EDITORIAL COMMENTARY

Xenodiagnosis for Posttreatment Lyme Disease Syndrome: Resolving the Conundrum or Adding to It?

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(See the Major Article by Marques et al on pages 937–45.)

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The first reports of Lyme disease in Connecticut in the mid-1970s, followed by the discovery of its tick-borne etiologic agent, *Borrelia burgdorferi*, in 1983, has spawned one of the longest controversies in the history of biomedical research [1]. Whereas some clinical signs, such as the hallmark skin lesion erythema migrans (EM), can be explained by the inflammatory response elicited by spirochetes, other features remain enigmatic. Impressive generalized symptomatology can accompany EM and last for weeks to months following therapy and resolution of clinical signs. A minority of people (<10%) continue to experience fatigue, musculoskeletal pain, and/or cognitive dysfunction, a condition called posttreatment Lyme disease syndrome (PTLDS) [2]. The issue at the heart of the current, often acrimonious debate is whether persistent infection drives protracted symptomatology.

Four randomized, placebo-controlled trials have evaluated whether extended courses of antimicrobials ameliorate symptoms, ostensibly by eliminating persistent organisms [3–5]. The first 2 trials enrolled seropositive subjects with a previous episode of Lyme disease and seronegative subjects with physician-documented EM [3]. These were the most rigorous, as they evaluated therapeutic responses in a large number of subjects and also sought evidence of infection in blood and cerebrospinal fluid using culture and polymerase chain reaction (PCR). The treatment regimen (1 month of intravenous ceftriaxone followed by 2 months of oral doxycycline) was selected because both agents have good tissue penetration (including the central nervous system) and well-documented in vitro and in vivo activity against *B. burgdorferi*. The results of the intervention were clear: No evidence was obtained for persistent spirochetes, and antimicrobials provided no benefit over placebo. The remaining 2 trials showed either a similar lack of efficacy after 10 weeks of ceftriaxone [4] or only improvement in fatigue after a 4-week course [5], with an unacceptable rate of treatment-associated adverse events.

Critics countered that the lack of therapeutic response reflected the persistence of spirochetes in a state that renders them insensitive to antimicrobials. Investigators turned to animal models to examine this possibility (reviewed in [6]). A study in dogs infected using adult ticks found culture positivity and persistence of arthritis in 3 of 12 treated animals [6, 7]. A follow-up study, however, failed to replicate these findings; only DNA was detected and dogs remained culture negative even after prednisone immunosuppression [6, 8]. The first study to use xenodiagnosis, the use of uninfected ticks to detect low-level infection, was conducted in mice [9]. Rare, avirulent spirochetes were visualized by immunofluorescence in tick midgut contents 3 months posttreatment but not thereafter. A different group reported that nymphs molted from xenodiagnostic larvae transmitted DNA, but not spirochetes, to severe combined immunodeficiency (SCID) mice—a truly perplexing result [10]. A rhesus macaque study [11], hailed by some for providing definitive evidence of persistent infection, has evoked considerable skepticism [12]. In 1 of 2 experiments, all monkeys had positive skin biopsy cultures assessed during the first 4 weeks of untreated infection, but postmortem, only 1 of 12 monkeys in each of the treated and control groups was culture positive. A second experiment used xenodiagnosis to detect infection in 5 monkeys. Prior to treatment, infection status was examined by both culture and PCR of skin biopsies but only PCR was positive, even though monkeys were

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inoculated with an inordinately high dose of spirochetes (3×10^8/animal). Rare spirochetes of unproven viability were visualized in xenodiagnostic ticks at 2 months in 2 of 3 treated monkeys. Paradoxically, xenodiagnosis at all time points yielded negative results for both spirochetes and DNA in the 2 untreated animals. A recent study using xenodiagnosis in conjunction with 2-photon intravital microscopy has yielded valuable insights regarding the meaning of posttreatment PCR positivity [13]. In the vast majority of treated mice, only DNA was detected in xenodiagnostic ticks, whereas live imaging revealed just spirochetal remnants. One take-home message from these collective studies seems clear—detection of borrelial DNA by xenodiagnosis is not tantamount to detection of viable spirochetes.

