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Case report

Anaplasmosis: An emerging tick-borne disease of importance in Canada

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ABSTRACT

Human Granulocytic Anaplasmosis (HGA) is an infection caused by the intracellular bacterium *Anaplasma phagocytophilum*. As a tick-borne disease, the public health impact of HGA continues to increase with range expansion of the disease vector. The clinical presentation of HGA is often a non-specific febrile illness. The presence of leukopenia, thrombocytopenia, and mild hepatic injury are frequently noted on laboratory investigations, which can be important diagnostic clues in attaining an appropriate diagnosis. Herein we present three cases of HGA, highlighting the spectrum of disease by which HGA can manifest. Although each case has their unique features, we outline important shared clinical elements to facilitate an empiric diagnosis while definitive laboratory investigations are pending. Our case series further serves to highlight the critical importance of prompt antimicrobial treatment to reduce morbidity and potential mortality.

Introduction

Human Granulocytic Anaplasmosis (HGA) is a rickettsial infection of granulocytes caused by the intracellular bacterium *Anaplasma phagocytophilum* [1]. As a tick-borne disease, its transmission results in human illness through bites of the blacklegged tick (*Ixodes scapularis*) [2]. This same vector is implicated in the transmission of other known pathogens including *Borrelia burgdorferi* and *Babesia microti*, the etiologic agents of Lyme disease and Babesiosis, respectively [3]. The public health impact of HGA continues to increase, largely attributed to the expanding geographic range of the disease vector [4]. Since Manitoba became the first and only province in Canada to provincially mandate HGA reporting in 2015, the number of confirmed HGA cases, in addition to *A. phagocytophilum* prevalence among field-collected blacklegged ticks continues to rise [5]. Despite this, recent human seroprevalence data suggest the true incidence and prevalence of HGA remains underestimated, with possible subclinical presentation of illness or missed clinical opportunities of disease recognition [6].

The clinical presentation of HGA is often non-specific, with most patients presenting with fever, chills, myalgias, arthralgias, headache and gastrointestinal symptoms [4]. Tick-exposure, if known, usually precedes symptom development by one to two weeks. In contrast to some other tick-borne diseases, the presence of rash is uncommon. Although complications, such as sepsis, heart failure, renal failure, neurologic disease and rhabdomyolysis remain infrequent [4], almost half of patients with HGA require

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hospital admission [7]. Severe clinical presentations, although less common, can be life threatening and require critical care support, particularly amongst the elderly and immunocompromised or those with significant comorbidities [7]. With *B. burgdorferi* co-infection rates with HGA ranging from 2.3 to 10% [8], consideration of concurrent infectious processes in the appropriate clinical context must be given. Clinical overlap with features of thrombocytopenia, elevated hepatic enzymes and anemia may be observed in Babesiosis; however, in many cases, the presence of the pathonogmonic "Maltese cross" formation of the parasite on peripheral blood smear is observed with marked gastrointestinal symptoms [9]. Leukocytosis and thrombocytopenia are uncommon with Lyme disease [9].

Herein we present three cases of HGA, presenting to an urban tertiary care center, highlighting the spectrum of disease by which HGA can manifest. Although each case has unique features, we outline important shared clinical elements to facilitate an empiric diagnosis. Our case series further highlights the critical importance of prompt recognition and antimicrobial treatment to reduce morbidity and potential mortality.

Cases

Case 1: A 68-year old previously well male farmer, presented with complaints of fatigue, myalgias and confusion. He reported a tick bite two weeks prior to symptom onset. Fever was noted, with an otherwise unremarkable physical examination. Laboratory investigations revealed lymphopenia (lymphocyte count 0.40 [normal 1.3–3.2] $\times 10^9$/L), thrombocytopenia (platelet count 16 [normal 140–440] $\times$ 10^9/L), hepatocellular injury (AST 840 [normal 10–32] U/L; ALT 189 [normal 0–25] U/L), rhabdomyolysis (creatine kinase 57,624 [normal 20–215] U/L) and acute kidney injury (creatinine 3.77 [normal 0.40–1.10] mg/dL). Polymerase chain reaction (PCR) testing on whole blood for *A. phagocytophilum* DNA was positive. A peripheral blood smear demonstrated a neutrophil containing intracytoplasmic inclusions (morulae) suspicious for *A. phagocytophilum* (Fig. 1). The patient was promptly started on empiric oral doxycycline 100 mg twice daily prior to receipt of confirmatory testing, with complete resolution of symptoms and normalization of laboratory aberrations, without complications.

