Clinical Manifestations, Epidemiology, and Laboratory Diagnosis of Human Monocytotropic Ehrlichiosis in a Commercial Laboratory Setting

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Clinical, epidemiological, and laboratory diagnostic issues of human monocytotropic ehrlichiosis (HME) were investigated in a retrospective case study conducted at a national reference laboratory (Focus Technologies, formerly MRL Reference Laboratory), and at the University of Texas Medical Branch at Galveston, Texas, during 1997 and 1998. Standard questionnaires were sent to physicians for each laboratory-diagnosed patient 2 days to 2 weeks after immunofluorescent antibody assay results were available. Among the 41 cases for which data were obtained, 32 (78%) were definite cases of HME, and 9 (22%) were probable cases of HME. Tick bite or exposure to ticks was recorded in more than 97% of cases. The most prominent clinical findings were fever, abdominal tenderness, and regional lymphadenopathy. There was an association between age and severity of illness. The main laboratory findings included leukopenia, thrombocytopenia, and elevated aspartate aminotransferase and alanine aminotransferase. Clinical and laboratory findings were nonspecific and were not good predictors of the severity of illness. The 90% of patients who received doxycycline treatment underwent rapid clinical improvement with a favorable outcome. The usual duration of effective treatment with doxycycline was 7 to 10 days. This retrospective study is unique because it was based in a commercial reference laboratory setting that receives specimens from different geographic locations. The clinical and laboratory information from 41 patients provides insight into the epidemiological, clinical, and laboratory characteristics of HME.

Ehrlichiae are obligately intracellular bacteria that reside in a phagocytic vacuole and have evolved in close association with an arthropod vector and a zoonotic host (12, 27, 29, 30). Human monocytotropic ehrlichiosis (HME) was first described in 1987 in the United States (22). Four years later, the etiologic agent Ehrlichia chaffeensis was isolated from a patient and classified in the genus Ehrlichia based on 16S rRNA subunit gene sequence analysis (2, 11). In 1994, a second human ehrlichiosis was described and was named human granulocytotropic ehrlichiosis (HGE) due to the cell tropism of the infectious agent (8). The etiologic agent has been renamed Anaplasma phagocytophilum, and therefore the human disease should be designated human anaplasmosis (13). A second granulocytotropic ehrlichiosis described in 1999 is caused by Ehrlichia ewingii, which seems to have a milder course than HME and more commonly affects immunosuppressed patients (5). HME, like many other infectious diseases, has a clinical spectrum that ranges from mild to life-threatening illness. In most cases the disease presents as nonspecific febrile illness associated with tick exposure. More than 700 cases had been documented by laboratory testing at the Centers for Disease Control and Prevention in 47 of the 50 United States as of 1997, and this figure is most likely an underestimate of the true incidence of the disease (23).

Although a large number of cases are diagnosed serologically at reference laboratories such as Focus Technologies and the Centers for Disease Control and Prevention, there are few published series that address clinico-epidemiological issues and laboratory diagnosis for HME (14, 16). The clinical and laboratory diagnosis of HME remains challenging. Clinical signs and symptoms and routine laboratory tests are rather insensitive and diagnostically nonspecific for HME.

We present in this report the results of a retrospective case study. All cases were diagnosed serologically at Focus Technologies, and clinico-epidemiological information, as well as laboratory data, was collected in standardized questionnaires that were completed by the attending physicians responsible for the patients’ care.

MATERIALS AND METHODS

Collection of data. Focus Technologies is a reference laboratory based in Cypress, Calif., that receives specimens from across the United States. Standardized questionnaires were sent to the physicians of patients who were diagnosed with HME based on laboratory diagnostic criteria. Questionnaires were sent to physicians 2 days to 2 weeks after immunofluorescent antibody assay (IFA) results were available. Questions included epidemiological variables, clinical signs and symptoms, laboratory data, complications, and treatment. When a response for a specific question was not available, the patient was excluded from the analysis for that particular variable. The protocol was reviewed and approved by the Institutional Review Board for Protection of Human Subjects at the University of Texas Medical Branch at Galveston.

Laboratory case definitions. The laboratory diagnostic criteria for serologic diagnosis for a probable case were an endpoint titer of 1:64 to 1:128 for immunoglobulin G (IgG) determinations or an endpoint titer of ≥1:20 for IgM determinations. A definite case was that of a patient with the presence of an endpoint titer of ≥1:256 for IgG determinations.

