REVIEW ARTICLE

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Is There a Role for Dark Field Microscopy in the Diagnosis of Lyme Disease? A Narrative Review

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ABSTRACT

The diagnosis of Lyme disease is becoming more common in Turkey. Nonetheless, some physicians are not aware of the diagnostic principles that should be followed when faced with a suspected patient and could use tests that are not recommended, such as darkfield microscopy. Dark field microscopy is a diagnostic technique to visualize the spirochetes that cause Lyme disease; however, it is not recommended for the diagnosis of Lyme disease. One of the main limitations of dark field microscopy is its low sensitivity. Another limitation is its high false-positivity rate, as other microorganisms and cellular debris can be mistaken for spirochetes, leading to a misdiagnosis that-may result in unnecessary treatment. Therefore, this study aimed to review the literature on the role of dark field microscopy as a diagnostic method for Lyme disease and inform physicians about recommended approaches in line with the recommendations of national or international guidelines. An electronic search of Pubmed, Scopus, and Web of Science was performed using the following medical subject headings (MeSH) search terms: Lyme borreliosis, Lyme disease, *Borrelia burgdorferi*, diagnosis, and microscopy. With this narrative review, we aimed to inform physicians better and improve patient care for patients with suspected Lyme disease.

Keywords: Lyme disease, Lyme borreliosis, dark field microscopy, diagnosis

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INTRODUCTION

Lyme disease, also known as Lyme borreliosis, is the most common vector-borne disease in Europe and North America, caused by the spirochete *Borrelia* species, which can be transmitted through the bite of an infected black-legged tick (1). The Centers for Disease Control and Prevention (CDC) data on Lyme disease in the United States showed a fluctuation in annual cases, with the highest reported in 2017 at 42,743, the lowest in 2020 at 18,000, and a mean annual rate of approximately 29,393 cases during the five years from 2017 to 2021 (2). The number of estimated cases is much higher, and the annual number of patients diagnosed and treated for Lyme disease is approximately 476,000 (3, 4). Conversely, Belgium, Finland, the Netherlands, and Switzerland exhibited the highest Lyme borreliosis incidences (>100 cases per 100,000), while the Czech Republic, Germany, Poland, and Scotland reported rates ranging from 20 to 40/100,000, with lower rates (<20/100,000) observed in multiple other European countries. Additionally, subnational areas displayed markedly elevated incidences, reaching a peak of 464/100,000 in specific locales across Europe (5). In Turkey, the studies reported on Lyme disease are mainly case reports, including a limited number of patients (6). On the other hand, there are difficulties in the diagnostic process and surveillance level. Lyme disease diagnosis is based on a combination of factors, including symptoms, medical history, findings upon physical examination, and laboratory tests.

Dark field microscopy is a diagnostic technique that can be used for the detection of various microorganisms; however, imaging techniques, such as immunofluorescence staining or cell sorting of cell wall-deficient or cystic forms of *Borrelia burgdorferi*, are not currently recommended for the diagnosis of Lyme disease because of certain limitations (7). This study aimed to conduct a literature review on the utility of dark field microscopy as a diagnostic tool for Lyme disease to provide physicians with valuable insights into recommended diagnostic approaches aligned with national and international guidelines.

Clinical and Research Consequences

An elaborate search was designed and conducted to present an evidence-based approach for the laboratory diagnosis of Lyme disease using direct microscopic visualization techniques like dark field microscopy. A structured literature search was conducted based on the question "What is the exact role of dark field microscopy in diagnosing Lyme disease?" We searched Pubmed, Scopus, and Web of Science with the medical subject headings (MeSH) search terms, including Lyme borreliosis, Lyme disease, *B. burgdorferi*, diagnosis, and microscopy. In addition, we analyzed selected national and international guidelines to assess the recommended diagnostic approach for Lyme disease. We did not filter the date field and included all types of articles. Studies that did not have an eligible full text, such as those that did not provide information on the use of dark field microscopy for diagnostic purposes in the context of Lyme disease, were excluded.

Dark Field Microscopy in the Diagnosis of Lyme Disease

Several publications were investigated in detail for information about the clinical manifestations and recommended approaches for diagnosing Lyme disease. The consensus statement of Spanish scientific societies points out that there are stains to demonstrate the presence of spirochetes in tissues. However, only immunohistochemistry is mentioned to be specific and could lead to the direct diagnosis via molecular biology techniques (8). According to the European Society of Clinical Microbiology and Infectious Disease (ESCMID) Study Group on Lyme borreliosis (ESGBOR), molecular methods can be used for the detection of *Borrelia* as supplementary diagnostic methods for particular indications, and the visual contrast sensitivity test cannot be recommended for diagnosis due to low specificity (9). Dark-field or phase-contrast microscopy is not recommended for Lyme borreliosis because of a lack of sensitivity and specificity in guidelines from the French scientific societies and CDC (7, 10, 11).

