www.nclinmed.com
Novelty in Clinical Medicine. 2023; 2(1): 11-23
https://doi.org/10.22034/NCM.2023.378654.1058

Narrative Review Open Access

Lyme Disease and New Molecular Biological Detection Methods

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Received: 12 December 2022 Accepted: 10 January 2023 e-Published: 9 February 2023

Abstract

Molecular biology plays an important role in understanding the structures, functions and internal controls within each cell. All this can be used to diagnose the diseases, effectively target new medicines and better understand cellular physiology. The bacteria that cause Lyme disease are difficult to observe directly in body tissues and too time-consuming to grow in the laboratory. Lyme disease can affect several body systems and produce a broad range of symptoms. Not everybody with Lyme disease has all the symptoms and many of the symptoms are not specific but may occur with other diseases. Common laboratory diagnostic methods have many false positive results in contaminated areas. These problems have made scientists think of finding accurate and fast methods to diagnose. The accuracy and precision of molecular biological methods have made an important field of research to identify the pathogen of this disease. *Borrelia Burgdorferi* is a species of the Spirochaeta order and Borrelia genus. This type of Gram-negative bacteria is the most important cause of Lyme disease. Five of these species, *Borrelia afzeli*, *Borrelia garinii*, *Borrelia bavariansis*, *Borrelia bergdorferi* senses strict, and *Borrelia spirmani*, have been described as causative agents of Lyme disease in humans. There are 36 known Borrelia species. In the Borrelia family, three species cause Lyme disease or borreliosis, the most important cause in USA is *Borrelia Burgdorferi* and the main cause in Europe are *Borrelia afzelii* and *Borrelia garinii.* Accurate molecular tests are designed for specific detection and isolation of strains. This study was conducted by reviewing 131 related articles from Scopus, ISI and PubMed databases. Finally, methods for designing accurate molecular tests to identify disease agents were reported.

Keywords: *Borrelia Burgdorferi,* Lyme Disease, New Molecular Biology, OspA and OspB, Genomic Borrelial DNA.

Introduction

Lyme disease is a bacterial infection that is caused by a spiral-formed *Borrelia burgdorferi* bacterium.[1] The human becomes infected after being bitten by infected tough-bodied ticks (Ixodes species). These occasionally known as deer, sheep, or woodland ticks in the forest regions, moorland and parks; but less frequently discovered in cone-bearing forests.[2] The most medical signs and symptoms related with the lyme disease include facial palsy, a viral-like meningitis, and different nerve injure or arthritis.[3] The various typing systems based on molecular and immunological distinctiveness have divided Borrelia burgdorferi into several specific groups.[4] In *Borrelia burgdorferi*, the genes encoding major outer membrane proteins; OspA and OspB are positioned on a 49 kb linear plasmid.[5] Immunochemical and biochemical research of the OspA protein of *Borrelia burgdorferi* have discovered differences in apparent molecular mass and reactivity with monoclonal antibodies.[6] This heterogeneity has been exposed to be more prominent amongst European isolates than North American isolates.[7] Furthermore, in each the European and the North American *Borrelia burgdorferi* isolates, the OspA-B protein exposed more strain variability.[8] OspA is protective antigen of this microorganism and induces a protecting immunity against spirochete infection in mice.[9] The most important vectors of the spirochete are ticks of the *Ixodes ricinus* complex.[10] The genus Borrelia consists of two most important phylogenetic groups of pathogenic spirochetes: the etiologic agents of relapsing fever and Lyme disease (now known as Lyme borreliosis). Both groups of Borrelia have been considered in several