Serological testing. The IFA method for serological testing employed E. chaffeensis (Arkansas strain) cultivated in DHB2 cells as the antigen (Focus Technologies). Antibody endpoint titers were expressed as the reciprocal of the highest serum dilution at which a 1+ fluorescence intensity was observed. The
sera were diluted in twofold increments from 1:64 to 1:1,024, and if reactivity stronger than 1+ was still observed, the titers were expressed as ≥1:1,024. Endpoint titers were determined for both IgG and IgM. IgM determinations were made after the sera were absorbed with anti-human IgG antibodies. All determinations were made with nonpaired serum samples. The IFA method was also used for the detection of IgG and IgM directed against A. phagocytophilum. The antigen substrate consisted of the A. phagocytophilum strain cultivated in HL-60 cells (Focus Technologies). The IFA procedure used for A. phagocytophilum antibody detection was otherwise the same as the E. chaffeensis method described above.

PCR for human anaplasmosis and HME. DNA was extracted from 100 μl of a patient’s serum with an IsoQuick extraction kit (ORCA Research, Bothell, Wash.). Five to ten microliters of extracted DNA was amplified for the detection of E. chaffeensis or A. phagocytophilum 16S ribosomal DNA. E. chaffeensis-specific DNA was detected using EC1 (5′-TCGTATACTCCTGCCGAT-3′) and EC2 (5′-GTACCTGTCATTATCCCTCAT-3′) primers that amplified a 381-bp fragment. The specificity of the amplicon for E. chaffeensis was confirmed using a specific 23-mer oligonucleotide probe (5′-TTAGAAATGATGGGTAATACTGR-3′). The A. phagocytophilum DNA was amplified using HGE1 (5′-ATTAGATACCCCTGGTAGTCCAC-3′) and HGE2 (5′-CGCGCTTAACCCCTGGGCAAC-3′) primers corresponding to nucleotides 734 to 755 and 1091 to 1072 of the 16S rRNA gene, respectively. After 50 cycles at 94, 55, and 72°C, the resulting 337-bp amplicon was probed with a 27-mer A. phagocytophilum-specific oligonucleotide (5′-ATTAGATCTCTTAAACGGAAGGGCGC-3′). All probes were labeled with 32P, and the hybridized products were detected using 6% acrylamide gels and autoradiography. The analytical sensitivities of the PCR assays range from 10 to 25 copies of the genomic target per reaction for both E. chaffeensis and A. phagocytophilum. Analytical specificity studies of the A. phagocytophilum primers showed no cross-reactivity with Borrelia burgdorferi, E. chaffeensis, Ehrlichia canis, and Babesia microti. Analytical specificity studies for the E. chaffeensis primers showed no cross-reactivity with B. burgdorferi, A. phagocytophilum, and Babesia microti. The primers and probes were designed at Focus Technologies.

Statistical analysis. All patient information was entered into the Access 97 and Excel 97 software packages (Microsoft Corp., Redmond, Wash.) and SigmaStat version 2.0 (SPSS Inc., Chicago, Ill.). The information was analyzed by the chi-square test, t tests, and the Fisher exact test, where indicated.

RESULTS

Demographic data. A total of 486 cases were diagnosed serologically during 1997 and 1998. Forty-one questionnaires were completed by physicians who were directly involved in the care of patients diagnosed with HME. Thirty-two cases (65%) were hospitalized. Thirty-seven patients (90%) who were enrolled in the study received treatment with doxycycline. Age and time to defervescence were compared, and there was no statistically significant association between these two variables (P = 0.24). Likewise, duration of illness after treatment was compared to age, and no statistically significant association was found (P = 0.09).

The most common signs and symptoms were fever, photophobia, abdominal tenderness, regional lymphadenopathy, and confusion (Table 1). Central nervous system (CNS) manifestations included stupor in three patients, hallucinations in four patients, and both seizures and coma in one patient. Renal failure occurred in two patients. No fatalities were documented for any of the patients enrolled in the study.

Hematologic laboratory results included leukopenia in 21 cases (54%) and thrombocytopenia in 28 cases (70%). High serologic endpoint titers for IgG determinations (≥1:256) by IFA correlated with the presence of leukopenia (P < 0.01). However, there was no statistically significant difference in the IFA titers in relation to the presence or absence of thrombocytopenia (P = 0.5). The presence of leukopenia was strongly associated with the presence of thrombocytopenia (P < 0.01). Neutropenia was present in 28% of the cases, and increased quantities of bands were seen in 21% of cases. Absolute lymphocytosis was present in 44% of cases, and atypical lymphocytes were detected in 8% of cases. The same percentage of

