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Tick-borne Infections in New Hampshire: An Evaluation of the Diagnostic Process in a Local Patient Population

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Abstract

Overall, approximately 95 percent of reported cases of vector-borne disease were associated with ticks, making these the most medically important group of arthropods in the United States.[1] Despite the prevalence of tick-borne infections, the process for the diagnosis of this condition is not well studied. This study aims to analyze data from a pool of 100 patients who underwent testing for tick-borne disease in the same institution in Dover, New Hampshire during the most recent peak tick season of 2019. Information utilized in this study included: patient age, sex, location of testing (inpatient versus outpatient), diagnostic testing methods used pertaining to investigation of tick-borne disease, results of tick-borne panel testing, number of days to obtain tick panel results, symptomology, treatments pertaining to the investigation of tick-borne disease, and record of follow-up visits. Analyses of these data points revealed a trend that suggests the current diagnostic process for tick-borne disease is unnecessarily burdensome for patients and medical facilities. There is a need for a faster turnaround time in testing to decrease the need for supplemental tests and follow-up visits pertaining to the investigation of tick-borne diseases. This study also suggests that recognition of symptoms associated with positive results is paramount to improve the detection of tick-borne illnesses. Further investigation of our current methods and possible future adaptations to them are critical if we are to conquer the diverse array of challenges presented by tick-borne diseases.

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Introduction

Ticks transmit the most diverse array of infectious agents of any arthropod vector. Both ticks and the microbes they transmit are recognized as significant threats to human and veterinary public health. Greater than 60% of human infectious diseases emerging between 1940 and 2004 were zoonotic, resulting in significant global morbidity, mortality, and economic costs.[2] During 2013, 48,821 cases of autochthonous, nationally notifiable, vector-borne disease were reported to the United States Centers for Disease Control and Prevention (CDC). Overall, approximately 95 percent of reported cases of vector-borne disease were associated with ticks, making these the most medically important group of arthropods in the United States.[1] Lyme disease alone accounted for almost 75 percent of all reported cases of indigenously acquired vector-borne disease.[1] Lyme disease is the most commonly reported vector-borne illness in the United States. Since the institution of Nationally Notifiable surveillance efforts for Lyme disease in the United States in 1991, there has been a consistent increase in the number of reported cases. Thus, the need for targeted prevention strategies is underscored.[3] Additionally from 2000 to 2007, the incidence of infections caused by *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis,* two tick-borne pathogens, increased linearly from 0.80 to 3.0 and 1.4 to 3.0 cases per million population, respectively.[1] Nonetheless, the true incidence of tick-borne disease is likely greatly underestimated, as patients with presumed infections are rarely tested for the full range of tick-borne agents, and only a fraction of positive cases are properly reported.[4]

Lyme disease, babesiosis, anaplasmosis, ehrlichiosis, and Powassan virus infection are the most prevalent diseases transmitted by ticks in the United States, all of which are endemic in the New England and upper Midwestern regions.[3,5] Diagnosis of these conditions is largely reliant on blood testing panels that utilize a whole blood sample to detect the presence of genetic material of a list of organisms. The Accutix panel offered by Imugen, a reference laboratory operating in Norwood, Massachusetts, consists of *Babesia microti* DNA detection, *Anaplasma phagocytophilium* DNA testing, *Ehrlichia chaffeensis* DNA detection, *Borrelia* species DNA detection, and Lyme Antibody analysis.[6]

Babesia are malaria-like protozoans that parasitize and reproduce within mammalian red blood cells. They have a complex life cycle involving several different stages and physical forms and are maintained in nature primarily via exchange between Ixodes ticks and various mammals, such as deer and mice.[7,8] In the United States, the primary agent of human babesiosis is *Babesia microti*, which is transmitted by the bite of *Ixodes scapularis*, the same tick species that vectors Lyme disease. Cases of babesiosis caused by *B. microti* occur in southern New England and the northern Midwest. Although primarily transmitted by tick bite, babesiosis can also be acquired via blood transfusion and maternal-fetal transmission.[8] *Babesia* infection can range from asymptomatic to life threatening. Risk factors for severe babesiosis include asplenia, advanced age, and impaired immune function. Severe cases can be associated with marked thrombocytopenia, disseminated intravascular coagulation (DIC), hemodynamic instability, acute respiratory distress, renal failure, hepatic compromise, altered mental status, and death.[5] Symptoms commonly seen in *Babesia* infection include malaise, headache, fatigue, fever, chills,

sweats, gastrointestinal symptoms (anorexia and nausea), and in some occurrences mild splenomegaly, mild hepatomegaly, and jaunice.[5] Laboratory findings might indicate decreased hematocrit due to hemolytic anemia, thrombocytopenia, elevated serum creatinine and blood urea nitrogen (BUN), and/or mildly elevated hepatic transaminase values.[5] Primary diagnostic methods are the identification of intraerythrocytic *Babesia* parasites via light microscope of blood smear, *Babesia* polymerase chain reaction (PCR), isolation of *Babesia* parasites from a whole blood specimen by animal inoculation, and antibody detection by indirect fluorescent antibody (IFA) testing for total immunoglobulin (Ig).[5] The non-specific symptomology and unremarkable physical presentation can make babesiosis difficult to detect. Physicians should be alert to test for this condition with the presentation of flu-like symptoms in the summer months. Limitations in testing are plentiful. It may be difficult to detect in early stages in a peripheral blood smear, IFA antibody detection does not necessarily indicate active infection since antibodies can be found in serum long after infection, and PCR may not be able to detect the organism in an early infection if the titer is not high enough. Coinfection with Lyme disease or anaplasmosis may also complicate the clinical presentation and predispose the patient to more severe disease.[8]