approaches, which includes the microbiological, immunological, epidemiological, and ecological views. Among the strange things, the presence of linear DNA with hairpin telomeres for prokaryotes.[11] Borrelia is unique among prokaryotes in having a small linear chromosome of 1 mega base pairs.[12] Currently, *Borrelia bissettii* and *Borrelia mayonii* were defined as the cause of Lyme borreliosis in USA and Canada.[13-15] The incidence of lyme disease has increased in USA and Europe. In 2011, the prevalence of cancers in England and Wales, and in Scotland have been 1.73 and 4.36 per 100000 overall populations, respectively.[16,17] The diagnostic tests presented to confirm the human lyme disease, the sensitivity and specificity of the variable vary depending on the level of the infection, for that reason it is essential to observe the literature on available tests for lyme disease to support those tests that complete the most correctly and address concerns about the overall performance of non-validated tests and test protocols the usage of evidence-informed techniques for decision creation.[18,19] currently in Canada and USA, a two-tiered serology protocol is the only validated diagnostic approach for lyme disease analysis advised via USA, CDC, and the general public health organization of Canada.[18,19] This two-tiered test is an enzyme immunoassay (EIA) to discover IgM or IgG antibodies to *Borrelia burgdoferi* in serum and if the sample is positive or equivocal at the screening assay, then a western blot is used to detect serum IgM or IgG antibodies to *Borrelia burgdorferi.* Use of IgM testing is usually recommended at some stage in the first 30 days of infection, after which only IgG tests for Lyme disease be used. Currently, simplest serology exams had been certified to be used via the FDA and The Health Canada Medical Gadgets Branch (HC) for lyme disorder checking out.[20,21] other direct recognition tests which includes PCR can be commercially available, however they have got no longer been certified for use via a governing body. There are some of ELA kits licensed by means of FDI or HC are commercially available and use both complete cell education of *Borrelia burgdorferi* or purified recombinant or chimeric antigens.[22] Different EIAs mentioned in the literature had been evolved inside the reporting laboratory and feature no longer been commercialized or beneath-long past licensing and may be called in-residence developed tests.[23] EIA shows good sensitivity 30 days after infection, but usually has low specificity. In 1995, the Centers for Disease Control and Prevention (CDC) adopted standards for interpreting western blot results for Lyme disease, and most commercially available tests follow these guidelines.[23] The aim of this study was to investigate the molecular detection of the Lyme disease pathogen *Borrelia burgdorferi.*

1- Borrelia Phylogenetic

Borrelia, like other spirochetes, is a spiral bacteria, containing a protoplasmic cylinder, a peptidoglycan-cytoplasmic membrane complex, a flagellum and a periplasmic space which is covered by two layers of fat of the outer membrane.[24] Interestingly, flagellar filaments are absent from the outer membrane in most bacteria, but inserted at the end of the protoplasm and fully contained in the periplasmic space.[25,26] It is the rotational motion of these flagella that drives the left-handed corkscrew motion that distinguishes Borrelia.[27] Over 100 protein species have been detected by *Borrelia burgdorferi* sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).[28] Labeling with 125 I or biotin identified 13 major surface proteins with apparent molecular weights of 22, 24, 29, 31, 34, 37, 39, 41, 52, 66, 70, 73, and 93 kDa.[28] The 31 kDa and 34 kDa outer surface proteins (OSPs) are called OSP-A and OSP-B, respectively.[29] The two outer surface proteins (OSP-A and OSP-B) of USA isolates are more uniform than those of European strains when analyzed by Western blotting. The 31 kDa and 34 kDa outer surface proteins (OSPs) are called OSP-A and OSP-B, respectively.[30] The spirochete Lyme disease complex includes at least 20 genotypes.[31,32] Five of these species, *Borrelia afzeli, Borrelia garinii, Borrelia bavariansis, Borrelia bergdorferi senses strict*, and *Borrelia spirmani,* have been described as causative agents of Lyme disease in humans.[33] Other Borrelia species (*Borrelia lusitaniae, Borrelia bissettii, Borrelia valaisiana*) are rarely or never isolated from humans and their pathogenicity remains unknown. *Borrelia bergdorferi* is a typical spirochete, motile, host-associated, and requires cultivation. Borrelia has a highly unusual genome, containing a linear chromosome (approximately 910 kbp) and several linear and circular plasmids containing over 600 kbp of DNA.[27] All previously analyzed members of the Lyme disease spirochete have linear chromosomes of similar size to those of the *B31* strain.[34] Linear replicons have covalently closed telomeres.[31,35] Genes encoding various lipoproteins and expressed in the outer membrane are on plasmids, and most housekeeping genes are on chromosomes. The plasmid content varies between Borrelia strains and is naturally found in Borrelia. Several parts of the Borrelian genome are unique to Borrelia. For example, a chromosome with a single gene encoding 16S rRNA (rrs) separated from a pair of randomly repeated 23S (rrlA and