| Table 1. Selected signs and symptoms observed in 41 patients with laboratory-confirmed acute human monocytotropic ehrlichiosis |
|-----------------|----------------|----------------|
| Sign or symptom | No. of patients | % of patients with manifestation |
| Fever (>37.5°C) | 39 | 95 |
| Regional lymphadenopathy | 12 | 29 |
| Abdominal tenderness | 11 | 27 |
| Photophobia | 11 | 27 |
| Confusion | 9 | 22 |
| Rash | 77 | 17 |
| Hallucinations | 4 | 10 |
| Stupor | 3 | 7 |
| Meningitis | 3 | 7 |
| Coma | 1 | 2.4 |
| Seizures | 1 | 2.4 |
| Hepatomegaly | 1 | 2.4 |
| Splenomegaly | 1 | 2.4 |

* Fever was usually accompanied by malaise and headaches. The rash was described as maculopapular in four cases and petechial in three cases.
cases (8%) also had monocytosis. Anemia was present in 59% of males and 38% of females. Aspartate aminotransferase (AST) was elevated in 86% of cases and alanine aminotransferase (ALT) was elevated in 83% of cases. Elevations of transaminases were up to 12 times the normal value, and in no case did they exceed a concentration of 600 U/liter. Cerebrospinal fluid (CSF) was obtained from three patients who presented with a clinical picture of aseptic meningitis. Counts of white blood cells, most of which were lymphocytes, were elevated in the CSF of two patients (70 and 205 cells/μL). CSF protein concentrations were elevated in all three patients, with values of 60, 81, and 105 mg/dl (see Table 2 for details of all laboratory values).

Hospitalized and nonhospitalized patients were compared by age; magnitude of fever; duration of symptoms after treatment; white blood cell count; neutrophil, lymphocyte, monocyt, and platelet counts; hemoglobin concentration; and AST and ALT concentrations. There was no statistically significant difference between the two groups except for age (Table 3). The mean (±SD) age for the hospitalized patients was 54 years (±16.2 years), and for nonhospitalized patients the mean age was 39 years (±20.1 years) (P = 0.05). Comparisons between probable and confirmed cases using the same clinical and laboratory parameters revealed no statistically significant differences, except for the proportion of patients who were hospitalized, which was a more common occurrence for patients with confirmed cases than for patients with probable cases (P < 0.01).

Data regarding the duration of treatment and defervescence were available for 34 patients: 17 patients (50%) received doxycycline for 7 to 10 days, 15 patients (44%) received the drug for 11 to 21 days, and 2 patients (6%) received the drug for more than 21 days. There was no statistically significant difference between the first two groups when the duration of treatment until defervescence was analyzed. These data suggest that relatively short treatment protocols are highly effective against E. chaffeensis, although well-designed clinical trials are needed to address this important issue.

**IFA results.** The geometric mean for IgG reciprocal endpoint titers was 278. The distribution of endpoint titers for IgG was as follows: 7 patients (17%) had endpoint titers below 1:64, 9 patients (24%) had endpoint titers of 1:64 to 1:128, 9 patients (24%) had endpoint titers of 1:256 to 1:512, and 16 patients (42%) had endpoint titers of ≥1:1,024. The IgM endpoint titer distribution was as follows: 11 patients (27%) had titers below 1:20, 7 patients (17%) had titers of 1:20 to 1:40, and 23 patients (56%) had titers between 1:80 and 1:320. The geometric mean for IgM reciprocal endpoint titers was 66.5. Thirteen patients (32%) were positive (≥1:64) by IFA for IgG alone, 7 patients (17%) were positive (≥1:20) for IgM alone, and 21 patients (51%) were positive for both IgG and IgM. Only one patient had titers of 1:64 for IgG and 1:20 for IgM. The presence of high endpoint titers by IFA for IgM was associated with the presence of high endpoint titers for IgG (P < 0.001).

Serological cross-reactions with *A. phagocytophilum* occurred in eight cases (20%). In all these cases the endpoint titers obtained by IFA for HME were three- to fourfold higher than the titers obtained for *A. phagocytophilum*. Cross-reactions were seen with both IgG and IgM. The geographic distribution of these cross-reactive cases was as follows: two each from Virginia and New Jersey and one each from North Carolina, South Carolina, New York, and Maryland.

PCR was performed for *E. chaffeensis* and *A. phagocytophilum* on the 16 serum samples that contained IgM antibodies to *E. chaffeensis* and for which a sufficient sample was available. Only one serum sample yielded *E. chaffeensis* DNA.