Case 2: A 63-year old male, with a past medical history notable for coronary artery disease, presented with fever, dysarthria, and dysphagia in the context of multiple recent tick-bites sustained at a vacation home. On physical exam, dysarthria and dysphagia were noted in addition to a unilateral cranial nerve seven palsy. Laboratory investigations demonstrated lymphopenia (lymphocyte count 0.34×10^9/L), thrombocytopenia (platelet count 18×10^9/L) and hepatocellular injury (AST 118; ALT 71). Additional findings included an elevated serum triglyceride level (2.3 [normal <1.7] mmol/L), hyperferritinemia (ferritin 13 806 [normal 20–200] ug/L), and splenomegaly. PCR testing of whole blood and cerebrospinal fluid for *A. phagocytophilum* DNA confirmed the diagnosis of HGA. Initial serologic testing for *B. burgdorferi* co-infection was negative. Oral doxycycline was empirically initiated prior to receipt of confirmatory testing. The patient rapidly improved with complete resolution of neurologic symptoms over three days, followed by normalization of all hematologic and laboratory aberrations. Repeat serologic testing one month later for IgG antibodies to *A. phagocytophilum* demonstrated seroconversion.

Case 3: A 72-year old male presented with generalized weakness resulting in a fall. He endorsed a two week history of fevers and chills, with findings of progressive confusion and respiratory distress noted at the time of initial assessment. On collateral history, an unengorged tick had been removed from his body one week prior to illness. Laboratory investigations demonstrated thrombocytopenia (platelet count 39×10^9/L), anemia (hemoglobin 98 [normal 130–170] g/L), hepatocellular injury (AST 110 U//L; ALT 59 U/L), elevated serum triglycerides (2 mmol/L) and hyperferritinemia (4740 μg/L). Given the suspicion of a tick-borne infection, doxycycline 100 mg orally twice daily was initiated empirically. Given concern for evolving acquired hemophagocytic lymphohistiocytosis (HLH) [10,11], intravenous immunoglobulin and dexamethasone were concurrently administered. Review of peripheral blood smear demonstrated neutrophils containing morulae. Subsequent, whole blood *A. phagocytophilum* PCR confirmed a diagnosis of HGA. With appropriate antimicrobial therapy following receipt of confirmatory testing, the patient's condition quickly improved.

Discussion

HGA is an emerging infectious disease of public health concern in Canada. With demonstrated geographic range expansion of the disease vector, the incidence of HGA disease has followed in parallel. In Manitoba, the number of confirmed HGA cases has steadily increased since 2013. Fig. 2 demonstrates the number of IgG positive cases (defined as a four-fold titer increase to $\geq$ 1:256 over two to four weeks for paired samples) in Manitoba. The number of reported cases has also increased in other geographic regions of Canada. Populations of *I. scapularis* with *A. phagocytophilum* have established in parts of southern Manitoba, northwestern, southern and eastern Ontario, southern Quebec, southern New Brunswick, and regions of Nova Scotia [12,13]. Awareness and recognition of this highly treatable disease and its increasing prevalence is critical among health care providers. The majority of

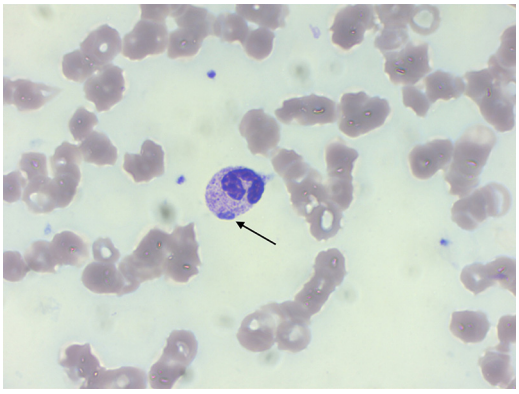

Fig. 1. Peripheral blood smear with Giemsa stain demonstrating the presence of an *Anaplasma phagocytophilum* morula within a neutrophil (arrow) Magnification x1000.

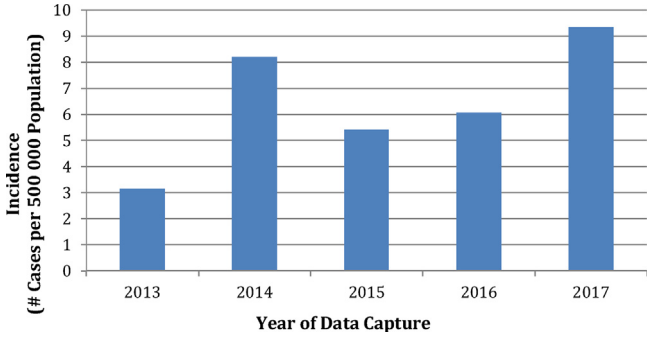

Fig. 2. Incidence of *Anaplasma phagocytophilum* positive cases, from 2013 to 2017 in Manitoba, by means of IgG indirect fluorescence assay (titre $\geq$ 1:256). Data obtained from National Microbiology Lab, Winnipeg, Manitoba.