Another tick-borne pathogen endemic to the Northeast is *Anaplasma phagocytophilum*. This organism is an obligate gram-negative, intracellular bacterium that causes an acute febrile illness known as anaplasmosis or human granulocytic anaplasmosis (HGA), formerly known as Human Granulocytic Ehrlichiosis (HGE).[5] The organism is genetically related to *Rickettsia* and is transmitted *by Ixodes scapularis* in the northeast United States and *by Ixodes pacificus* in California. The vector also transmits other organisms responsible for diseases such as Lyme,

babesiosis, ehrlichioses and Powassan encephalitis.[9] *A. phagocytophilum* infection is acquired through a tick bite and disseminates to the bone marrow and spleen where it can evade neutrophil antimicrobial functions.[9] Severe and life-threatening illness is less common with anaplasmosis compared to other rickettsial diseases, such as Rocky Mountain spotted fever (RMSF) or *E. chaffeensis* ehrlichiosis. While the case-fatality rate among patients who seek care for the illness is <1%, predictors of a more severe course include advanced age, immunosuppression, comorbid medical conditions, and delay in diagnosis and treatment.[5] Anaplasmosis generally presents with nonspecific symptoms such as fever, chills, malaise, headache, and myalgias. On rare occasions, a rash may be present. The patient may also report nonspecific gastrointestinal (GI) or respiratory symptoms.[9] In the first weeks of infection laboratory findings may include a mild anemia, thrombocytopenia, leukopenia, and/or elevations in hepatic transaminases. Diagnosis is primarily accomplished by PCR analysis, IgG antibody titer by IFA, and immunohistochemical staining of organism from skin, tissue or bone marrow biopsies. Since antibody titers are often negative in first 7-10 days after infection and PCR is most sensitive during first week of infection, there are limitations in our current standards of testing for this condition.[5]

Ehrlichia chaffeensis is another prevalent pathogen transmitted by *Ixodes* ticks. *E. chaffeensis* is an obligate intracellular Gram-negative bacterium that causes Human Monocytic Ehrlichiosis (HME). Signs and symptoms of ehrlichiosis typically begin within 1-2 weeks after the bite of an infected tick. The most common symptoms include a fever with headache, myalgia, and malaise, and a rash can be observed in up to 33% of patients.[10] Gastrointestinal, respiratory,

or central nervous system involvement also may occur in more serious manifestations. Currently most infections are not diagnosed, but HME can be a life-threatening disease, with hospitalization in 41-63% of recognized cases. Severely affected patients can develop acute respiratory failure, renal failure, meningoencephalitis, coagulopathy, and GI bleeding. Untreated disease may progress to death as early as the second week of illness.[10] Available diagnostic tests include IFA (some cross reactivity with other Ehrlichia species is possible), western blot, PCR, visualization of morulae (intraleukocytic clusters of bacteria) in a blood smear, immunohistochemical staining, and isolation.[10] As with other diseases detected by these methods, the diagnosis of *E. chaffeensis* is complicated by the possibility of false positives if the infection level is below sensitivity parameters, particularly in microscopic and PCR methods. Seeing as this infection is carried by the same vector as several other tick-borne pathogens, diagnosis can also be affected by coinfection. Coinfecting pathogens may cause competition in the PCR reaction, since significantly higher concentrations of one pathogen compared with the others can result in the detection of only one organism.[11]

The spirochetes include several human pathogens, including *Treponema pallidum* (agent of syphilis), *Leptospira interrogans* (leptospirosis), and several *Borrelia* spp. that cause relapsing fever.[11] The spirochete *Borrelia burgdorferi* is a tick-borne obligate parasite whose normal reservoir is a variety of small mammals. Whereas infection of these natural hosts does not lead to disease, infection of humans can result in Lyme disease, as a consequence of the human immunopathological response to *B. burgdorferi.* The organism has a distinctive morphology that includes a spiral or wavelike body and flagella (organs of motility) enclosed between the outer

and inner membranes. Ticks of the genus *Ixodes* transmit *B. burgdorferi* between hosts and are the only natural agents through which humans have been shown to become infected. In the northeastern and midwestern United States, the primary tick species for human disease is *Ixodes scapularis* (the black-legged tick) and in the western states *I. pacificus* (the western black-legged tick) is the main agent of dissemination.[11] In 2015, 95% of Lyme disease cases were reported from 14 states: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and Wisconsin.[5] Ticks most frequently acquire spirochetes from infected rodents during their larval feeding. Both nymphs and adults occasionally feed on humans, but the small size of the nymphs makes them difficult to detect and, hence, more likely to feed long enough to transmit the spirochete and cause Lyme disease.[11] In the localized stage of Lyme disease, Erythema migrans (EM), a red ring-like or homogenous expanding rash; is a classic indicator of *Borrelia* infection but may not always be present. More common symptoms are flu-like, such as malaise, headache, fever, myalgia, arthralgia, and lymphadenopathy. During the localized (early) stage of illness, Lyme disease may be diagnosed clinically in patients who present with an EM rash. Serologic tests may be insensitive at this stage. During disseminated disease, however, serologic tests should be positive.[5] The disseminated stage is a later stage that may cause multiple secondary annular rashes, more severe flu-like symptoms, and lymphadenopathy. Rheumatologic, cardiac and neurologic manifestations are also possible at this point in infection.[5] Most notable laboratory findings include an elevated erythrocyte sedimentation rate, mildly elevated hepatic transaminases, and microscopic hematuria or proteinuria. In Lyme meningitis, cerebrospinal fluid typically shows lymphocytic pleocytosis, slightly elevated protein, and normal glucose.[5] Diagnosis

