SPECIAL SECTION: EMERGING INFECTIONS

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Human Granulocytic Ehrlichiosis

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Human granulocytic ehrlichiosis is a recently recognized tick-borne infectious disease, and to date >600 patients have been identified in the United States and Europe. Most patients have presented with a non-specific febrile illness occurring within 4 weeks after tick exposure or tick bite. The risk for serious illness or death increases with advancing age and delayed onset of therapy. Routine laboratory testing may reveal reduced white blood cell and platelet concentrations and mildly elevated hepatic transaminase activity in peripheral blood. A high index of suspicion is necessary to arrive at a timely clinical diagnosis. Patients suspected of having human granulocytic ehrlichiosis (HGE) should be treated with a tetracycline-class antibiotic while awaiting the outcome of confirmatory laboratory testing.

Ten years have passed since the first patient with clinically recognizable human granulocytic ehrlichiosis presented for admission to St. Mary's Medical Center in Duluth, Minnesota [1, 2]. Since that time, an amazing amount of clinical and laboratory research has been conducted in the United States and Europe, and cumulative evidence suggests that HGE is a significant emerging tick-borne infection. HGE may well occur at prevalence rates that rival those of Lyme borreliosis in selected areas. In this article, we will address the following questions of relevance to clinicians: what are the incidence and prevalence rates of HGE in areas of endemicity, which are the signs and symptoms of HGE in the acute phase of illness, which laboratory analyses will help the clinician establish a working diagnosis, which laboratory tests will confirm the diagnosis, how do you treat HGE, and what is the acute and long-term prognosis of HGE?

Microbiology

Ehrlichia species are gram-negative bacteria that infect phagocytic bone marrow–derived cells in mammalian hosts [3–5]. These bacteria measure from 0.5 to 2 μm in diameter and lead an obligate intracellular existence inside cytoplasmic vacuoles, where they divide by binary fission to form clusters of bacteria called morulae. Many *Ehrlichia* species spend part of their nor-

mal life cycle in an arthropod host, most commonly a hard-shell tick [3, 5, 6]. Transovarial transmission of *Ehrlichia* species appears to be inefficient in ticks, and mammalian hosts are therefore presumed to play an important role in the maintenance and propagation of *Ehrlichia* species in nature.

Ehrlichioses are zoonoses, and characteristic infectious syndromes have been recognized in animal species, such as dogs, sheep, goats, cattle, and horses [3]. *Ehrlichia phagocytophila* and *Ehrlichia equi* cause infections in ungulates and equines, respectively [5]. HGE is an illness caused by an *Ehrlichia* species that is closely related to or conspecific with these species, but final species designation for the HGE agent awaits description [2, 6]. As the name implies, the preferred host cells for the HGE agent are the granulocytic leukocytes, but nonhematologic cell lines may also become infected in severe disease [1, 5–10].

Epidemiology

There is now substantial evidence to suggest that *Ixodes* ticks are the principal tick vectors for the HGE agent [1, 11–16]. *Ixodes scapularis,* or the deer tick, is the principal vector in the northeastern and upper midwestern states of the United States [16–19], and *Ixodes pacificus,* the black-legged tick, is the primary vector in the Pacific western states [20, 21]. *Ixodes ricinus,* the sheep tick, is the candidate tick vector in western Europe [22–24]. Seroepidemiologic investigations of mammals have detected HGE agent–specific DNA and antibodies in blood from many different species. It is currently believed that *Peromyscus leucopus,* the white-footed mouse [14, 25–27], and *Odocoileus virginianus,* the white-tailed deer [28–30], are the most important reservoir host mammals in the eastern and midwestern United States. Therefore, the life cycle of the HGE agent, with

its specific requirements for tick and mammal hosts, appears to be similar to that of *Borrelia burgdorferi,* the agent of Lyme disease [31]. It is thus hardly surprising that the geographic areas where HGE has been reported to occur overlap closely with the geographic areas where Lyme disease is endemic [6, 32, 33]. Variations in the number of cases of tick-borne illnesses reported from year to year partially reflect a complicated relationship between the density of the tick vector and the mammal reservoir hosts. Ostfeld [31] has suggested that the annual incidence rates of Lyme borreliosis and HGE vary directly with the availability of mammal food sources and the host mammals' ability to survive harsh winters.

HGE has now been diagnosed in >600 patients from 13 states in the United States, and most patients have been infected in Connecticut, Minnesota, New Jersey, New York, or Wisconsin [34]. A few patients have been described from California [35, 36]. Furthermore, laboratory-confirmed HGE has been reported to occur in patients from Slovenia [37], Holland [38], and Sweden [39]. HGE is a reportable illness in only 19 US states [34]; therefore, exact figures about disease incidence or prevalence rates on the North American continent are incomplete [40]. Although data are limited, studies in the Upper Midwest have estimated that the annual incidence rates of HGE vary from 1 to 58 cases per 100,000 population [41]. Epidemiological surveys have demonstrated the presence of antibodies that react with the HGE agent or *E. equi* in sera of 3% of the residents of Long Island, New York [42], and 14.9% of the residents of northwestern Wisconsin [43]. Prevalence rates of antibody to the HGE agent in sera from European patients who have had confirmed Lyme borreliosis have ranged from 7.5% to 24% [44–50].