rrlB) and 5S rRNA genes (rrfA and rrfB). This unique rRNA gene organization is a target for molecular analysis of Borrelia.[36] Ten genomic groups related to the *Borrelia burgdorferi sensu lato* complex have been identified worldwide.[37-39] European and Asian groups such as *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, *Borrelia garinii (type NT29)*, *Borrelia afzelii*, *Borrelia valaisiana (group VS116)*, *Borrelia lusitaniae* (*group PotiB2)*, *Borrelia japonica*, *Borrelia tanukii*, *Borrelia turdae*, but Groups *Borrelia burgdorferi s.s.*, *Borrelia andersonii (group DN127)*, *21038*, *CA55*, and *25015* were found in USA. In Japan, discovery of *Borrelia japonica* is not pathogenic to humans. Additionally, group VS116 (*Borrelia valaisiana*) has not reported pathogenic potential.[40]

2- Pathogenesis

Borrelia burgdorferi is the causative agent of Lyme disease, and failure of several systems is determined by a wide range of clinical symptoms. Initial clinical manifestations are usually localized skin disease, spread of Borrelia to various organs, early spreading erythema migrans (Lyme neuroborreliosis, Lyme carditis, erythema migrans multifocals, Borrellian lymphoma) or persistent sexually transmitted diseases (chronic Lyme arthritis, chronic acrodermatitis, late neurological symptoms).[33] Different species of Borrelia lead to different clinical manifestations, some species being *Borrelia afzeli* with cutaneous manifestations, *Borrelia garinii* with central nervous system disorders, and *Borrelia bergdorferi senses* strict with Lyme arthritis.[33] *Borrelia burgdorferi* is found in the midgut of ticks. When fed to adult worms, the bacteria begin replicating, causing changes in gene expression. As a result, lipoprotein expression is altered, leading to increased colonization and chemotaxis. For example, *Borrelia burgdorferi* expresses outer surface protein A (OspA) in the midgut of ticks. When ticks begin feeding, OspA expression is down-regulated and OspC is up-regulated.[40] OspA promotes bacterial binding at the tick midgut OspA receptor, whereas OspC is a potential plasminogen receptor.[41] It plays an important role in the colonization of host tissues. Approximately 36 hours after the tick first feeds, the bacteria travel to the salivary glands and finally reach the host via saliva.[42] In *Borrelia burgdorferi*, two factors, migration and adhesion, are critical for host release and initiation of infection.[43,44] They can swim within the host matrix, penetrate between cells, and enter capillaries. In addition, *Borrelia burgdorferi* can colonize large joints, heart, and other tissues of the host.[45]

3- Geographic distribution and gene bank

GenBank storage was used as a reference of Table-1 was used in the study.

