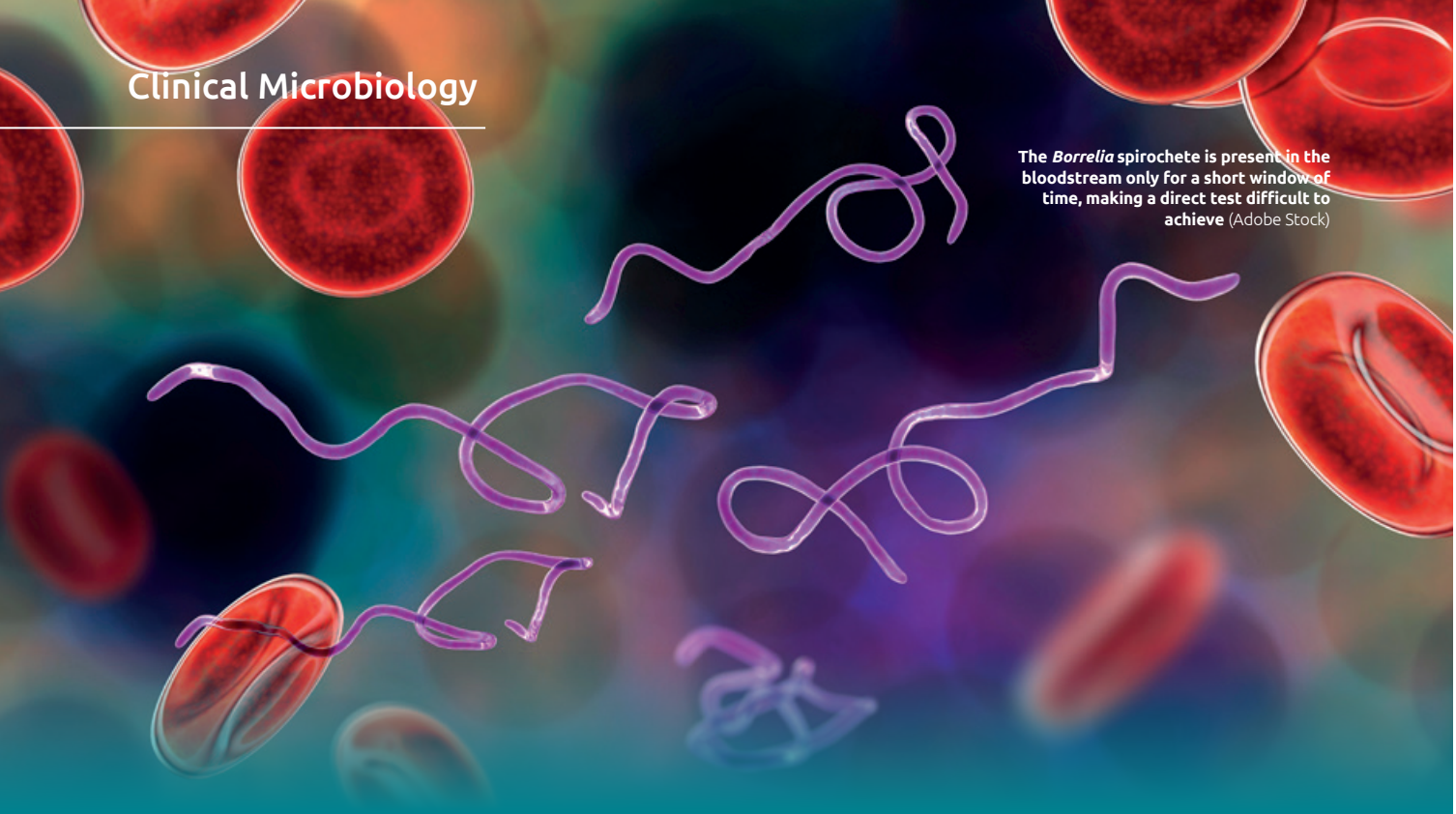


The *Borrelia* spirochete is present in the bloodstream only for a short window of time, making a direct test difficult to achieve (Adobe Stock)

Single-tier testing improves diagnosis of Lyme disease

Lyme disease can be treated simply and effectively if caught early. However, significant challenges are associated with current tests. CLI chatted to Prof. Monica Embers (Tulane National Biomedical Research Center, Tulane University, New Orleans, LA, USA) to discover more about her single-tier test process that allows effective diagnosis at early stages of the disease.