A few patients appear to have HGE and Lyme borreliosis at the same time [41, 51]. Thus, patients who are bitten by deer ticks may be placed at risk for becoming infected by ⩾1 of the infectious agents that are associated with this tick vector (*B. burgdorferi*, HGE agent [41, 51]—and *Babesia microti* [P. Krause, personal communication]). Tetracycline-class drugs are active against *B. burgdorferi* but not against *B. microti.* Careful clinical and laboratory follow-up during the treatment period is therefore strongly advised, to ensure that the prescribed therapy leads to resolution of the infectious process.

Most cases of HGE have been reported between May and August [1, 5, 13, 40, 52], which is also the period when nymphal stage deer ticks are questing [53]. School recess and vacation time jointly result in peak human outdoor activity during the summer months, which increases the risk for tick exposure. In addition, the diminutive size of the nymphal tick and the short period (3–4 days) required for nymphal ticks to feed to repletion make this stage of deer tick difficult to detect while taking its blood meal from a human host. Adult ticks primarily quest during the autumn months, and because of their larger size, they are easier to detect and remove early after attachment [50]. For these reasons, it is currently believed that nymphal stage deer ticks are the most important vectors for the transmission of the HGE agent to humans.

Most patients diagnosed with HGE have acquired their infection after exposure in areas where ticks are endemic, and ~60% of patients recall a preceding tick bite [13, 41]. Although tick bites are thought to represent the main method of transmission of the HGE agent to most patients, we have recently seen several butchers develop HGE shortly after cutting large quantities of fresh deer carcasses [54]. None of the butchers described any preceding tick bites. It is therefore possible that exposure to infected blood represents an occupational hazard to persons who process large quantities of fresh deer meat, by direct inoculation of the HGE agent through cuts on skin or contamination of mucous membranes.

HGE: Clinical Illness

Previously reported clinical reviews have indicated that patients have the signs and symptoms of HGE for 4–8 days before presenting to their physician [1, 13, 41]. The incubation period for HGE following a tick bite is 7–10 days, and infected patients develop an acute nonspecific febrile illness characterized by high-grade fever (temperature, >39°C), rigors, generalized myalgias, severe headache, and malaise [1, 13, 41, 52]. Many patients also have complained of anorexia, arthralgias, nausea, and a nonproductive cough (table 1). Thirteen (10.9%) of 119 patients have been described to have a nonspecific rash, with the clinical description varying from a erythematous to pustular appearance [13, 41, 52]. We have seen a nonspecific erythematous rash in 2 (1.1%) of 181 patients with HGE in the Upper Midwest (authors' unpublished data) and believe that a rash is not part of the clinical picture of HGE, except when patients are coinfected with Lyme borreliosis. Thus, the presence of a rash should raise suspicion toward other febrile infectious diseases, some of which include viral illnesses, meningococcemia, disseminated gonorrhoea, and Rocky Mountain spotted fever.

The duration of illness may last only a few days, but some patients have been ill for as long as 60 days in the absence of appropriate antibiotic therapy. Approximately one-half of the

Table 1. Frequency of presenting signs and symptoms reported by patients who had laboratory-confirmed human granulocytic ehrlichiosis in New York [13] and the upper Midwest [41].

Sign or symptom	New York (n = 18)	Upper Midwest (n = 41)
Fever (temperature, >38.3°C)	94	100
Rigors	39	98
Malaise	NA	98
Headache	61	85
Nausea	NA	39
Anorexia	NA	37
Arthralgias	78	27
Cough	NA	29
Confusion	NA	17
Rash	11	2

NOTE. Data are percentage of patients. NA, not available.

patients who have been seen in our practice were ill enough to require hospitalization for an average period of 5.8 days ([55, 41]). The severity of illness of 41 patients with HGE was directly proportional to age, which was reflected by the higher mean age for hospitalized patients than for patients who were treated in the outpatient setting (73 vs. 53 years, respectively) [41]. On the basis of previously reported studies [1, 6, 13, 41, 52] and personal experience, we estimate that the fatality rate of HGE is <1%. On the basis of retrospective analysis of data for a limited number of patients [41, 56], poor prognostic indicators appear to include advanced age, concomitant chronic illnesses (such as diabetes mellitus, collagen-vascular diseases, and diseases requiring immune-modulating therapies), and lack of diagnosis recognition or delayed onset of specific antibiotic therapy.