is primarily achieved through demonstration of diagnostic IgM or IgG antibodies in serum. A two-step testing protocol is recommended, the first step being serological antibody analysis and the second being confirmation via western blot.[5] Coinfection with *B. microti* and/or *A. phagocytophilum* should be considered in patients who present with initial symptoms that are more severe than are commonly observed with Lyme disease alone, especially in those who have high-grade fever for more than 48 hours despite appropriate antibiotic therapy or who have unexplained leukopenia, thrombocytopenia, or anemia. Coinfection should also be considered in patients whose erythema migrans skin lesion has resolved but have persistent flu-like symptoms.[5] As will all aforementioned tick-borne pathogens, testing limitations are abundant. In serological testing the sensitivity and specificity are not always high enough to be detected, and cross-reacting immunoglobulins can be a problem in many cases. Genetic detection by PCR is successful only during acute infection when levels of the analyte are found at high enough concentrations in a blood specimen. Also, given the organism's spirochete morphology, it can be difficult to observe using traditional microscopic techniques and may require special staining and dark microscopy.

Even with the true incidence of tick-borne disease likely being greatly underestimated by epidemiological data, studies of trends over the last few decades have demonstrated that this group of infections have emerged or re-emerged in many geographical regions.[12] Yet the precise diagnosis of many of these diseases still remains a major challenge because of the lack of comprehensive data available on accurate and reliable diagnostic methods.[12] The diagnostic process is further complicated by the many limitations on the available testing strategies in

current practice. Identification of pathogens in biological samples has been dominated by the use of culture-dependent methods, conventional molecular approaches, and serological tests.[12] In regards to tick-borne infections, these methodologies suffer from major limitations. Microscopy remains an important part of laboratory testing for the diagnosis of most tick-borne diseases, especially in resource-limited settings, but it is highly subjective and dependent on experience and training.[12] In a modern diagnostic laboratory setting, the time constraint and expertise that microscopy requires prevents this method from being used on most samples unless specifically mandated by a physician. Cell culture procedures are time consuming, and isolation of pathogens is not always successful. The specificity and the sensitivity of serological tests are not always optimal, and cross-reactions are a common problem.[12] With the upward trends the health community is seeing in this group of infections, it will become imperative to adapt our current standards of diagnosis to more accurately and quickly treat affected patient populations. To better understand these issues, this study aims to analyze data from a pool of 100 patients who underwent testing for tick-borne disease in the same institution in Dover, New Hampshire during the most recent peak tick season of 2019. It is here that we quantify and elaborate on the biggest challenges in detecting tick-borne disease with the goal of identifying possible pitfalls in current tick-borne diagnostic practices.

Materials and Methods

This study was based on results obtained through the electronic medical records of 100 random, fully de-identified patients who received tick panel testing through Wentworth-Douglass Hospital in Dover, New Hampshire from April 1st to July 31st of 2019. Approval of the retrieval of this de-identified information was granted from the Institutional Review Boards of the University of New Hampshire (IRB #8122) as well as Wentworth-Douglass Hospital in August 2019. From the time period of August 2019 to November 2019, data collection was performed on the WDH campus from a computer within the hospital network. Here, information was extracted, organized, and de-identified from the electronic medical records. The Safe Harbor method for de-identification of protected health, a HIPAA compliant way to remove specific identifiers from a data set, was used in the collection of data.[13] The electronic medical records were obtained through multiple hospital software systems. Soarian Clinicals was used to access information on the testing results from all departments within the laboratory as well as outside, such as cardiology and radiology. Soarian Electronic Documents Manager was utilized to view all documentation under the patient profile, this includes intake forms, triage nurse notes, hospitalist notes, and treatment notes. NextGen is the software containing information on most outpatients such as phone call records, drug prescriptions, and follow-up appointments. SoftLab is the laboratory software which provided the results of the tick panel for each patient as well as all other laboratory testing. From a hospital device on the WDH campus, the medical history of 100 random patients was accessed and manually transferred and simultaneously de-identified to an Excel spreadsheet on a private device outside the hospital network. This relevant information included: patient age, sex, location of testing (inpatient versus outpatient), diagnostic testing

methods used pertaining to investigation of tick-borne disease, results of tick-borne panel testing, number of days to obtain tick panel results, symptomology of the patient, treatments pertaining to the investigation of tick-borne disease, and record of follow-up visits pertaining to initial symptoms of tick-borne disease. After collection of each of these data points on all 100 patients, analyses and generation of figures was done using the original Excel spreadsheet where data was stored. Figures were generated using formulas in Excel and charting tools within the software.

Results

Patient Demographics

The total number of patients analyzed (100) was partitioned into age groups. The age groups (in years) were under 21, 21-30, 31-40, 41-50, 51-60, 61-70, and 71+. Each age group was then further divided into males and females. The under 21 age group comprised a total of 7 patients, 5 of them being female and 2 being males. The 21-30 age group totaled 18 patients, 12 female and 6 male. The 31-40 age group comprised a total of 9 patients, 4 female and 5 male. The 41-50 age group consisted of 13 patients, 11 female and 2 male. The 51-60 age group comprised a total of 23 patients, 8 female and 15 male. The 61-70 age group totaled 17 patients, 5 female and 12 male. The 71+ age group consisted of 13 patients, 5 female and 8 male (Fig. 1A).

Data on each of the 100 patients included the location which the initial visit occurred. All the locations were simplified into three categories: inpatients, outpatients, and emergency room patients. The number of patients in each group was 11, 49, and 40 respectively (Fig. 1B).