Table-1. Geographic distribution and gene bank

Genospecies	Geographic origin	Accession No
B. burgdorferi sensu stricto	United States	*AY586362*
B. burgdorferi sensu stricto	United States	*AY586363*
B. burgdorferi sensu stricto	Holland	*AY586364*
B. burgdorferi sensu stricto	Switzerland	*AY586365*
B. burgdorferi sensu stricto	France	*AY586366*
B. burgdorferi sensu stricto	France	*AY586367*
B. burgdorferi sensu stricto	France	*AY586368*
B. burgdorferi sensu stricto	Switzerland	*AY586369*
B. garinii	Japan	*AY586370*
B. garinii	Japan	*AY586371*
B. garinii	Switzerland	*AY586372*
B. garinii	Germany	*AY586373*
B. garinii	Switzerland	*AY586374*
B. garinii	Japan	*AY586375*
B. garinii	Russia	*AY586376*
B. garinii	Holland	*AY586377*
B. afzelii	Switzerland	*AY586384*
B. afzelii	Denmark	*AY586383*
B. afzelii	Holland	*AY586384*
B. afzelii	Sweden	*AY586385*
B. afzelii	Germany	*AY586386*
B. lusitaniae	Portugal	*AY586378*
B. lusitaniae	Portugal	*AY586379*
B. lusitaniae	Portugal	*AY586380*
B. valaisiana	Switzerland	*AY586381*
B. valaisiana	England	*AY586382*
B. Burgdorferi sensu lato	United States	*AY586383*
B. japonica	Japan	*AY586387*
B. japonica	Japan	*AY586388*
B. bissettii	United States	*AY586389*
B. bissettii	United States	*AY586390*
B. bissettii	United States	*AY586391*
B. andersoni	United States	*AY586392*

4- Whole Genome-Based Genotyping

4-1- Whole Genome Based Restriction

A large restriction fragment pattern (LRFP) is required for growth in Borrelia cultures. It is based on whole-genome restriction analysis.[51] Various restriction enzymes

can be used to restrict genomic DNA, including MluI, ApaI, KspI, SmaI, and XhoI. MluI-based constraints are most commonly used in relation to decision potential in Borrelia species.[52] Large DNA molecules are separated by periodic electrical changes in Lyme disease, while constrained genomic DNA is separated using pulsed Phil-Lime disease gel electrophoresis. MluI-based restriction allows the detection of Borrelia species and the definition of subgroups within the species. MluI-LRFP from Borrelia afzelii isolates show a fairly uniform restriction pattern. The majority of isolates belong to the subgroup Borrelia afzeli Mla1 (>99%) and a minority to *Borrelia afzeli Mla2*, *Mla3* and *Mla4.*[51] On the other hand, *Borrelia garinii* and *Borrelia burgdorferi sensu stricto* isolates show a restricted and heterogeneous pattern, divided into 7 (Mlg1-7) and 15 (Mlb1-15) subgroups, respectively.[51] In terms of abundance, these subgroups were more closely related to reservoir host, geographic location, and clinical presentation. Due to the required approach, there are few studies on this subject.[51] *Borrelia valaisiana, Borrelia lusitaniae,* and *Borrelia spielmanii Borrelia valaisiana, Borrelia lusitaniae,* and *Borrelia spielmanii*, with two subgroups identified in each species, have a very uniform restriction pattern, but a large number of strains were analyzed and many strains were not worked.[51]

4-2- Plasmid Analysis

Determination of plasmid Specifications requires growing borrelial culture by using the gel insert method previously described, Genomic borrelial DNA can be detached.[51-54] Generally, bacterial cells are embedded in agarose blocks at a density of 109 ml, lysed with lysozyme, and digested with proteinase K.[51–54] Pulsed-field gel electrophoresis (PFGE) is used for 0.9–3 seconds with a run time of 37 hours to separate chromosomal and plasmid DNA.[55,56] The relative molecular size of a given linearized plasmid should be calculated based on the appropriate pulse markers. PFGE isolates only linear plasmids and has problems with circular Borrelia plasmids. Based on the number of plasmids in each cell and the molecular weight of the plasmids, the linear plasmid profile of a particular strain is determined.[57] The number and size of linearized plasmids vary among Borrelia species.[58] The presence of multiple plasmids of the same molecular weight that could only be distinguished by PCR was also evident. Additionally, some plasmids can be lost during long-term culture.[59] If the copy number of a given plasmid is low and below PFGE sensitivity, the plasmid cannot be detected and plasmid profiling becomes more difficult.[58] Several publications reported that each strain harbored one large plasmid and many small plasmids, while others reported strains harboring multiple large plasmids or plasmid dimers is reporting. Several publications reported that each strain harbored one large plasmid and many small plasmids, while others reported strains harboring multiple large plasmids or plasmid dimers.