Routine Laboratory Testing for HGE

Routine laboratory testing of blood for HGE often demonstrates nonspecific changes in blood cell counts and chemistry parameters. Many patients have had 2- to 4-fold increases in concentrations of hepatic transaminases (aspartate aminotransferase and alanine aminotransferase), and the C-reactive protein concentration is generally elevated [41]. Most patients also have been noted to have decreased concentrations of total WBCs and platelets at the time of the initial evaluation [13, 41, 52], but the likelihood of finding blood cell counts below normal depends on the time of testing relative to the duration of illness [55, 57, 58]). Nadir values of total WBCs and platelets have been observed around the seventh day of illness; thereafter cell counts return toward the normal range even when clinical symptoms persist. Serial determinations of complete blood cell counts for 131 of our patients with laboratory-confirmed HGE showed that 75% developed leukopenia (WBC count, $\leq 4.5 \times 10^9$/L) on ≥ 1 days during the course of their illness [55]. All of our patients had platelet counts $< 150 \times 10^9$/L on ≥ 1 days during the same period [55, 57]. Thus, caution is advised against dismissing HGE from the differential diagnosis if blood cell counts are normal at the time of the initial visit, especially for patients who have been ill for >1 week. Although total WBC, absolute neutrophil, and lymphocyte counts may be below normal, patients with HGE frequently have a left shift during the first week of illness [55, 57, 58]. During the second week of illness, neutrophil counts gradually return to normal accompanied by relative and absolute lymphocytosis.

Light microscopic examination of peripheral blood smears treated with Wright's stain may reveal morulae in the cytoplasm of neutrophils during the acute illness and thus provide immediate evidence in support of the diagnosis [1, 5, 13, 41]. Morulae may take on many different shapes and sizes, but the texture of the inclusion is often coarser, more stippled, and stained darker blue than the adjacent neutrophil chromatin [1, 5, 41]. The success in finding morulae varies directly with the experience of the microscopist and also with the duration of

illness, since morulae tend to be detected less frequently after the first week of illness (J. S. Bakken, unpublished data). Approximately 62% of patients in the Upper Midwest who were evaluated within the first week of illness had detectable morulae in 0.1%–42% of their peripheral blood neutrophils (table 2) [55, 34]. Absence of morulae in the peripheral blood smear does not exclude the diagnosis of HGE for individuals who present with a nonspecific febrile illness and have a history of recent tick exposure.

Recently the CAFÉ (Consensus Approach for Ehrlichiosis) Society, which consists of epidemiologists, infectious disease clinicians, microbiologists, and pathologists, met to define the case definitions for HGE. Their conclusions were reported in the American Society for Rickettsiology Newsletter [60], and a modified summary of the case definitions for possible, probable, and confirmed HGE is shown in table 3. The diagnosis of possible HGE is founded on the combination of an adequate exposure history, symptoms suggestive of HGE, documented fever and absence of specific findings during physical examination, and changes in results of routine laboratory blood tests that are suggestive of HGE. Patients who meet the epidemiological, clinical, and laboratory criteria for HGE should begin specific antibiotic treatment and undergo further laboratory testing to confirm the clinical diagnosis retrospectively.

Confirmatory Laboratory Tests for HGE

Specific laboratory tests may be used to confirm the clinical diagnosis of HGE [1, 13, 41, 60]. Serology by indirect fluorescent antibody (IFA) testing has been used most frequently to confirm the clinical diagnosis, and testing of serum is currently available at several commercial laboratories in the United States. An antibody titer ≥ 80 is considered reactive (positive) and reflects recent or past exposure to the HGE agent [4–6, 13, 41, 60]. Con-

Table 2. Outcomes of confirmatory laboratory testing of blood or serum samples from patients who were diagnosed with human granulocytic ehrlichiosis (HGE) at the St. Mary's–Duluth Clinic (SMDC) Health System (Duluth, MN) from June 1990 through December 1999 ([55, 41]).

Laboratory test or HGE marker	No. of positive patients/ total no. tested (%)
Blood smear microscopy[a]	83/134 (62)
PCR analysis[b]	56/83 (67)
Culture[c]	11/20 (55)
IFA indicated by seroconversion[d]	126/127 (99)
IFA titer ≥ 80[e]	168/169 (99)

NOTE. IFA, indirect fluorescent antibody.
[a] Detection of morulae in neutrophil leukocytes in peripheral blood smears treated with Wright's stain [1].
[b] PCR analysis of acute-phase blood specimens with use of primers *ge9f* and *ge10r* [41].
[c] Inoculation of acute-phase blood specimens into cell cultures containing HL-60 human promyelocytes [59].
[d] Four-fold or greater increase in titer of antibody to *Ehrlichia equi* or the HGE agent in acute- and convalescent-phase serum samples obtained at least 2 weeks apart [41].
[e] Antigenic marker of *E. equi* or the HGE agent [41].

Table 3. Proposed case definitions for human granulocytic ehrlichiosis (HGE).

Diagnosis category, definition criteria
Possible HGE
History of tick exposure
Nonspecific febrile illness (temperature, >37.6°C)
Nonrevealing physical examination
Probable HGE
Above criteria
Single IFA titer ≥80,[a] neutrophil morulae,[b] or positive PCR analysis[c]
Confirmed HGE
Above criteria
Four-fold or greater increase in IFA titer,[a] positive PCR analysis and morulae,[b] or blood culture positive for HGE agent[d]

NOTE.   Data are modified from [60]. IFA, indirect fluorescent antibody.
[a] Revealed by IFA test with *Ehrlichia equi* or the HGE agent.
[b] Revealed by microscopic examination of peripheral blood smear treated with Wright's stain.
[c] Positive PCR analysis of blood with use of HGE agent–specific primer sets.
[d] Isolation of the HGE agent in culture of blood.

firmation of the diagnosis of HGE that is supported by specific laboratory testing, as recommended by the CAFÉ Society, includes the following positive test outcomes: a ≥4-fold increase in IFAs that react with the HGE agent or *E. equi* in acute- and convalescent-phase serum samples [13, 41, 52, 60–69] and/or successful isolation of the HGE agent in culture of blood [59, 60]. Amplification of HGE agent–specific DNA in blood from infected patients by PCR analysis [60, 70–72] and the presence of morulae in peripheral blood neutrophils or an elevated titer of antibody to the HGE agent also meet the criteria established for laboratory confirmation of the diagnosis.