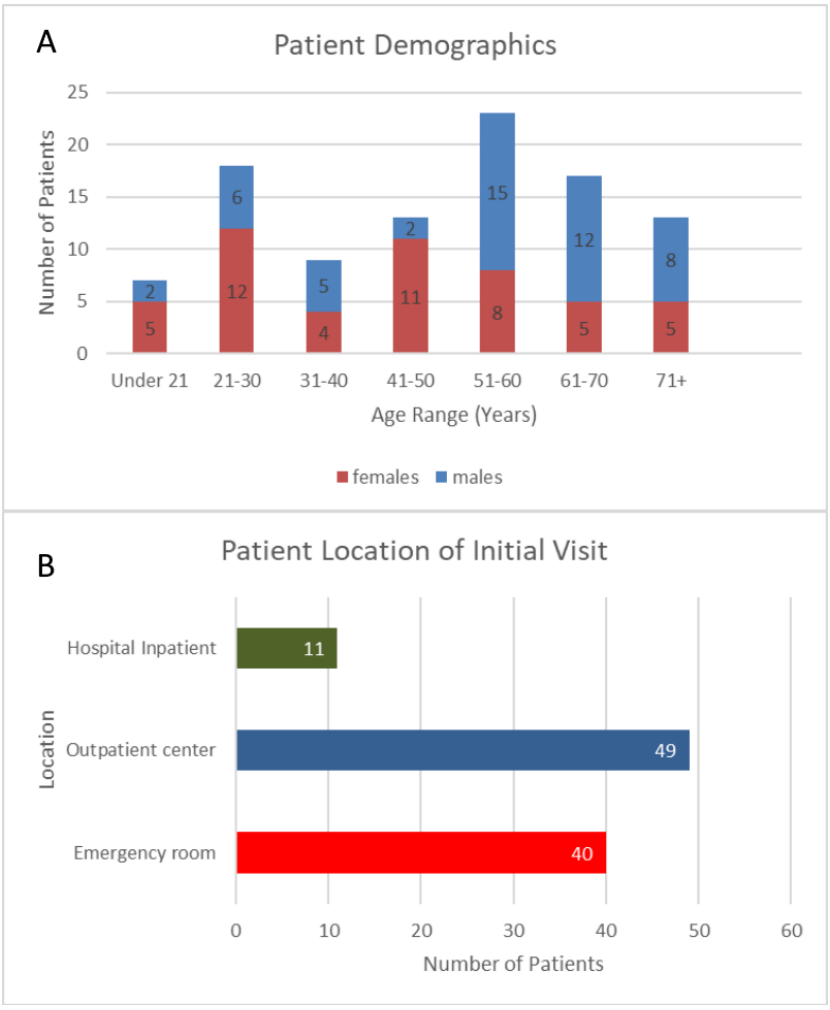

Figure 1. Patient Demographics. A. The age ranges for the 100 patient pool spans from under 21 years to 71+ years. Both males and females are represented in each age range. **B.** The location of the initial visit for each patient is shown via three groups, Hospital inpatients, outpatients, and emergency room patients.

Clinical Presentation

Within the 100 patient pool the most commonly reported symptoms were determined. They included: fatigue, dizziness, vomiting, nausea, headache, myalgia, fever, and erythema

migrans/rash/tick bite. For each symptom the total number of patients reporting it were determined as well as the amount of those patients that were positive for any organism detected via PCR and positive for any immunoglobulins detected by serology. Seeing as patients could report more than one symptom, the number of patients reporting should not be considered as part of the total 100 but rather part of the group of all patients reporting the symptom. Nine patients reported fatigue, 2 of them PCR positive and 1 serology positive. Dizziness was reported by 6 patients, only 1 of which was positive for PCR. Vomiting was reported in 3 patients, each were negative in PCR and serology testing. Nausea afflicted 11 patients, 2 PCR positive and 1 serology positive. Twenty patients reported headaches, 5 were PCR positive and 2 were serology positive. Forty-five patients reported myalgia as a symptom, of which 3 were PCR positive and 8 were serology positive. Fever was reported by 27 patients, 5 were PCR positive and 3 were serology positive. Twenty-eight patients reported having erythema migrans, a rash, or a tick bite, 4 were PCR positive and 5 were serology positive (Fig. 2A).