A previous systematic review of 'direct microscopy of human tissues' emphasized that the modified dark-field microscopy technique should not be used for diagnosis and *Borrelia* detection by microscopy can only be used for research purposes (12). Laane et al. performed a modified dark field microscopy technique with a 66% (21/32) positivity for

Borrelia in blood samples of patients with non-specific symptoms (13). On the other hand, Aase et al. revealed an 85% false positivity for *Borrelia* and/or *Babesia* among 41 healthy controls and a 66% positivity in the patient group that had previously supposedly tested positive for *Borrelia* or *Babesia* by the microscopy method (14). In addition, the structures interpreted as *Borrelia* and *Babesia* by this method could not be confirmed by the polymerase chain reaction (PCR) method. Therefore, the modified dark field microscopy method was determined to be invalid and unfit for clinical use (14).

Summarized Recommendations in the Diagnostic Process for Lyme Disease by Several National and International Guidelines

The evidence-based guidelines and the CDC currently recommend the two-tier serology, which is first based on an immunoenzymatic technique (ELISA) and then, if positive or equivocal, on a confirmatory immunoblot test (western blot, WB) for the laboratory diagnosis (15-18), so-called standard two-tier testing. In addition, the U.S. Food and Drug Administration (FDA) cleared several serologic assays, allowing for an enzyme immunoassay (EIA) rather than WB as the second test in the testing algorithm (19), referred to as modified two-tier testing. Selected national and international guidelines are presented in Table 1 regarding the recommended diagnostic approach for Lyme disease.

The diagnosis of Lyme borreliosis should be based on the patient's epidemiological history (residence or recent travel to endemic areas, engagement in outdoor activities in high-risk environments, exposure to potential tick habitats, a history of tick bites, consideration of seasonal factors, and involvement in outdoor occupations), clinical symptoms and signs, and microbiological findings. Although seropositivity rates ranging from 2% to 44% have been reported for *B. burgdorferi*, the actual prevalence of Lyme disease is not fully understood in Turkey (20).

Patients in Turkey often receive non-recommended tests (such as dark field microscopy and lymphocyte transformation tests) during the diagnostic process because recommended diagnostic methods are not available in many centers. As a consequence, we came across a group of patients who were misdiagnosed with Lyme disease through dark field microscopy on a blood sample in which live *Borrelia* spirochaetes were supposedly observed, similar to the experiences of our colleagues in Norway (21). Therefore, we believe that this review will be helpful for our colleagues in correctly diagnosing Lyme disease, guiding treatment and management, and improving patient care of patients suspected of Lyme disease.

Branda et al. reviewed the laboratory diagnosis of Lyme disease and emphasized that direct visualization of *Borreliae* in blood or other infected tissues easily leads to misinterpretation. Direct visual detection is less sensitive or practical than a first-line diagnostic or adjunctive test because of the low in vivo organism burden of primary tissue samples (22). Lohr et al. also reviewed the diagnostic utility of direct microscopy for Lyme disease and mentioned the limited clinical utility because of the sparseness of organisms in samples (23).

There are also several publications about spirochete detection by electron microscopy, silver staining with light microscopy, or focus-floating microscopy in various samples. However, these methods also had poor sensitivity and high rates of false positivities (12, 24-26).

The guidelines have recommended serologic tests as the primary diagnostic (erythema migrans, which is more a clinical diagnosis, excluded) approach using standard 2-tier testing or modified 2-tier testing. Emerging technologies using biomarkers may be helpful in early Lyme disease, but more data is needed to recommend these newer methods (27).

The literature clearly shows that the sensitivity of dark field microscopy is low, and it may produce false negative and positive results. In addition, factors such as the technician's expertise and the specimen's quality should also be considered. Therefore, direct detection of *Borrelia* from patient material using dark field or focus floating microscopy is not recommended for diagnostic purposes (28).

In terms of the limitations of our study, it was conceived as a comprehensive review rather than a systematic one, and not every guideline pertaining to Lyme disease could be encompassed. The select-

Table 1. Recommended approach for the diagnosis of selected Lyme disease manifestations.