Currently, these tests are offered only at a few research centers and commercial laboratories. Test outcomes may be influenced by poor sample quality as a result of delays inherently associated with shipping of samples, and specific laboratory test results are seldom available to the clinician during the acute illness. Furthermore, there are remaining concerns about the sensitivity and specificity of these procedures because of lack of standardization, and negative test results do not exclude the diagnosis of HGE, since none of the tests are 100% sensitive. The outcomes of confirmatory laboratory testing for patients who have been treated for HGE through the St. Mary's–Duluth Clinic (SMDC) Health System (Duluth, MN) are shown in table 2. Detection of morulae in acute-phase blood specimens is a specific but not very sensitive confirmatory laboratory test for HGE in our hands. Seroconversion indicated by IFA in acute- and convalescent-phase serum samples appears to be the most sensitive confirmatory laboratory test for HGE at present. Clinicians eagerly await US Food and Drug Administration approval of rapid tests that can be used for diagnosis confirmation during the acute illness (dot-blot tests and EIA) in the near future.

Antibiotic Treatment of HGE

In vitro cultivation of the HGE agent is attempted only in a few public health and research laboratories, and only 1 previously reported study has evaluated the in vitro susceptibility of the

HGE agent to antibiotic drugs to date [73]. Recommendations for antibiotic treatment of HGE have therefore been empirically based on the clinical experience with drugs that work well in vivo [1, 5, 13, 40, 41, 74, 75]. Both tetracycline hydrochloride and doxycycline hyclate have demonstrated marked activity against the HGE agent in vitro, as well as in vivo, although the latter has been the most preferred because of a better pharmacokinetic profile and better patient tolerability.

Doxycycline may be administered to patients aged ≥8 years for 14 days, to assure adequate duration of treatment for HGE, as well as possible concomitant Lyme borreliosis. Children aged <8 years may be treated with a shorter course of doxycycline, according to the same guidelines as those issued for Rocky Mountain spotted fever [75, 76]. Patients who have intolerance or specific contraindications to a tetracycline drug may be treated with rifampin for 7–10 days; however, previously reported experience with rifampin is limited [74, 75, 77], and close clinical follow-up is recommended to ensure that patients respond as expected to therapy. We recently treated a 6-year-old girl who had HGE with rifampin, and her infection promptly resolved. A recent report reviewed the clinical courses of 2 women with HGE who were treated with rifampin during their third trimesters of pregnancy [78]. There were no documented ill effects on their offspring. Chloramphenicol appears to be inactive against the HGE agent in vitro, and previously reported clinical information about the efficacy of chloramphenicol has been conflicting [59, 73, 74]. It therefore seems prudent to avoid the use of this agent for the treatment of HGE [74, 75].

It is currently impossible to prospectively identify those patients who are likely to do well when they initially present with clinical signs and symptoms of HGE. We therefore recommend that all patients who are diagnosed with probable or confirmed HGE undergo treatment with an antibiotic agent with proven activity against the HGE agent. The therapeutic effect of tetracycline drugs and rifampin on the course of HGE becomes apparent by disappearance of fever and marked clinical improvement within 24 h [13, 41, 74, 75]. Consequently, an alternative diagnosis should be sought for patients who continue to have fever and clinical symptoms after 48 h of tetracycline therapy.

Insecticide skin sprays and treatment of clothing with tick repellents are important means to reduce the risk of tick attachment after exposure to ticks [31, 74]. Transmission of the HGE agent from the feeding tick to the human host during a blood meal requires a minimum period of attachment of at least 24 h before it becomes effective [79]. The risk of transmission of the HGE agent from the tick vector to the human host can therefore be reduced substantially by performing daily inspections of all skin areas and by promptly removing attached ticks after outdoor exposure.