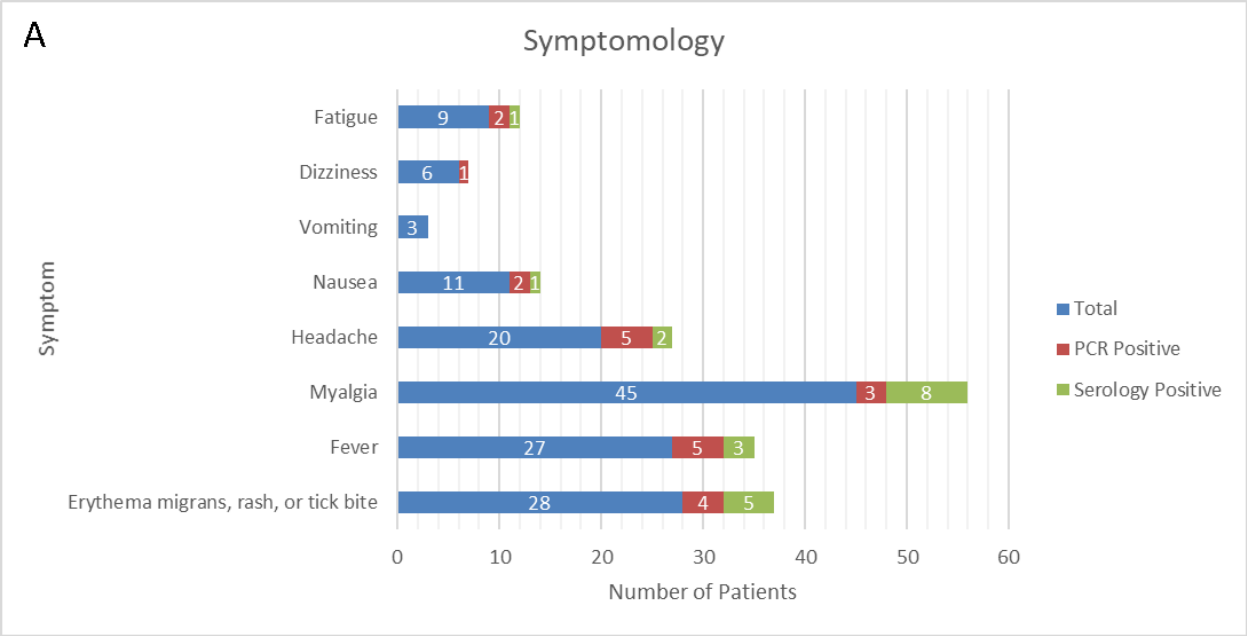

Figure 2. Clinical Presentation. A. The most common symptoms as reported by a nurse or physician along with the total number of patients that reported that symptom, the number of PCR positive patients with that symptom, and the number of serology positive patients with that symptom.

Diagnostic Process

As part of the diagnostic protocol, patients underwent testing other than an Accutix panel. Within the hospital, patients received testing results from radiology, cardiology and the laboratory. Within each of these departments, the most reported testing procedures were identified. In radiology, patients could have undergone a CT scan, an MRI, other specialty imaging, ultrasounds, or an X-ray. In cardiology, some patients received cardiac analysis from a specialist. In the laboratory, cultures of blood, urine, and other body fluids could have been done,

in addition to Flu testing and the Monospot which detects Epstein-Barr virus. In radiology, 14 patients had a CT scan, 2 had MRI's, 3 had specialty imaging, 1 had an ultrasound, and 1 had an X-ray. Three patients saw a cardiac specialist in the cardiology department. In the laboratory, 9 patients had cultures, 2 got tested for the flu, and 4 were reported as having done a Monospot test (Fig. 3A).

All 100 patients obtained results from an Accutix panel test, consists of *Babesia microti* DNA detection, *Anaplasma phagocytophilium* DNA testing, *Ehrlichia chaffeensis* DNA detection, *Borrelia* species DNA detection, and Lyme Antibody analysis. All DNA detection was performed via PCR, and antibody analysis was done via serology. The results of the panel were as follows: negative PCR for all organisms, positive PCR for 1 organism, positive PCR for 2 organisms, and positive Lyme antibody serology. The number of patients with those results was 89, 11, 0, and 16 respectively. An additional result of note is a positive *Borrelia* PCR and positive Lyme antibody serology, which only 1 patient had (Fig. 3B).

The Lyme antibody serology analysis detected three immunoglobulins, IgG, IgA, and IgM. A positive serology result could be positive for IgM, IgG, IgA, or a combination of IgM and IgG, IgM and IgA, IgG and IgA, or all three immunoglobulins. The number of patients with positive IgM only was 11, 3 patients were positive for only IgG, and 2 were positive for only IgA. Patients with both IgM and IgG totaled 2, while the other two combinations had no patients. One patient was positive for all three immunoglobulins (Fig. 3C).

Each of the 100 patients had an Accutix panel tested at a reference laboratory with results sent electronically back to the hospital laboratory. For each patient, the time (in days) between the blood sample draw and the testing on the sample was calculated. Most patients (33) had a 4 day time, 22 patients had 5 days, 20 had 3 days, 10 patients had 2 days, 8 patients had 6 days, 4 patients had 7 days, 2 patients had 8 days, and 1 patient had a 1 day time until testing was done (Fig. 3D).

