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Comparison of clinical and laboratory characteristics of patients fulfilling criteria for proven and probable human granulocytic anaplasmosis

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Abstract

To assess the value of clinical definitions for human granulocytic anaplasmosis (HGA) epidemiological, clinical and laboratory findings in 50 adult patients with proven HGA (*Anaplasma phagocytophilum* isolated from blood, and/or positive PCR result, and/or seroconversion or ≥4−fold change in serum IFA antibody titres to *A. phagocytophilum*) and 46 patients with probable HGA (demonstration of serum antibodies to *A. phagocytophilum* in titres ≥1:256) were compared. Patients with proven HGA were older (55 versus 43.5 years; $p = 0.001$), were more often treated with doxycycline (31/50, 62% versus 11/46, 23.9%; $p < 0.001$), more frequently reported chills (40/50, 80% versus 17/46, 36.9%; $p < 0.001$), myalgia (37/50, 74% versus 21/46, 45.7%; $p = 0.005$) and cough (10/50, 20% versus 2/46, 4.4%; $p = 0.02$), and had more often abnormal laboratory findings such as thrombocytopenia (45/50, 90% versus 22/46, 47.8%; $p < 0.001$), abnormal liver function test results (45/50, 87% versus 22/46, 47.8%; $p < 0.001$), leukopenia (38/50, 76% versus 21/46, 45.7%; $p = 0.002$) and elevated serum CRP concentration (48/50, 96% versus 31/46, 67.4%; $p < 0.001$). The dissimilarities imply that in some patients fulfilling criteria for probable HGA the signs and symptoms most likely are not the result of a recent infection with *A. phagocytophilum* and indicate that clinical definitions used in the present study have a distinctive value.

Keywords: Human granulocytic anaplasmosis; Clinical diagnosis; Proven; Probable; Slovenia; *Anaplasma phagocytophilum*

1. Introduction

Human granulocytic anaplasmosis (HGA), caused by *Anaplasma phagocytophilum*, has been recognized as an emerging tick-borne disease in the United States and Europe [1,2]. The first European proven case of HGA was diagnosed in Slovenia and was published in 1997 [3].

Until 2015, >100 cases of HGA had been reported from Europe but several did not fulfill the European criteria for proven HGA [4]. The majority of patients contracted their illness in Slovenia [5], Scandinavia (predominantly Sweden) [6,7], and France [8] but reports on individual cases or small case series originated also from several other European countries [9–22].

HGA in humans is an acute disease. Its clinical presentation is unspecific and usually consists of fever, headache, malaise, myalgias and/or arthralgias, and is often accompanied by leukopenia, thrombocytopenia, elevated activity of hepatic enzymes and elevated concentration of C-reactive protein. Early in the course of HGA the disease can be diagnosed by the presence of morulae in granulocytes, demonstration of *A. phagocytophilum* by polymerase chain reaction (PCR) in whole blood and/or isolation of *A. phagocytophilum* from

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blood. Serological tests are commonly used but are often negative during the first few days of the disease [1].

To assess the value of clinical definitions for HGA we compared epidemiological, clinical and laboratory findings in patients with proven HGA and in those fulfilling criteria for probable HGA; all the patients were diagnosed in a prospective study on the etiology of febrile illness occurring after a tick bite or tick exposure. We hypothesized that the findings in the two groups will be dissimilar.

2. Patients and methods

The approach used in the present study and some patients included in the study (i.e. 13 with proven and 21 with probable HGA) have been reported previously [23].

2.1. Selection of patients

Patients >18 years old who participated in a prospective study on the etiology of febrile illness occurring within 30 days after a tick bite or tick exposure, with no alternative explanation for their illness (such as urinary infection, respiratory infection, erysipelas, pyogenic skin and soft tissue infection), in whom infection with *A. phagocytophilum* was established, qualified for the present report. The study was performed at the Department of Infectious Diseases, University Medical Center Ljubljana, Slovenia, from January 1996 to December 2014. A detailed epidemiological and medical history was taken from all patients, physical examination was performed, and several laboratory tests were carried out. Follow-up evaluation and serum collection was performed 14 days, 6–8 weeks, and 6 months and 12 months after the first visit.

2.2. Laboratory examinations

From each patient blood samples were obtained for a complete blood count and chemistry profile: concentration of C-reactive protein, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatinine phosphokinase (CPK). At the first visit, Giemsa-stained peripheral blood smears were examined by light microscopy for the presence of morulae within leukocytes.

2.3. Serological testing

All the patients were evaluated for the presence of infection with several tick borne agents. Acute and convalescent serum samples were tested by immunofluorescent assay (IFA) for the presence of IgG antibodies to *A. phagocytophilum* antigens as previously described [5]. Endpoint titres for *A. phagocytophilum* were recorded as the reciprocal of the last serial dilution at which specific apple-green fluorescence of inclusion bodies was focally located in the cytoplasm of the infected cells. Reciprocal IFA titres of ≥1:256 to *A. phagocytophilum* were interpreted as a positive result. The cut-off value 1:256 was based upon findings in patients with proven HGA and a healthy control population, and was additionally validated in relation to case definitions of probable and proven HGA [23].

All patients included in the present study were tested by IFA for the presence of serum IgM and IgG antibodies against *Borrelia burgdorferi* sensu lato (whole cells of local isolate of *Borrelia afzelli*) [24] and for IgM and IgG antibodies against *Ehrlichia chaffeensis* (FOCUS Diagnostics, Cypress, California, USA), while antibodies against tick-borne encephalitis (TBE) virus were detected using Enzygnost Anti-TBE/FSME Virus IgG, IgM reagents (Siemens, Marburg, Germany).

2.4. Nucleid acid amplification

DNA was extracted from leukocytes separated from blood in the buffy coat and used as a template for PCR assays detecting DNA of *A. phagocytophilum* genogroup, as described elsewhere [25]. The primers used were 16S rRNA gene primers Ehr521 and Ehr790 and nested primer set (HS1/HS6 followed by HS43/HS45) that target the *gro*ESL operon of *Ehrlichia*. All amplicons of the *gro*ESL operon and of the 16SrRNA gene were further sequenced [26].

2.5. Case definitions

A case of HGA was defined as proven in a patient who developed an acute febrile illness within 30 days after a tick bite or tick exposure, with laboratory findings of a positive PCR result with subsequent sequencing of the amplicons to demonstrate specific DNA and/or isolation of *A. phagocytophilum* from blood and/or seroconversion or a ≥4-fold increase in antibody titre to *A. phagocytophilum*.

A probable case of HGA was defined as a patient who developed an acute febrile illness within 30 days after a tick bite or tick exposure, with a positive IFA titre ≥1:256 in acute and convalescent-phase serum samples without demonstrating a 4-fold change in titre and with PCR testing found negative or not performed [23].

2.6. Data analysis

Categorical data were summarized with frequencies and percentages, numerical with medians and ranges. The association between HGA (proven or probable) and each of the clinical or laboratory covariates was assessed using univariate logistic regression with Firth's correction [27]. Results were summarized with odds ratios (OR) and their 95% confidence intervals (CI) and with Wald's P values. A logistic regression model with Least Absolute Shrinkage and Selection Operator (lasso) penalization was used to analyze the association of HGA with all the clinical and laboratory variables. This type of model is preferable to regular logistic regression when the data include many covariates; it performs variable selection (shrinking the OR to exactly 1 for some of the variables) and reduces the problem of overfitting. The complexity parameter, which determines the amount of shrinkage, was estimated with