What is Lyme disease?

Lyme disease is the most common tick-transmitted infectious disease, which is caused by *Borrelia burgdorferi* spirochetes transmitted through hard ticks (of the *Ixodidae* family). It's prevalent throughout North America and throughout Europe and we're also seeing it on other continents. Lyme disease has become more prevalent and it's become very commonly diagnosed in the USA, with close to half a million cases per year.

Lyme disease typically affects multiple organs as it's a systemic infection. The bacteria tend to cause problems in and around the joints, so it can cause arthritis. It can get into the brain and cause neuroborreliosis, which has a variety of manifestations such as cognitive impairments (some people call it brain fog) for example difficulty with solving problems, and massive fatigue. Then also the heart can be affected, so you have cardiac Lyme disease. So those are the three main organ systems that are found to be affected by Lyme disease: the heart, the brain and the joints.

Often Lyme disease starts with a skin rash – in North America we have *B. burgdorferi* sensu stricto which causes erythema migrans at the site of the tick bite, which is the classical expanding red 'bullseye' rash. Unfortunately, it doesn't always look like that and not everyone sees the rash. And so those people who have the rash can go to the doctor and readily become diagnosed, but those who have something that looks a little bit different or they don't see it. Once diagnosis is prolonged, then long-term problems can ensue.

In the UK and Europe, Lyme disease is caused by *B. burgdorferi* from the sensu lato group, most commonly *B. afzelii* and *B. garinii* (and less commonly by *B. bavariensis* as well as *B. burgdorferi* sensu stricto). *B. afzelii* can cause multiple skin lesions or rashes, whereas *B. garinii* is more neurotropic, so neuroborreliosis is more prevalent with that infection.

In terms of the disease, if diagnosis is delayed or treatment is incomplete, patients can have what we call post-treatment

Lyme disease, and this is typically characterized by, ironically, the same primary symptoms that are seen in long Covid, which are neurocognitive difficulties, extreme fatigue and musculoskeletal pain. Patients who have this chronic version of Lyme disease have long-term impairment in terms of their morbidity, in terms of their functionality in life, so it's very problematic.

I would tentatively compare it to cancer where the earlier you're treated the more likely it is that you will be able to return to health. The frontline drug treatment is typically doxycycline or amoxicillin for anywhere from 10 to 21 days if it's caught early. After that, once the neurological, cardiac manifestations or arthritic manifestations start to develop, patients sometimes need intravenous antibiotics. We've also found that the bacteria have the capability to persist in the presence of doxycycline, and so we need to start thinking about treating patients with multiple drugs at the same time in the same way that we treat tuberculosis. We have found that in mice that this kind of multidrug treatment regimen does actually work a lot better in terms of clearing the infection.

How is diagnosis of Lyme disease normally done?

The classic signs and symptoms of Lyme disease that occur in the first 3–30 days after a tick bite include:

- fever, chills, headache, fatigue, muscle and joint aches and swollen lymph nodes, which may occur in the absence of rash; and/or
- erythema migrans rash (which may or may not necessarily enlarge into the typical 'bull's eye' appearance.

If the patient's symptoms meet the clinical case definition according to the Centers for Disease Control and prevention (CDC) recommendations for diagnosis of Lyme disease, the physician can make a diagnosis and begin treatment.

As mentioned above, early treatment is ideal to prevent development of chronic Lyme disease, which can be extremely debilitating.

The challenges are that the symptoms can be highly variable: a rash may or may not be present, and if it is, it might not develop into the classic bull's eye appearance. Also, even if the patient has the characteristic bull's eye rash, the criteria are that it should be greater than 5 cm in diameter. So if the patient has a 4.5 cm, there are probably clinicians who will say that they don't have Lyme disease. Also, there are certainly differences between the sexes in terms of how the rash presents. Other variables include where on the body you're bitten, how long the tick feeds and if there are other co-infections. So there are a lot of variables that go into using the rash only as a clinical diagnostic.