In a provocative pilot investigation published in the current issue of *Clinical Infectious Diseases*, Marques and colleagues explored the feasibility of xenodiagnosis for addressing the PTLDS conundrum [14]. The study population, 36 subjects in all, consisted of 10 seronegative healthy controls, 1 "control" subject with recent-onset EM on antimicrobial therapy, 5 subjects recently treated for EM, 10 seropositive, asymptomatic subjects after a documented episode of Lyme disease, and 10 seropositive subjects with PTLDS. Xenodiagnosis was considered positive if either spirochetes *or* borrelial DNA was detected in the ticks or in SCID mice fed upon by the ticks or inoculated with tick lysates. Midway through the study, the authors added a novel, highly sensitive PCR assay that employs isothermal amplification to enrich for *Borrelia* DNA prior to PCR for 8 gene targets, which then are identified by electrospray ionization mass spectroscopy (IA/PCR/ESI-MS) [15]. Live spirochetes were not recovered from skin biopsies of the 26 subjects with recent or past Lyme disease or from SCID mice, which also were negative by PCR. Only 2 subjects—the control EM subject and 1 person with PTLDS—tested positive, but only for DNA amplified from cultures of the tick or from the tick itself (no spirochetes were seen). Repeat xenodiagnoses months later were positive only in a single tick examined by IA/PCR/ESI-MS from the PTLDS subject. The authors cautiously speculated that the DNA detected in these 2 individuals might be due to viable organisms. This interpretation seems highly unlikely given their inability to recover or visualize live spirochetes.

The authors concluded that xenodiagnosis can be performed safely and is generally well tolerated. We agree, with the caveat, based on their own experience, that this is far from a user-friendly technique. We have reservations, however, about the use of xenodiagnosis to address the question of *B. burgdorferi* persistence in humans. The first concerns how one defines a positive xenodiagnosis. As discussed above, animal data and the current human study indicate that detection of DNA alone is not sufficient for positivity. Indeed, the use of highly sensitive PCR tests such as IA/PCR/ES-MS may compound the issue by enhancing detection of rare DNA fragments. In our opinion, recovery of live spirochetes is the only reliable criterion for a positive xenodiagnosis. The question then becomes whether the method has utility in human Lyme disease research. Xenodiagnosis was developed for Chagas disease, a blood-borne parasitic infection with *Trypanosoma cruzi* transmitted by reduviid insects. Xenodiagnosis can detect low-level parasitemia in patients with chronic Chagas disease [16]. In contrast, Lyme disease spirochetes are only transiently blood-borne and are acquired by ticks from the skin [17]. Xenodiagnosis works in inbred mice because spirochetes disseminate from the feeding site to distal skin where, as in the natural reservoir, the white-footed mouse, they persist [17]. The consensus among entomologists is that humans are not reservoir-competent hosts and, thus, are biological dead ends for *B. burgdorferi* [18].

In our opinion, for xenodiagnosis to answer the question of borrelial persistence posttreatment, the method needs further investigation. To properly interpret a negative result in PTLDS subjects, which is the main finding in the Marques et al study, one must know whether spirochetes would have been present prior to treatment. In addition to subjects with EM, asymptomatic seropositive subjects from endemic areas who have not been treated for Lyme disease (a group for which there is no evidence-based guideline for management) should be included. People who present with late Lyme disease (eg, arthritis), for whom a few days' delay in therapy is unlikely to engender risk or change the clinical outcomes, would be another useful group. Studies using xenodiagnosis prior to treatment might do more than just buttress the rationale for its use in PTLDS; they have the potential to yield critical insights into the natural history of borrelial infection in humans. Ironically, a method employed to seek evidence for the persistence of spirochetes in PTLDS may actually provide evidence against the biologic plausibility of a hypothesis that has fueled controversy for nearly 30 years.

Notes

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