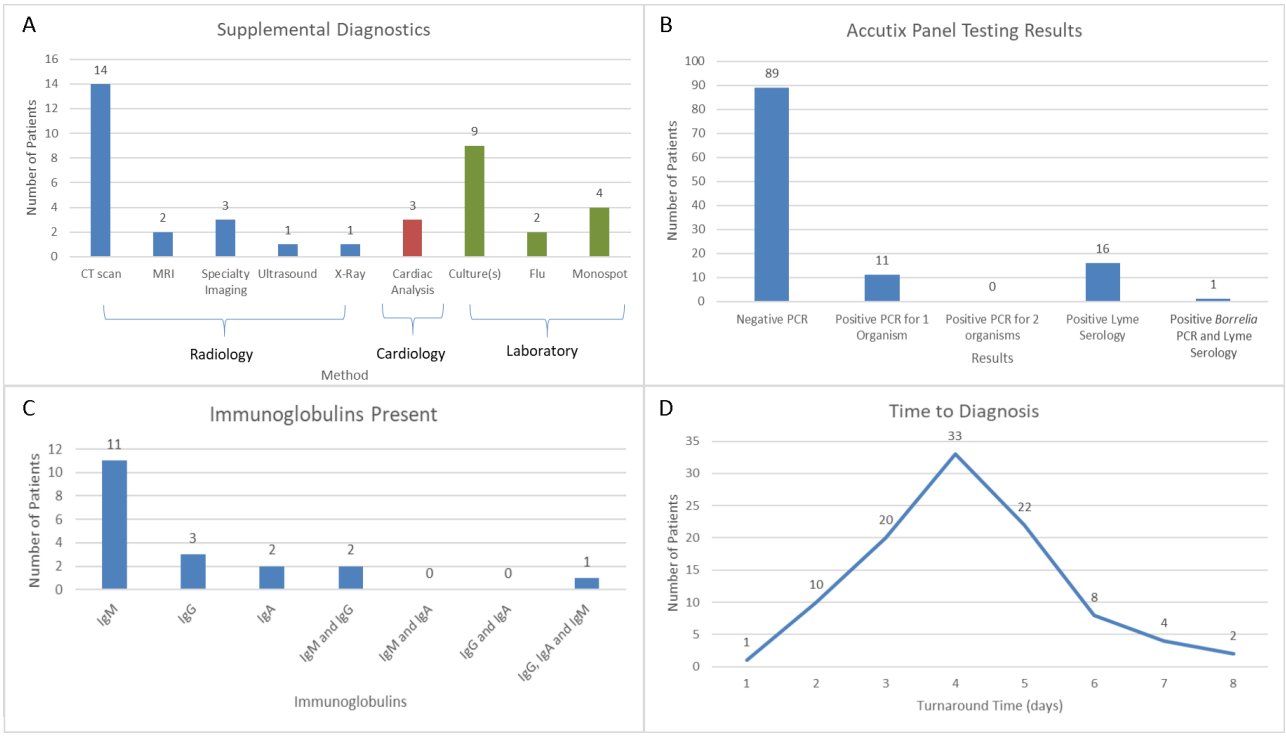

Figure 3. Diagnostic Process. A. The most common reported supplemental methods of diagnosis grouped by departments radiology, cariology, and laboratory, and the number of patients whose records indicated diagnostic procedures in each. **B.** Number of patients with each possible result from testing in both serology and PCR analysis. **C.** The number of patients with each combination

results of immunoglobulins IgG, IgA, and IgM. **D.** The time (days) between when the patient sample was sent for testing and the test was performed in a reference laboratory.

Treatment Management

The medications used to treat each of the 100 patients that were reported in the patient documentation can be categorized into analgesics, antibiotics, antihistamines, steroids, and other substances not belonging to any category. The number of patients in each was quantified. In the analgesics, 2 patients were given aspirin, 14 were recommended ibuprofen, 6 were given ketorolac, 30 received acetaminophen, and 4 were given Zofran. Of the antibiotics, 2 patients took amoxicillin, 2 had ceftriaxone, 35 took doxycycline, and 2 had vancomycin. The only antihistamine was Benadryl, which was given to 3 patients. Prednisone, the only steroids, was prescribed to 4 patients. Fluids were administered to 22 patients (Table 1).

Treatment	Number of Patients
Analgesics	
Aspirin	2
Ibuprofen/Advil/Motrin	14
Ketorolac	6
Tylenol/Acetaminophen	30
Zofran	4
Antibiotics	
Amoxicillin	2
Ceftriaxone	2
Doxycycline	35
Vancomycin	2
Antihistamines	
Benadryl	3
Steroids	
Prednisone	4
Other	
Fluids	22

Table 1. Treatment Management. The most common reported substances for treatment categorized into drug classes analgesics, antibiotics, antihistamines, steroids, and other substances along with the number of patients who received that treatment.

Incidence of Follow-Up

Based on patient visit information on the hospital software, the incidence of follow-up visits at facilities in the hospital network pertaining to the investigation of tick-borne disease was quantified. Of the 100 patients, 42% did have a documented follow-up visit and 58% did not (Table 2).

Follow-up Indicated	Percentage of Patients
Yes	42%
No	58%

Table 2. Incidence of Follow-up. The percentage of patients whose record indicated a follow-up visit pertaining to the investigation of tick-borne disease compared to the percentage of patients whose record did not indicate a follow-up.

Discussion

The pool of 100 patients who underwent testing related to a possible tick-borne disease consisted of 50 men and 50 women ranging in age from under 21 to 71+ years. Most of the patients, 66%, were 41 years and older, and the category with the lowest number of patients was the under 21 group. This is not surprising considering the group most at-risk in acquiring tick-

borne diseases are older patients with weaker immune systems.[14] The majority of patients (23%) were ages 51-60, and within this category was also the highest number of patients from a single gender, 15 males. The high number of patients in this age range could be suggestive of an at-risk lifestyle in this population. People between 51-60 are at or close to retirement age and likely own their own homes, meaning their summers may largely be spent on lawn care. Patient in this age range are particularly at-risk in acquiring tick-borne infections since they are still active enough to become exposed, but may not have strong enough immunity to protect themselves from infection.[14]

When a patient begins the process to receive tick panel testing on their blood sample, they are often in one of three locations. They are either a hospital inpatient, an outpatient at a clinic, or an emergency room patient. Hospital inpatients are the least healthy of all these populations seeing as they are already in the hospital being cared for due to a different cause. Not surprisingly, this group of patients totals only 11 out of the 100 studied. This is most likely because these patients are not outside becoming exposed to ticks, but still do have symptoms that might warrant tick panel testing. It is the outpatients that make up a total of 49%, the most of any population, with emergency room patients having just a slightly lower total of 40%. These two populations present the most risk considering they are more likely to be spending their days outside becoming exposed to ticks. One reason outpatients might outnumber emergency room patients would be based on the preference of most patients. Most people prefer to visit a walk-in clinic rather than face a trip to the emergency room which is usually more expensive.[15] If a patient has only headaches or a mild rash, it is unlikely they treat this as an urgent enough issue

and make a trip to the hospital where longer wait times and expensive bills are real consequences.