Often the disease is called the summer flu; if a patient presents with a mild fever and malaise in the absence of a rash then testing for Lyme disease is necessary, particularly if they're in an area where the disease is endemic.

How is testing for Lyme disease usually done and what are the limitations of these methods?

The most commonly used test is the two-tier serologic test. The first tier or the first phase is an enzyme immunoassay (typically an ELISA) and the second tier is an immunoblot (Western blot). Additionally, along with the standard two-tier test, we also have the modified two-tier test, which consists of two different ELISAs, one experimental and one confirmatory.

These are antibody-based tests and so they reflect exposure to the pathogen but it can sometimes take time for the antibody responses to develop. Lyme disease typically progresses through three phases.

1. The early, localized phase occurs 3–30 days after the tick bite, with the symptoms described above, and infection is limited to the site of the bite.
2. The early disseminated phase occurs approximately 3–12 weeks after the bite, with bacteria spreading through the bloodstream causing rashes, fatigue, dizziness, headaches and muscle/joint pain, as well as potential neurological and/or cardiac symptoms.
3. The late disseminated phase occurs months or even years later and can include the chronic issues mentioned above: arthritis/joint pain, neurological and cardiac issues.

The standard two-tier or modified two-tier, antibody-based tests, therefore, tend to perform poorly in the early stages of infection and in the later stages of stages of infection – they are most reliable or sensitive in the early disseminated phase of the infection.

The limitations of these tests, therefore, are that they exclude a lot of patients as they are only really useful in one phase of the disease. Additionally, the tests don't necessarily reflect the breadth of the >>

It is useful to test for both IgG and IgM antibodies when testing for Lyme disease (Adobe Stock)

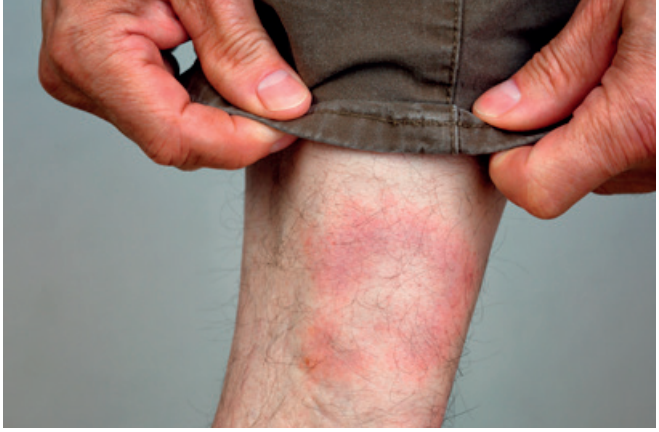
>> antibody response in patients because it can be highly variable and they rely solely on antibody responses. The criteria for such high specificity, which has been set at 99%, has made the sensitivity very low. Something that we've seen with patients is that those who have Lyme arthritis, classic Lyme arthritis have very bold, broad antibody responses and they're going to test positive by the tests. However, if they have neuroborreliosis or Lyme carditis, they don't necessarily have those antibodies and so are not going to test positive. So having such a high bar set for specificity means that, if you include all phases of disease, sensitivity is less than 60%; this means that four out of 10 patients who have the infection will not test positive.

So what we would consider the Holy Grail of Lyme disease testing would be a direct test, something where we can prove that an individual has an active *Borrelia* infection. Unfortunately that has been very, very difficult to achieve because once the tick bites and transmits the bacteria into the bloodstream, those bacteria very rapidly disseminate out of the bloodstream and into the tissues, and it's hard to find even remnants of the bacteria in the blood. So the idea of developing a direct test really means that you have to be able to capture the bacteria in a narrow window of time after the tick bite, but it's certainly achievable at that point. It wouldn't cover all phases of infection. On occasion, physicians might take a skin biopsy and try to culture that if they see a rash that doesn't look completely like a bullseye rash, but it's very unreliable.