Acute and Long-Term Prognosis of HGE

Seroepidemiologic studies have suggested that most patients who become infected with the HGE agent develop a mild or even
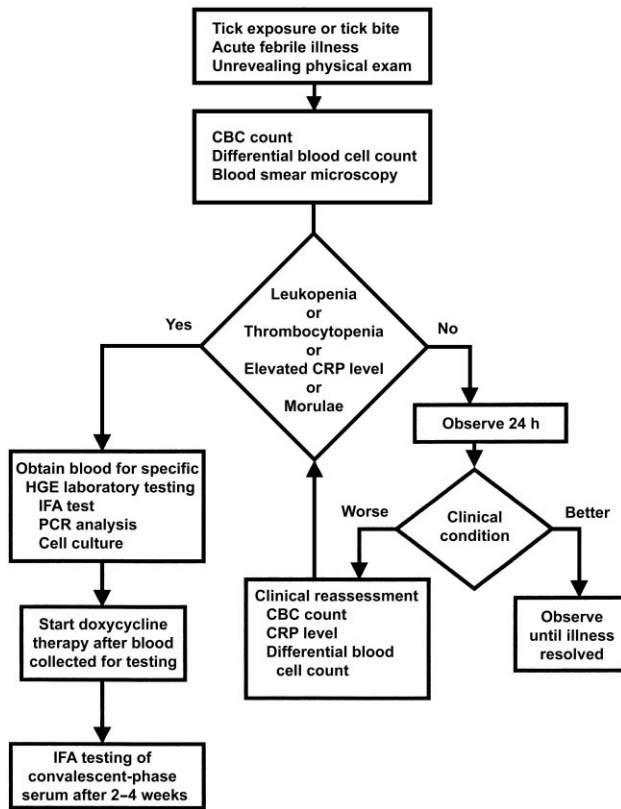
Figure 1. Suggested clinical, laboratory, and treatment approach for patients with a history of recent exposure to ticks or a confirmed tick bite who present with a nonspecific febrile illness. CBC, complete blood cell; CRP, C-reactive protein; IFA, indirect fluorescent antibody.

a subclinical illness, which resolves spontaneously without specific antibiotic therapy [11, 40, 42–45]. Approximately 50% of infected patients have maintained elevated titers of antibody to the HGE agent for as long as 3 years after infection [41, 80, 81]. A patient from New York became reinfected with the HGE agent following a new tick bite 2 years after his initial infection [82]. At the time of the second infection, the previously elevated serum titer of antibody to the HGE agent had fallen from 1280 to 80. There are no other known occurrences of reinfection with the HGE agent. Thus, it is likely that patients who develop and maintain high serum titers of antibody to the HGE agent during the weeks to months following HGE may be protected against HGE upon future tick bite challenges. There is currently no clinical information to suggest that patients who are simultaneously infected with *B. burgdorferi* and the HGE agent have more severe illness than do patients who are infected with one of these agents alone (G. Wormser, personal communication).

Patients who present with a nonspecific febrile illness after exposure in areas where ticks are endemic during the summer months should be evaluated with routine laboratory tests for changes suggestive of HGE and be considered for treatment with an antibiotic agent that includes the HGE agent in the therapeutic spectrum. A laboratory testing and treatment algorithm for patients with presumed HGE that is currently in use at the SMDC Health System (Duluth, MN) is shown in figure 1. Convalescent-phase serum samples for paired IFA analysis should be collected from patients 2–4 weeks after the initial serum sample in those instances where PCR analysis or cell culture was either unavailable or inconclusive.

Most patients who have had clinically recognized HGE have recovered completely within 2 months, regardless of whether they received appropriate antibiotic treatment [13, 41, 52]. Two patients are known to have developed chronic neurological sequelae following HGE [8, 9]. Both patients received appropriate antibiotic therapy, and in each case, irreversible neurological damage was believed to be caused by the acute inflammatory response elicited by the ehrlichial infections rather than by persistent infection. There is currently no clinical evidence in the published literature to suggest that untreated HGE may evolve into a chronic illness as has been seen with Lyme borreliosis. Persistently elevated antibody titers should therefore be interpreted as evidence of past infection rather than as proof of an ongoing unresolved infectious process. The long-term prognosis after HGE appears favorable, and infected patients should be expected to make a complete recovery. Unlike Lyme borreliosis, there does not appear to be a chronic form of HGE.

Acknowledgment

References

1. Bakken JS, Dumler JS, Chen SM, VanEtta LL, Eckman MR, Walker DH. Human granulocytic ehrlichiosis in the United States: a new species emerging? JAMA **1994**;272:212–8.
2. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. J Clin Microbiol **1994**;32:589–95.
3. Rikihisa Y. The tribe ehrlichia and ehrlichial diseases. Clin Microbiol Rev **1991**;4:286–308.
4. Walker DH, Dumler JS. Emergence of ehrlichioses as human health problems. Emerg Infect Dis **1996**;2:18–29.
5. Dumler JS, Bakken JS. Ehrlichial diseases of humans: emerging tick-borne infections. Clin Infect Dis **1995**;20:1102–10.
6. Dumler JS, Bakken JS. Human ehrlichioses. Newly recognized infections transmitted by ticks. Annu Med Rev **1998**;49:201–13.
7. Jahangir A, Kolbert C, Edwards W, Mitchell P, Dumler JS, Persing DH. Fatal pancarditis associated with human granulocytic ehrlichiosis in a 44-year-old man. Clin Infect Dis **1998**;27:1424–7.
8. Horowitz HW, Marks SJ, Weintraub M, Dumler JS. Brachial plexopathy associated with human granulocytic ehrlichiosis. Neurology **1996**;46:1026–9.
9. Bakken JS, Erlemeyer SA, Kanoff RJ, Silvestrini TC, Goodwin DD, Dumler JS. Demyelinating polyneuropathy associated with human granulocytic ehrlichiosis. Clin Infect Dis **1998**;27:1323–4.
10. Shea KW, Calio AJ, Klein NC, Cunha BA. *Ehrlichia equi* infection associated with rhabdomyolysis. Clin Infect Dis **1996**;22:605.
11. Pancholi P, Kolbert CP, Mitchell PD, et al. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis **1995**;172:1007–12.

