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 normal 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 Lyme 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 myalgia, 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].

<table>
<thead>
<tr>
<th>Sign or symptom</th>
<th>New York (n = 18)</th>
<th>Upper Midwest (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (temperature, &gt;38.3°C)</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Rigors</td>
<td>39</td>
<td>98</td>
</tr>
<tr>
<td>Malaise</td>
<td>NA</td>
<td>98</td>
</tr>
<tr>
<td>Headache</td>
<td>61</td>
<td>85</td>
</tr>
<tr>
<td>Nausea</td>
<td>NA</td>
<td>39</td>
</tr>
<tr>
<td>Anorexia</td>
<td>NA</td>
<td>37</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>78</td>
<td>27</td>
</tr>
<tr>
<td>Cough</td>
<td>NA</td>
<td>29</td>
</tr>
<tr>
<td>Confusion</td>
<td>NA</td>
<td>17</td>
</tr>
<tr>
<td>Rash</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

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 \( \geq 1 \) days during the course of their illness [55]. All of our patients had platelet counts \( < 150 \times 10^9/L \) on \( \geq 1 \) 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 \( \geq 1 \) 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 \( \geq 80 \) is considered reactive (positive) and reflects recent or past exposure to the HGE agent [4–6, 13, 41, 60].

**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]).**

<table>
<thead>
<tr>
<th>Laboratory test or HGE marker</th>
<th>No. of positive patients/ total no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood smear microscopy(^a)</td>
<td>83/134 (62)</td>
</tr>
<tr>
<td>PCR analysis(^b)</td>
<td>56/83 (67)</td>
</tr>
<tr>
<td>Culture(^c)</td>
<td>11/20 (55)</td>
</tr>
<tr>
<td>IFA indicated by seroconversion(^d)</td>
<td>126/127 (99)</td>
</tr>
<tr>
<td>IFA titer ( \geq 80 )(^d)</td>
<td>168/169 (99)</td>
</tr>
</tbody>
</table>

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].
Probable HGE

- History of tick exposure
- Nonspecific febrile illness (temperature, >37.6°C)
- Nonrevealing physical examination

Confirmed HGE

- Four-fold or greater increase in IFA titer, positive PCR analysis and morulae, or blood culture positive for HGE agent

Possible HGE

- History of tick exposure
- Nonspecific febrile illness (temperature, >37.6°C)
- Nonrevealing physical examination

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.

firmedation of the diagnosis of HGE that is supported by specific laboratory testing, as recommended by the CAE 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. Serocconversion 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
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 of these agents alone (G. Wormser, personal communication). More severe illness than do patients who are infected with one of these agents alone (G. Wormser, personal communication).

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.

References