The symptomology of each patient was based on reports from nurse practitioners and doctors who assessed the patient and recommended or administered treatment. The most common symptom reported was myalgia, or muscle pain, which comprised a total of 45 patients. The group of patients who reported this symptom also had the highest number of serology positive people, 8 total, and had 3 people who were PCR positive. The next highest reported symptom was erythema migrans/rash/tick bite which had 28 patients total. Five of these patients were serology positive and 4 were PCR positive. This is interesting seeing as erythema migrans has long been considered a hallmark symptom of Lyme disease,[16] and yet only 11 patients who reported this symptom and others like it were serology or PCR positive. This study may beg the question as to whether this clinical presentation should be given the weight that it currently holds in the diagnostic process. The third most commonly reported symptom was fever, which occurred in 27 patients, 3 being serology positive and 5 being PCR positive. Patients with fever and patients with a headache (20 total) were tied for most PCR positive patients at a total of 5. The lowest reported symptom was vomiting with 3 patients (none positive) and dizziness with 6 patients (1 PCR positive). This information can be helpful in differentiating the likelihood of positive results based on symptomology. Patients presenting with myalgia were mostly serology positive, which is not likely reflecting a current infection but perhaps a prior infection that has been treated. Patients with PCR positivity indicating an acute infection were mostly comprised of those presenting with fever and/or headache. Fatigue was only reported in 9 patients with 2 testing PCR positive and 1 serology positive. Considering that fatigue or malaise is a chief

symptom of tick-borne disease,[16] it is interesting that it was reported in so few cases. This may indicate that more attention should be put on those suffering myalgias and fevers than those who are fatigued, nauseous, vomiting, or experiencing dizziness regarding tick-borne diseases.

In addition to submitting a blood sample for Accutix panel testing, many of the 100 patients were reported as having other supplemental diagnostic testing done in hospital departments. The most commonly utilized departments were radiology, cardiology, and the hospital laboratory. Of the testing done in radiology, CT scans were reported by the most patients of all the departments at 14%. In the laboratory, 9 patients had other cultures performed, 2 had flu testing, and 4 had a Monospot test. Three patients underwent cardiac analysis in cardiology. The total percentage of patients who had reported supplemental testing was 39%, meaning that a considerable amount of people underwent additional testing while their Accutix results were pending. This trend creates a large burden for the testing facility and the patients undergoing testing. Seeing as this testing is often done in the days before the reference laboratory performs the Accutix testing, this extra cost could theoretically be eliminated if the results were returned at a faster rate.

Seeing as the Accutix panel is comprised of PCR analysis of four different organisms and a Lyme antibody serology analysis, there are several kinds of testing results that patients could obtain. The result belonging to most patients, 89, was negative PCR analysis. The remaining 11 patients out of the 100 were positive in PCR for one organism. There were no patients who tested positive in PCR for 2 or more organisms. Of the 100 patients, 16 total tested positive in Lyme antibody serology. There was only one patient who tested positive for *Borrelia* species PCR and Lyme serology, which is interesting considering people with *Borrelia* infection are expected to

have positive results in both of these analyses.[17] This number could indicate that it is not often that patients are going to reflect both active infection and immune response during testing. Most patients are either in an acute phase where DNA is present and antibodies are not yet detectable, or they are in a recovered phase where antibodies are still present but the DNA of the organism is not detectable.[17] This information could be valuable in assessing the window period of infection and comparing it to when patients are most likely going to see symptoms that prompt a visit to their clinic for testing.

In the patient population who tested positive in Lyme antibody serology, there are three immunoglobulins for which a person could be positive, IgG, IgM and IgA. The sole presence or combination of any (or all) of these immunoglobulins provides important diagnostic information which helps determine what stage of infection the patient might be in.[17] In the pool of 100 patients, most people who tested positive in serology had IgM (11 patients). IgG was only found in 3 patients and IgA was found in 2. Since IgM is the first antibody produced upon infection, this could indicate that most people who tested positive were in an early response stage of infection, which often occurs at least ten days after the initial exposure.[18] The low prevalence of IgG immunoglobulins in this patient population indicates that there were not many cases of recurring infection, where IgG is expected to be found in higher concentrations than IgM.[18] These figures offer a valuable insight as to the stage of infection that most patients present with at the time of testing.

Since the Accutix panel testing is done at a reference laboratory outside the hospital, there is a wait time between when the specimen is collected and when it is tested. This time is expressed in days and is calculated from the laboratory reports from each of the 100 patients.

About one third of patients, 33 of them, had a 4 day wait time for their Accutix testing. There were 22 patients who had a wait time of 5 days and 20 patients who waited 3 days. Seeing as most patients had more than a couple days to wait for their results, it is likely that in this time patients underwent other testing and further investigation of their symptoms pending their panel results. This presents a greater economic burden to the patient, and the testing might not ultimately be necessary depending on the actual diagnosis the patient receives. The time and resources of the different departments being handed these patients while they are waiting for off-site results is also significant and presents a challenge for the facility.

Of the treatment that was most commonly reported amongst the 100 patients, most can be categorized by drug class. These include analgesics, antibiotics, antihistamines, steroids, and other substances. The most noteworthy treatments were the analgesics Ibuprofen and Acetaminophen which were used by 14 and 30 patients respectively, and the antibiotic doxycycline which was prescribed to 35 patients. Fluids were either recommended or administered via IV to a total of 22 patients. This data reveals that the way tick-borne testing is treated is mostly palliative care and low-grade pain management. The most common antibiotic used is doxycycline which is almost always written as a 100 mg pill taken twice per day for at least ten days. Although doxycycline has been shown to be effective against tick-borne disease,[19,20] the problem of over-prescription must be considered. Since many patients given this antibiotic are prescribed in leu of Accutix results being released, there are a considerable number of patients taking a course of antibiotic they likely don't need. The consequence of over-use of antibiotics has long been investigated as a cause of antibiotic resistance.[21] If the medical community relies too heavily on doxycycline, it is simply a matter of time before the organisms

we are attempting to combat debut a mutation that allows resistance in the next tick season. With the prevalence of tick-borne disease today, it is likely that our treatment for this type of infection will have to become more targeted if we are to prevail over these pathogens in the future.