**DISCUSSION**

By applying strict criteria for serologic diagnosis, we have detected a total of 32 definite cases (78%) and 9 probable cases (22%). HME seems to affect every age group but is diagnosed more frequently in persons over 50 years of age, a fact that has been reported previously in other series of both HME and
human anaplasmosis (1, 3, 14–16, 18, 31). However, the gender distribution of nearly equal incidences of disease in males and females was surprisingly different from what was found in other series in which HME was reported to affect males more often than females. The distribution by state is rather broad, involving areas in the Northeast, the Mid-Atlantic, the central Midwest, and the South. In our series, some states known to have a high incidence of HME (e.g., Arkansas, Missouri, Georgia, and Oklahoma) do not have a high number of cases, most likely reflecting a referral bias of samples to the commercial laboratory from different markets or a low index of suspicion by clinicians in states where the disease is endemic as reported by Carpenter et al. (6). Evidence of *E. chaffeensis* DNA has been detected in states where the prevalence of HME is thought to be low, such as the northeastern coast in states such as Connecticut and Rhode Island (19).

The history of tick bite exposure and the time interval between tick bite and the appearance of symptoms are also consistent with previous reports, as is the occurrence of the disease during months in which ticks are most active and seeking a blood meal (14–16). In our series, however, the history of tick bite or exposure was documented in an even larger proportion (97%) of cases.

The most common clinical findings were nonspecific signs and symptoms. Therefore, diagnostic pitfalls can occur, and a high index of suspicion is required in order to diagnose these infections. A history of tick bite or exposure is of the utmost importance in order to suggest the diagnosis, although it is not absolutely necessary to request laboratory testing for confirmation. It is worth mentioning that a rash was present with only 17% of patients, compared to what occurred in other series, in which more than 30% of patients developed a rash at some point during the disease process. The previously reported percentage of patients with a rash at the beginning of the disease is even lower (only 6%). Therefore, rash is not helpful as an early sign to stimulate suspicion of the diagnosis. The presence of signs and symptoms related to the CNS in 22% of cases also requires emphasis. Previous series have described that the occurrence of CNS-related manifestations is more frequently associated with HME than HGE (19). CSF studies were performed for only three patients with CNS manifestations, and all revealed pleocytosis and/or abnormal protein levels. It is very possible that the other cases in which CNS anomalies were present would have shown abnormal CSF results if they had been evaluated.

Regarding treatment of HME, current recommendations call for at least 7 days of therapy with tetracyclines. The vast majority of patients in our series were treated with doxycycline. Defervescence occurred rapidly in most of the cases. Furthermore, the duration of other symptoms after treatment did not have any statistical correlation with the duration of treatment. However, we do not have any long-term follow-up data to document possible relapses or recurrences after shortened treatment. *E. chaffeensis* is very susceptible to tetracycline antibiotics such as doxycycline in cell culture and in retrospective clinical studies (4, 15), and this would explain the excellent clinical response even for patients who received a short course of treatment. The total duration of illness was shorter in our series, and the defervescence occurred later than in other series (14, 16).

Laboratory findings are also nonspecific, although they can provide invaluable clues in the diagnostic process of a possible case of human ehrlichiosis. Leukopenia and thrombocytopenia are two findings that occur in up to 70% of the cases. In our series, thrombocytopenia occurred more frequently than leukopenia. Leukocytosis was documented in only one case and was not marked (12.6 × 10^9 cells/liter). Therefore, the presence of either leukopenia or thrombocytopenia, along with a clinical and epidemiological history consistent with HME, should raise the possibility of a diagnosis of HME. It is also noteworthy that the presence of thrombocytopenia was clearly associated with leukopenia (*P < 0.01*). Other interesting findings relating to the peripheral blood of patients with HME included an increased number of bands, monocyteosis, lymphocytosis, and the presence of atypical lymphocytes, all of which have been reported in other series and usually occur during the second week of illness (14). The mechanisms mediating these changes are unknown at this time. In our series there was a strong association between high IgG endpoint titers and the presence of leukopenia. This finding most likely reflects the average time at which both findings appear in the course of the disease; rising antibody titers usually occur after 7 days of disease, a time at which the leukopenia is frequently still present and the thrombocytopenia is resolving. Concentrations of hepatic enzymes in sera were also evaluated for most patients. However, liver failure was not observed in any case. The transaminase elevations were mild to moderate, never exceeded 600 U/liter, and most likely reflected subclinical damage to hepatocytes. A few pathological studies have documented the presence of histologic hepatic abnormalities, such as granulomas and the focal death of hepatocytes (24). There was no correlation between levels of transaminases and other laboratory abnormalities, such as cytopenias and IFA titers. Another interesting finding for which we have no clear explanation is the absence of IgM antibodies in one-third of the patients in whom IgG antibody titers were detected at high levels. This finding reflects the difficulty in interpreting IgM titers in clinical situations.