Clinical manifestations	References	Primary diagnostic testing	Supporting testing and findings
Erythema migrans	ECDC (15)	Testing is conducted on the basis of history and visual inspection of the skin lesion. Serological testing is recommended if the lesion is atypical, acute-phase, or convalescent phase.	Culture or PCR is not needed for routine clinical practice.
	ESGBOR (11)	Does not require serological confirmation.	
	IDSA, AAN, ACR (16)	Clinical diagnosis rather than laboratory testing is recommended.	In atypical erythema migrans, antibody testing is performed on an acute and convalescent phase serum sample rather than currently available direct detection methods such as PCR or culture performed on blood or skin samples.
	GDS (17)	If a typical erythema migrans is present, no further laboratory diagnostic confirmation (serological, cultural, molecular, biological) needs to be performed.	Direct detection of *Borrelia* in patient samples using light microscopy is currently not recommended.
	NMS (18)	Serum antibody testing can be analyzed.	PCR or culture of skin biopsy can be analyzed.
Lyme neuroborreliosis	ECDC (15)	Pleocytosis and demonstration of synthesis of intrathecal antibodies to Lyme *Borrelia*. Serological testing is usually positive at the time of presentation; if negative, convalescent-phase sera should be tested.	Detection of *B. burgdorferi* s.l. by culture or PCR in CSF, intrathecal synthesis of total immunoglobulin.
	ESGBOR (11)	Specific CSF/serum antibody index.	
	IDSA, AAN, ACR (16)	Antibody testing by obtaining simultaneous samples of CSF and serum to determine a CSF: serum antibody index, carried out by a laboratory using validated methodology.	Recommend against routine PCR or culture of CSF or serum.
	NMS (18)	Antibody testing by obtaining simultaneous samples of CSF and serum to determine a CSF: serum antibody index.	PCR or culture of CSF can be analyzed.
Lyme arthritis	ECDC (15)	Serological testing. As a rule, high concentrations of specific serum IgG antibodies are present.	Detection of *B. burgdorferi* s.l. by culture or PCR in synovial fluid.
	ESGBOR (11)	Detection of antibodies to *B. burgdorferi* via serum IgG.	
	IDSA, AAN, ACR (16)	Serum antibody testing over PCR or culture of blood or synovial fluid/tissue.	In seropositive patients, PCR for synovial fluid or tissue rather than *Borrelia* culture of those samples.
	NMS (18)	Serum antibody testing can be analyzed.	PCR or culture of synovial fluid or tissue can be analyzed.
Acrodermatitis chronica atrophicans (ACA)	ECDC (15)	Serological testing. As a rule, high concentrations of specific serum IgG antibodies are present.	Histology, culture, or PCR are not needed for routine clinical practice.
	ESGBOR (11)	Detection of antibodies to *B. burgdorferi* via serum IgG.	
	GDS (17)	When ACA is clinically suspected, the diagnosis shall be confirmed through a serological test. High IgG antibody values in the screening test, combined with a broadband pattern in the IgG immunoblot test, indicate a suspected clinical diagnosis.	When the clinical picture is ambiguous, further diagnostic clarification through biopsy and subsequent histological testing should be done. When the findings are unclear, direct detection by culture and molecular biology is recommended. Direct detection of **Borrelia** in patient samples using light microscopy is currently not recommended.
	NMS (18)	Serum antibody testing can be analyzed.	PCR or culture of skin biopsy can be analyzed.

IDSA: Infectious Diseases Society of America, **AAN:** American Academy of Neurology **ACR:** American College of Rheumatology, **ECDC:** European Centre for Disease Prevention and Control, **GDS:** German Dermatology Society, **ESGBOR:** European Society of Clinical Microbiology and Infectious Diseases Study Group for Lyme Borreliosis, **NMS:** National Microbiology Standards: Infectious Diseases Laboratory Diagnosis Guide (Republic of Turkey Ministry of Health, Public Health Institution), **PCR:** Polymerase chain reaction, **CSF:** Cerebrospinal fluid.

ed guidelines were succinctly summarized, highlighting recommended and unrecommended tests based on the clinical presentation in the diagnostic process of Lyme disease.

CONCLUSION

Clinicians should be aware of the diagnostic tests' sensitivities and specificities and combine them with the patients' epidemiologic factors and clinical signs and symptoms to accurately diagnose Lyme disease. Based on our findings, dark field microscopy is not recommended as a diagnostic method for Lyme disease due to low sensitivity and high false positivity rates.

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