12. Reed KD, Mitchell PD, Persing DH, Kolbert CP, Cameron V. Transmission of human granulocytic ehrlichiosis. JAMA 1995;273:23.

13. Aguero-Rosenfeld ME, Horowitz HW, Wormser GP, et al. Human granulocytic ehrlichiosis: a case series from a single medical center in New York State. Ann Intern Med 1996;125:904–8.

14. Telford SR, Dawson JE, Katavolos P, Warner CK, Kolbert CP, Persing DH. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proc Natl Acad Sci USA 1996;93:6209–14.

15. Schwartz I, Fish D, Daniels TJ. Prevalence of the rickettsial agent of human granulocytic ehrlichiosis in ticks from a hyperendemic focus of Lyme disease. N Engl J Med 1997;337:49–50.

16. Des Vignes F, Fish D. Transmission of the agent of human granulocytic ehrlichiosis by host seeking *Ixodes scapularis* in southern New York State. J Med Entomol 1997;34:379–82.

17. Daniels TJ, Falco RC, Schwartz I, Varde S, Robbins RG. Deer ticks *(Ixodes scapularis)* and the agents of Lyme disease and human granulocytic ehrlichiosis in a New York City park. Emerg Infect Dis 1997;3:353–55.

18. Magnarelli LA, Stafford KC, Mather TN, Yeh M-T, Horn KD, Dumler JS. Hemocytic *Rickettsia*-like organisms in ticks: serologic reactivity with antisera to *Ehrlichia* and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. J Clin Microbiol 1995;33:2710–4.

19. Rich SM, Caporale DA, Telford SR, Kocher TD, Hartl DL, Spielman A. Distribution of *Ixodes ricinus*–like ticks of eastern North America. Proc Natl Acad Sci USA 1995;92:6284–8.

20. Richter PJ, Kimsey RB, Madigan JE, Barlough JE, Dumler JS, Brooks DL. *Ixodes pacificus* as a vector of *Ehrlichia equi.* J Med Entomol 1996;33:1–5.

21. Barlough JE, Madigan JE, Kramer VL, et al. *Ehrlichia phagocytophila* genogroup rickettsia in ixodid ticks from California collected in 1995 and 1996. J Clin Microbiol 1997;35:2018–21.

22. Petrovec M, Sumner W, Nicholson WL, et al. Identity of ehrlichial DNA sequences derived from *Ixodes ricinus* ticks with those obtained from patients with human granulocytic ehrlichiosis in Slovenia. J Clin Microbiol 1999;37:209–10.

23. Von Stedink LV, Gürtelschmid M, Hanson HS, et al. The human granulocytic ehrlichiosis agent in Swedish ticks. Clinical Microbiology and Infection 1997;3:573–4.

24. Eliasson I, Björsdorff A. Does human ehrlichiosis exist in Sweden? Lakartidningen 1997;94:3487–8.

25. Walls JJ, Greig B, Neitzel DF, Dumler JS. Natural infection of small mammal species in Minnesota with the agent of human granulocytic ehrlichiosis. J Clin Microbiol 1997;35:853–5.

26. Nicholson WL, Muir S, Sumner JW, Childs JE. Serologic evidence of infection with *Ehrlichia* spp. in wild rodents in the United States. J Clin Microbiol 1998;36:695–700.

27. Stafford KC, Massung RF, Magnarelli LA, Ijdo JW, Anderson JF. Infection with the agents of human granulocytic ehrlichiosis, Lyme disease, and babesiosis in wild white-footed mice in Connecticut. J Clin Microbiol 1999;37:2887–92.

28. Dawson JE, Ewing SA, Little SE, Telford SR. Reservoir competence of white tailed deer for the agent of human granulocytic ehrlichiosis [abstract 416]. In: Program and abstracts of the 45th meeting of the American Society of Tropical Medicine and Hygiene (Baltimore). Washington, DC: American Journal of Tropical Medicine and Hygiene, 1996.

29. Belongia EA, Reed KD, Mitchell PM, et al. Prevalence of granulocytic *Ehrlichia* infection among white-tailed deer in Wisconsin. J Clin Microbiol 1997;35:1465–8.

30. Walls JJ, Asanovich KM, Bakken JS, Dumler JS. Serologic evidence of a natural infection of white-tailed deer with the agent of human granulocytic ehrlichiosis in Wisconsin and Maryland. Clin Diagn Lab Immunol 1998;5:762–5.

31. Ostfeld R. The ecology of Lyme disease risk. Am Sci 1997;85:338–46.

32. Kitron U, Kazmierczak JJ. Spatial analysis of the distribution of Lyme disease in Wisconsin. Am J Epidemiol 1997;145:558–66.

33. Centers for Disease Control and Prevention. Lyme disease: United States, 1995. MMWR Morb Mortal Wkly Rep 1996;45:481–4.

