGA is mostly a mild or even an asymptomatic infection.

In European medical literature, HGA diagnosis has most frequently been based on serologic criteria only. However, an important limitation for diagnostic purposes is that these latter tests are often negative during the initial phase of the disease: in the Slovenian cohort (Lotric-Furlan et al., 2006), specific antibodies were detected at initial examination in only 25% of the positive patients, whereas PCR test results were positive for 63% of the patients between days 2 and 15 following the onset of symptoms. These results show that PCR on EDTA anticoagulated blood could be a much more efficient tool for diagnosing the initial stages of HGA as compared to serology. Presence of morulae has been observed in only 3 European cases (Garcia et al., 2006; Remy et al., 2003; van Dobbenburgh et al., 1999). Morulae are usually detected in blood smears only during an acute febrile episode; sensitivity is at the highest during the first week of infection, but false-positive results may also occur due to the observation of toxic granulation or Dohle bodies (Dumler and Brouqui, 2004). This procedure may therefore lack sensitivity and specificity. Isolation of *A. phagocytophilum* in HL-60 cell cultures inoculated with acute-phase blood has never been reported in Europe (Blanco and Oteo, 2002). A combination of improved diagnostic tools and of better awareness of the disease by physicians and biologists has enabled the description of 3 new cases of HGA in northeastern France over only 1 year. Specific PCR assay on EDTA anticoagulated blood may be the diagnostic test of choice during the first 2 weeks following the onset of symptoms, allowing a faster diagnosis than serology. HGA should be included in the differential diagnosis of patients presenting febrile illness associated with bicytopenia, elevated rates of liver enzymes, increased CRP values, and whose medical history reveals recent exposure to ticks.

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References