What are the potential improvements that could be made?

We took an approach with research to identify which markers might be the most useful in identifying an infection throughout the different phases of Lyme disease. We started with non-human primates that were infected, and monitored how their antibody responses changed over a period of a year. This enabled us to put together a group of diagnostic antigens or biomarkers that could be used for all phases of disease. Where we fell short was in the early phase and so in a typical infection, you will have the development of IgM isotype antibodies and then the antibody responses mature into IgG antibodies. However, with Lyme disease you can get an IgM response persisting as well. So it became really important for us to include both IgG and IgM in the test. Then we started working with the Focus on Lyme Foundation (https://www.focusonlyme.org/), which had been funding different ways to try to diagnose Lyme disease in the early phases. They had looked at antibodies in patients with early Lyme disease using a big array of different peptides from the Lyme disease spirochete and identified proteins that could be used as diagnostic antigens in the early phase. So we put our data together and we developed a spectrum of biomarkers that could be used for all phases of disease. Then we also incorporated machine learning so that when we compare the data to a broad set of healthy controls or look-alike diseases, we can be very confident that when we say we have a positive test, it's a true positive. By incorporating the machine learning algorithm and the breadth of different diagnostic markers, we have been able to achieve over 90% sensitivity and specificity, even at the very earliest stages of the disease.

The beauty of it is that it uses a platform that's present in a lot of clinical labs already and it's a single test with a single result; no interpretation is needed by the clinician (unlike the immunoblot, which has led to so many problems in terms of how it's interpreted), and it has high sensitivity and specificity. So we're very excited for the for the potential of this test.



Lyme disease may not necessarily present with the typical 'bull's eye' rash shown here (Adobe Stock)

Future steps

We are pursuing FDA (US Federal Food and Drug Administration) approval here in the US and we will be pursuing approvals in Europe as well – ensuring that we can get our product out to Canada, the US and Europe. We're in the manufacturing process, making sure that we have validated the test in every possible way so that so that it is reliable, it gets FDA approved and we hope to be on the market by the end of this year or the beginning of next year. I'm grateful for all the support that we've had in in developing this test. And you know, there are already people who've heard about it and hope to get to be able to use it soon. I think I speak for the founders of Aces Diagnostics when I say that our number one priority is to have a reliable test that gets to patients as soon as possible.

The interviewee

Professor Monica Embers PhD
Professor of Microbiology and Immunology | Director of Vector-Borne Disease Research
Tulane National Biomedical Research Center, Tulane University, New Orleans, LA, USA

Email address:
members@tulane.edu

Bibliography

1. Branda JA, Body BA, Boyle J, Branson BM, Dattwyler RJ et al. Advances in serodiagnostic testing for Lyme disease are at hand. Clin Infect Dis. 2018;66(7):1133–1139 (https://doi.org/10.1093/cid/cix943). [Published in 2018, yet still no better tests are on the market.]
2. Waddell LA, Greig J, Mascarenhas M, Harding S, Lindsay R, Ogden N. The accuracy of diagnostic tests for Lyme disease in humans, a systematic review and meta-analysis of North American research. PLoS One. 2016;11(12):e0168613 (https://doi.org/10.1371/journal.pone.0168613). [Highlights the poor sensitivity of current tests.]
3. Embers ME, Hasenkampf NR, Barnes MB, Didier ES, Philipp MT, Tardo AC. Five-antigen fluorescent bead-based assay for diagnosis of Lyme disease. Clin Vaccine Immunol. 2016;23(4):294–303 (https://doi.org/10.1128/cvi.00685-15). [Serves as the foundation/platform for LymeSeek.]
4. Rebman AW, Crowder LA, Kirkpatrick A, Aucott JN. Characteristics of seroconversion and implications for diagnosis of post-treatment Lyme disease syndrome: acute and convalescent serology among a prospective cohort of early Lyme disease patients. Clin Rheumatol. 2015;34(3):585–589 (https://doi.org/10.1007/s10067-014-2706-z). [Demonstrates how poor early testing can be, and the need to include IgM.]