34. McQuiston J, Paddock CD, Holman RC, Childs JE. The human ehrlichioses in the United States. Emerg Infect Dis 1999;5:635–42.

35. Gewirtz A, Cornbleet P, Vugia DJ, et al. Human granulocytic ehrlichiosis: report of a case in northern California. Clin Infect Dis 1996;23:653–4.

36. Foley JE, Crawford-Miksza L, Dumler JS, et al. Human granulocytic ehrlichiosis in northern California: two case descriptions with genetic analysis of the ehrlichiae. Clin Infect Dis 1999;29:388–92.

37. Petrovec M, Lotric Furlan S, Avsic Zupanc T, et al. Human disease in Europe caused by a granulocytic *Ehrlichia* species. J Clin Microbiol 1997;35:1556–9.

38. Van Dobbenburgh A, van Dam AP, Fikrig E. Human granulocytic ehrlichiosis in western Europe. N Engl J Med 1999;340:1214–6.

39. Björsdorff A, Berglund J, Kristiansen BE, Soderstrom C, Eliasson I. Variable presentation and course in human granulocytic ehrlichiosis: twelve case reports of the new tick-borne zoonosis. Lakartidningen 1999;96:4200–4.

40. Walker DH, Dumler JS. Human monocytic and granulocytic ehrlichioses. Arch Pathol Lab Med 1997;121:785–91.

41. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. JAMA 1996;275:199–205.

42. Hilton E, DeVoti J, Benach JL, et al. Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the northeast United States. Am J Med 1999;106:404–9.

43. Bakken JS, Goellner P, Van Etten M, et al. Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. Clin Infect Dis 1998;27:1491–6.

44. Bakken JS, Krueth J, Tilden RL, Dumler JS, Kristiansen BE. Serological evidence of human granulocytic ehrlichiosis in Norway. Eur J Clin Microbiol Infect Dis 1996;15:829–32.

45. Dumler JS, Doteval L, Gustafson R, Granström M. A population-based seroepidemiologic study of human granulocytic ehrlichiosis and Lyme borreliosis on the west coast of Sweden. J Infect Dis 1997;175:720–7.

46. Björsdorff A, Brouqui P, Eliasson I, Massung RF, Wittesjö B, Berglund J. Serological evidence of *Ehrlichia* infection in Swedish Lyme borreliosis patients. Scand J Infect Dis 1999;31:51–5.

47. Brouqui P, Dumler JS, Lienhard R, Brossard M, Raoult D. Human granulocytic ehrlichiosis in Europe. Lancet 1995;346:782–3.

48. Sumption KJ, Wright JM, Cutler SJ, Dale BAS. Human ehrlichiosis in the UK. Lancet 1995;346:1487–8.

49. Nuti M, Serafini DA, Basetti D, et al. *Ehrlichia* infection in Italy. Emerg Infect Dis 1998;4:663–5.

50. Christova IS, Dumler JS. Human granulocytic ehrlichiosis in Bulgaria. Am J Trop Med Hyg 1999;60:58–61.

51. Nadelman RB, Horowitz HW, Hsieh T-C, et al. Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis. N Engl J Med 1997;337:27–30.

52. Wallace BJ, Brady G, Ackman DM, et al. Human granulocytic ehrlichiosis in New York. Arch Intern Med 1998;158:769–73.

53. Fish D. Environmental risk and prevention of Lyme disease. Am J Med 1995;98(Suppl 4A):2S–10S.

54. Bakken JS, Krueth J, Lund T, Malkovitch D, Asanovich K, Dumler JS. Exposure to deer blood may be a cause of human granulocytic ehrlichiosis. Clin Infect Dis 1996;23:198.

55. Bakken JS, Aguero-Rosenfeld ME, Tilden RL, et al. Serial measurements of hematological counts during the active phase of human granulocytic ehrlichiosis. Clin Infect Dis (in press).

56. Bakken JS, Tilden RL, Walls JJ, Dumler JS. Influence of occupation, preexisting illnesses, and chronic medication use on the severity of illness of human granulocytic ehrlichiosis. In: Raoult D, Brouqui P, eds. Rickettsia and rickettsial diseases at the turn of the third millennium. Paris: Elsevier, 1999:195–8.

57. Bakken JS, Krueth J, Tilden RL, Asanovich K, Dumler JS. Sequential changes in blood counts during the natural course of human granulocytic

ehrlichiosis [abstract K56]. In: Abstracts of the 37th International Congress of Antibiotic Agents and Chemotherapy (Toronto). Toronto, Canada: American Society for Microbiology, **1997**.

58. Hossain D, Aguero-Rosenfeld ME, Horowitz HW, et al. Clinical and laboratory evolution of a culture confirmed case of human granulocytic ehrlichiosis. Conn Med **1999**;63:265–70.

59. Goodman JL, Nelson C, Vitale B, et al. Direct cultivation of the causative agent of human granulocytic ehrlichiosis. N Engl J Med **1996**;334:209–15.

60. Walker DH. Consensus workshop on diagnosis of human ehrlichioses. American Society for Rickettsiology Newsletter **1999**;2:1–8.