The incidence of follow-up visits at facilities in the hospital network pertaining to the investigation of tick-borne disease was quantified. Of the 100 patients, 42% did have a documented follow-up visit and 58% did not. These follow-ups in 42 of the studied cases could have been a visit with a specialist, an appointment for scanning in radiology, a primary care physician visit, or an emergency room or outpatient facility visit for worsening symptoms. All of these types of visits pertain to the diagnostic investigation of tick-borne illness. It is important to consider that almost half of this small population of patients returned for a follow-up, and this figure is likely underestimated seeing as patients could have made an appointment outside of the hospital network. This high percentage of follow-ups present two problems; the first being a heavy financial burden on the patient and the second being the large amount of time and resources spent on these patients by the hospital. There are benefits to follow-ups where the treatment of these patients is discussed, for example when a physician is ensuring the patient how important it is to finish their course of antibiotics. However, these follow-ups can be detrimental to both the patient and the hospital facility if they are functioning as continued investigations in leu of tick-panel results. Supplemental testing being ordered and performed during these visits means there are more charges being added to the patient's bill and more resources being used on this patient by the facility. If there was a quicker methodology in

detecting these kinds of diseases, the savings on behalf of all parties involved would be significant.

Conclusions and Future Directions

The predominating conclusion of this study is that investigation of tick-acquired disease is burdensome to the patient and the hospital facility, and studying this process to better adapt to this challenge will be imperative in the future of health care. This is evidenced by data surrounding supplemental testing, time to diagnosis, and follow-up visits. Thirty-nine percent of the studied patient population had supplemental testing in addition to an Accutix blood analysis panel. Thirty-three percent of patients waited four days to receive the results of their panel, which is likely the same days they underwent additional testing. Finally, 42% of patients required a follow-up visit in the investigation of their tick-borne illness. Between supplemental testing and follow-up visits taking place in the hospital, there is a significant burden placed on the facility as these patients await their Accutix results. The days in which patients and physicians are waiting for definite diagnosis are often the same days in which further investigation is taking place. Ultimately, the financial obligations of the patient and testing facility are maximized in our current process. During the peak tick seasons in New England, it would be beneficial for more hospital facilities to study the flow of diagnoses in patients presenting with symptomology suggesting tick-borne disease. There is abundant room for improvement in this process that would protect the resources of both the patient and the hospital.

Another noteworthy trend that became apparent during this study was the immense challenge of navigating the clinical presentations of tick-borne diseases. The hallmark symptoms

in this category of infections are erythema migrans or bull's eye rashes, fever, fatigue and malaise. The bull's eye rash associated with Lyme disease is an observation that is rarely actually observed in clinical practice, and other "hallmark" symptoms like fatigue are so general to the scope of medical practice that they are virtually useless in a differential diagnosis. This study found that the most common reported symptom from patients who received positive test results for tick-borne infection was myalgia. This begs the question as to whether the medical community needs to re-visit its definition of the most common clinical presentations in tick-borne infections. More research to determine which symptoms are most closely related to positive test results is needed to best service clinicians and patients during peak tick season.

Despite the important implications of this study, the many limitations within it must be acknowledged in its consideration. The patient pool size of 100 is sufficient for a small-scale analysis, but future studies would be most effective with larger population sizes of thousands of patients. The geographic limitations of this study are also of note. Seeing as data in these analyses came from one single hospital and its affiliating locations, it is imperative that a larger range of facilities be studied across the Northeast and in other areas where tick-borne illnesses are endemic. Continuations of studies such as this should include investigation on alternative methods of diagnosis and their effectiveness as viable alternatives to the current process. For example, new companies such as Tick Report.com are surfacing which offer mail-in tick testing services. The idea behind this concept is based on testing the tick directly for pathogens before lengthy testing is done on the patient in a hospital setting. Patients who discover a tick can elect to mail it to company headquarters and receive an electronic report within 72 hours with a complete profile on their tick.[22] The information on the tick profile might indicate the presence

of a pathogen, at which point the patient can decide to seek further testing and possible treatment. Concepts such as this do have their own list of limitations, the most noteworthy being the small chance that a patient is able to recover a tick from the site of possible infection. However, the great opportunity for a more robust set of epidemiological data on the tick population in the Northeast is a valuable potential outcome for services such as these. Another option to decrease the turnaround time for tick-borne infection testing could be the development of adapted molecular assays. Many platforms already exist for the rapid detection of bacteria and viruses via polymerase chain reaction (PCR) amplification of nucleic acids. The GeneXpert system by Cepheid is an increasingly prevalent part of many hospital laboratories and features assays for Influenza, C. diff, Gonorrhea and Chlamydia, and others with turnaround times within a few hours[23]. With more molecular testing being made available at hospital level to eliminate the need for send-out tests, it may be worthwhile to explore adapting these methods for tick-borne pathogens. The ultimate goal is reducing the time to diagnosis in these patients and avoid over-testing, over-prescribing, and consequently over-paying for both the patient and the health care facility. Further investigation of our current methods and possible future adaptations to them are critical if we are to conquer the diverse array of challenges presented by tick-borne diseases.

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