HGA cases have been reported within the summer months, with a peak in cases reported in June and July, and a second peak observed in October and November [14].

While shared clinical features are observed, the above cases illustrate the spectrum of illness in which HGA may manifest, including sepsis, renal failure, neurologic disease and rhabdomyolysis. In each case, a non-specific febrile illness including fever, myalgias, and chills were noted, with thrombocytopenia, leukopenia, and biochemical evidence of hepatic injury. While both leukopenia and thrombocytopenia are present in many patients with HGA, these cytopenias often normalize within two weeks of infection [15], and may be absent depending on the timing of patient presentation. Two of the three cases had features suggestive, but not diagnostic of, acquired HLH [8], highlighting the importance of prompt antimicrobial treatment to reduce morbidity and potential mortality.

A definitive HGA diagnosis can be made by PCR amplification of *A. phagocytophilum* DNA from whole blood, the present gold-standard, or by acute and convalescent serologic testing. For the latter, a four-fold increase in IgG titer is required for paired samples drawn two to four weeks apart. A single positive IgG test for *A. phagocytophilum* is unreliable given the persistence of such antibodies from prior exposures, lasting upwards of three years following infection [16]. Visualization of morulae, intracytoplasmic inclusions of *A. phagocytophilum* within host granulocytes, on peripheral blood smear is highly suggestive but not pathognomonic of infection; however, the sensitivity of such a finding is too low to rule out active disease and even lower after initiation of doxycycline [17].

As suggested by seroepidemiologic data, HGA is most commonly a mild to asymptomatic infection, with the majority of patients recovering uneventfully without treatment [18]. Reassuringly, the case-fatality from HGA is low, less than 1% [19]. However, predicting those at increased risk for complicated or fatal disease is imperfect [7]. As such, prompt and empiric initiation of appropriate antimicrobial therapy is recommended in all suspected or confirmed symptomatic cases. *Anaplasma* species isolates have demonstrated uniform susceptibility to tetracyclines [20]. The preferred treatment for patients with HGA is doxycycline, in the absence of contraindications [14]. For adult patients, the recommended dose is 100 mg twice daily, orally or intravenously. For pediatric patients weighing less than 45 kg, doxycycline remains the antimicrobial of choice despite potential concerns of permanent teeth staining before the age of eight (dose of 2.2 mg/kg in two divided doses daily, orally or intravenously) [14][3]. Rifampin may be considered for treatment of HGA in pregnant patients; however, does not provide coverage against possible co-infection with other tick-borne diseases [20]. Rapid clinical response including defervescence is noted in the first 24–48 hours following initiation of antimicrobial therapy [4]. The consistency of this clinical response should prompt consideration of alternative diagnoses if not seen. The optimal duration of doxycycline has not been established; by convention 10 to 14 days is often prescribed, and 14 days is often selected when the risk for co-infection with *B. burgdorferi* is considered sufficiently high [13,14]. Given that convalescent serology for *B. burgdorferi* was not completed in case 2, to exclude the possibility of initial false positive testing, an extended course of antimicrobials was provided. Consideration of possible co-infection is necessary given co-infection rates of upwards of 10%, and disease transmissibility shared by the same disease vector, *I. scapularis* [8].

Conclusions

With increasing geographic range of *I. scapularis* populations, the incidence of HGA has followed in parallel, such that HGA is now endemic in several parts of Canada. A high index of clinical suspicion for HGA is required during tick season, in addition to other *I. scapularis* transmittable illnesses, such as Lyme Disease and Babesiosis as potential co-infections. For patients presenting with nonspecific fever and possible tick exposure, health care providers should consider tick-borne illnesses such as HGA. Prompt empiric treatment of symptomatic individuals with doxycycline is essential while awaiting confirmatory testing. Consideration should be given to making HGA notifiable in all Canadian provinces and nationally, allowing for accurate estimates of incidence and prevalence to inform targeted public health policies and effective messaging.

Conflict of interest

The authors declare that they have no competing interests. RZ receives salary and operating support from the Canadian Institutes of Health Research (CIHR) and holds the Lyonel G Irasels Professorship in Hematology from the University of Manitoba, Winnipeg, Manitoba, Canada. LJM has received a PhD Funding Award from the University of Manitoba Department of Internal Medicine and Department of Medical Microbiology and Infectious Diseases.

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Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Author contribution

Each author was involved in the writing of the manuscript. All authors approved the final manuscript.

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