61. Dumler JS, Asanovich KM, Bakken JS, Richter P, Kimsey R, Madigan JE. Serologic cross-reactions among *Ehrlichia equi, Ehrlichia phagocytophila,* and human granulocytic ehrlichia. J Clin Microbiol **1995**;33:1098–103.

62. Nicholson WL, Comer JA, Sumner JW, et al. An indirect immunofluorescence assay using a cell-culture derived antigen for detection of antibodies to the agent of human granulocytic ehrlichiosis. J Clin Microbiol **1997**;35: 1510–6.

63. Ijdo JW, Zhang Y, Hodzik E, et al. The early humoral response in human granulocytic ehrlichiosis. J Infect Dis **1997**;176:687–92.

64. Magnarelli LA, Ijdo JW, Anderson JF, Padula SJ, Flavell RA, Fikrig E. Human exposure to granulocytic ehrlichia and other tick-borne agents in Connecticut. J Clin Microbiol **1998**;36:2823–7.

65. Magnarelli LA, Ijdo JW, Dumler JS, Heimer R, Fikrig E. Reactivity of human sera to different strains of granulocytic ehrlichiae in immunodiagnostic assays. J Infect Dis **1998**;178:1835–8.

66. Ravyn MD, Goodman JL, Kodner CB, et al. Immunodiagnosis of human granulocytic ehrlichiosis by using culture-derived human isolates. J Clin Microbiol **1998**;36:1480–8.

67. Ijdo JW, Wu C, Magnarelli LA, Fikrig E. Serodiagnosis of human granulocytic ehrlichiosis by a recombinant HGE-44–based enzyme-linked immunosorbent assay. J Clin Microbiol **1999**;37:3540–4.

68. Walls JJ, Aguero-Rosenfeld ME, Bakken JS, et al. Inter- and intralaboratory comparison of *Ehrlichia equi* and human granulocytic ehrlichiosis agent strains for serodiagnosis of HGE by the immunofluorescent antibody test. J Clin Microbiol **1999**;37:2968–73.

69. Comer JA, Nicholson WL, Olson JG, Childs JE. Serologic testing for human granulocytic ehrlichiosis at a natural referral center. J Clin Microbiol **1999**;37:558–64.

70. Edelman DC, Dumler JS. Evaluation of improved PCR diagnostic assay for human granulocytic ehrlichiosis. Mol Diagn **1996**;1:41–9.

71. Sumner JW, Nicholson WL, Massung RF. PCR amplification and comparison of nucleotide sequences from the *groESL* heat shock operon of *Ehrlichia* species. J Clin Microbiol **1997**;35:2087–92.

72. Walls JJ, Caturegli P, Bakken JS, Asanovich KM, Dumler JS. Improved sensitivity of PCR for diagnosis of human granulocytic ehrlichiosis using *epank1* genes of *Ehrlichia phagocytophila*–group ehrlichiae. J Clin Microbiol **2000**;38:354–6.

73. Klein MB, Nelson CM, Goodman JL. Antibiotic susceptibility of the newly cultivated agent of human granulocytic ehrlichiosis: promising activity of quinolones and rifamycins. Antimicrob Agents Chemother **1997**;41:76–9.

74. Bakken JS, Dumler JS. *Ehrlichia* species. In: Yu V, Merrigan T, Barriere S, eds. Antimicrobial therapy and vaccines. Baltimore: Williams and Wilkins, **1998**:546–51.

75. Bakken JS, Dumler JS. Ehrlichiosis. In: Cunha BA, Maderazo EG, Spielman A, eds. Tickborne infectious diseases. New York: Marcel Dekker, **2000**: 139–158.

76. Abrahamson JS, Givner LB. Should tetracycline therapy be contraindicated for therapy of presumed Rocky Mountain spotted fever in children less than 9 years of age? Pediatrics **1990**;86:123–4.

77. Horowitz HW, Kilchewsky E, Haber S, et al. Perinatal transmission of the agent of human granulocytic ehrlichiosis. N Engl J Med **1998**;339:375–8.

78. Buitrago MI, Ijdo JW, Rinaudo P, et al. Human granulocytic ehrlichiosis during pregnancy treated successfully with rifampin. Clin Infect Dis **1998**; 27:213–5.

79. Katavolos P, Armstrong PM, Dawson JE, Telford SR. Duration of tick attachment required for transmission of granulocytic ehrlichiosis. J Infect Dis **1998**;177:1422–5.

80. Bakken JS, Krueth J, Tilden RL, Asanovich KM, Walls JJ, Dumler JS. Duration of IFA serologic response in humans infected with the agent of human granulocytic ehrlichiosis [abstract 73]. Clin Infect Dis **1997**;25:368.

81. Aguero-Rosenfeld ME, Kalantarpour F, Baluch M, et al. Serology of culture confirmed cases of human granulocytic ehrlichiosis. J Clin Microbiol **2000**; 38:635–8.

82. Horowitz HW, Aguero-Rosenfeld ME, Dumler JS, et al. Reinfection with the agent of human granulocytic ehrlichiosis. Ann Intern Med **1998**;129: 461–3.