The severity of HME apparently ranges from mild cases to life-threatening infections that require hospitalization and intensive care. The largest published series describes a disease that required hospitalization in more than 60% of patients (14). However, due to the selection method of the patients in the tertiary care center, the occurrence of mild cases might have been underrepresented. In this series the high percentage of hospitalized patients (56%) confirms the findings of older series. In a recently finalized prospective study in Cape Girardeau, Mo., we also found a similar portion of patients who required hospitalization (26). The patients were all enrolled in that study after being examined by primary care physicians in an ambulatory care setting. Therefore, the percentages of patients that require hospitalization seem to be similar in all of the studies. Hospitalization in the present series was strongly associated with older age. There was no association with other factors studied, such as white or red blood cell counts, platelet counts, the presence of hepatic enzymes, period of treatment until defervescence, or duration of illness. Although the adult population is overrepresented in most series and age seems to correlate strongly with severity of disease, the pediatric population is also at risk for severe disease (17, 20, 21).
For surveillance purposes, stringent criteria have been proposed for the diagnosis of HME, the “gold standard” being a fourfold rise in endpoint IFA titers obtained from samples collected from the patient at least 3 weeks apart (7). Unfortunately, no convalescent-phase samples were obtained from any of our patients because of the general situation in the clinical utilization of reference laboratory serologic services. However, the presence of high titers of IgG and IgM by IFA in a clinically compatible case is virtually diagnostic of HME (28). In our series, the presence of high IgM titers was associated with high IgG titers. The kinetics of the immune response to E. chaffeensis are largely unknown, but this observation suggests that by the time the IgM response is well developed, the IgG response is also under way. Serological cross-reactions with the HGE agent were observed in eight cases (20%), which is similar to what was found in other series (10, 25). Of all patients with probable cases (nine in total), only one had cross-reactive antibodies against A. phagocytophilum. In all cases, the endpoint titers were significantly lower when A. phagocytophilum was used as an antigen than when E. chaffeensis was used. Although this criterion has not been proven to be valid in ascribing etiology (HME versus human anaplasmosis) based on serological results, preliminary data based on PCR amplification of ehrlichial DNA suggest that comparison of serologic endpoint titers might be useful in making such a differentiation (9). Almost all patients with cross-reactive titers were from the region of the country where human anaplasmosis is also endemic, suggesting the possibility of prior infection with A. phagocytophilum. On the other hand, cross-reaction may have and probably did occur due to antigenic similarities of the two agents. In fact, certain protein families of these organisms have conserved amino acid sequences that might be responsible for such findings (32).

DNA from E. chaffeensis was amplified by PCR from only one patient’s serum. This low sensitivity is not surprising. A previous publication confirms the low sensitivity of serum as the specimen for PCR diagnosis of acute ehrlichial infections (10). It is therefore recommended that clinicians obtain EDTA anticoagulant-treated blood samples so that molecular tests such as PCR can be performed for the detection of ehrlichial DNA from whole-blood samples during the acute phase of the disease.

A potential pitfall of this series includes a selection bias towards more severely ill patients since these patients were ill enough to seek medical attention. Another potential pitfall of this series is that all our cases were diagnosed based on IFA titers obtained from a single serum sample or, stated conversely, based on the absence of convalescent-phase samples for IFA diagnosis. However, all patients presented with an acute febrile illness that fit the clinical case definition of acute ehrlichiosis. On the other hand, we considered endpoint titers of ≥1:256 as definitive for a diagnosis of acute HME and titers of 1:64 and 1:128 as indicative of probable cases, and thus we may well have missed some patients who had not developed antibodies at the time of sample collection. The use of the ≥1:256 cutoff value is based on recommendations by the Consensus Approach for Ehrlichiosis (CAFE) Society (28).

In summary, HME is a disease with a wide distribution in the United States that occurs most frequently during the warm months of the year and is usually associated with tick exposure or bite. The diagnosis requires a high level of suspicion because signs, symptoms, and laboratory findings are not specific. A clinical history of fever, a possible tick bite or tick exposure, and the presence of leukopenia or thrombocytopenia should raise the possibility of human ehrlichiosis. The disease spectrum ranges from mild to severe, with potentially fatal complications, and the disease is more severe in immunocompromised patients. In this series, more severe illness was associated with older age.

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