5- Next Generation Sequencing

Next-generation sequencing enables whole-genome sequencing of multiple bacterial isolates in a single sequence within a day. A major weakness of these methods, apart from cost, is the lack of data analysis and interpretation tools.[60-62] Multiple platforms are available for next-generation sequencing, including 454 (FLX Titanium), Illumina (HiSeq, MiSeq, GA), SOLiD (4, 5500), Helicos, Ion Torrent, PacBio, and Starlight. To date, complete or partially complete genome sequences of 42 *Borrelia burgdorferi sensu stricto*, 9 *Borrelia afzelii* and 40 *Borrelia garinii* isolates are available (NCBI Genome; http://www.ncbi.nlm. nih.gov/genome). Next-generation sequencing was also used in the study of Troy et al. During infection he gains insight into the spread of *Borrelia burgdorferi sensu stricto.*[63] They developed massively parallel sequences associated with transposon mutations to uncover the exact pathogenesis of *Borrelia burgdorferi* senses. The results confirm that *Borrelia burgdorferi sensu stricto* is a highly successful pathogen, capable of surviving population bottlenecks at the inoculation site and then causing fairly widespread and long-term infection indefinitely throughout the mammalian host.[63]

5-1- PCR-Based Typing

PCR-based restriction fragment length polymorphism (RFLP) analysis using PCR amplification of either rrsrrlA (16S-23S) or rrfA-rrlB (5S-23S rRNA) spacers, followed by restriction endonuclease digestion and fragmentation of PCR products by dissecting gel electrophoresis.[64,65] This technique can be used to input cultured spirochetes (one-step PCR) or crude spirochetes from clinical or tick samples (nested PCR). Amplification of the rrfA-rrlB spacer results in an amplicon size of 225-266 bp. Two restriction enzymes commonly used in RFLP; MseI and DraI. Mussels can be used to identify eight different species of *B. burfdorferi sensu lato.* Clarity of restriction digests can be visualized using gels containing 16% acrylamide - 0.8% bisacrylamide.[64] This method is often used to directly diagnose clinical specimens and identify strains, reservoir hosts, or ticks.[65–72] The rRNA spacer rrs-

rrlA is amplified with a logical 941 bp amplicon using nested PCR. RFLP analysis using HinfI or MseI distinguishes *Borrelia burgdorferi sensu stricto strains into three ribosomal spacer types, RST1, RST2 and RST3.*[73] *According to the literature, Borrelia burgdorferi sensu stricto RST* type is correlated with virulence.[73-77] A higher proportion of Lyme disease patients infected with the *RST1* strain had positive blood culture results, multiple erythematous migratory lesions, and more severe symptoms than those infected with *Borrelia burgdorferi sensu stricto RST2 or RST3* isolates.[76]

5-2- Outer Surface Protein C (OspC) Analysis

A gene for OspC known to be essential for *in vitro* growth, located in the single-copy circular plasmid cp26.[78] OspC is one of the most predominant antigens in the humoral IgM immune response. OspC causes infectious neck wounds in vertebrates and plays an important role in transmission of Borrelia from ticks to vertebrates.[79–82] Genotyping uses amplification and sequencing of a ~600 bp region of the OspC gene.[83] OspC typing groups *Borrelia burgdorferi sensu lato* strains into 21 different genetic variants. There is a correlation between different types of ribosomal spacers and OspC genotypes in *Borrelia burgdorferi sensu stricto.* RST1 corresponds to OspC genotypes A and B. RST2 to OspC types F, H, K, and N. RST3 corresponds to the remaining 10 OspC types including D, E, G, and I.[77,84-86] Comparing RTS and OspC genotypes of isolates, we observe that RST may miss differences within groups, whereas OspC genotypes may result in small groups. Both typing systems can be used to obtain more information about clinical correlations. OspC and other outer surface proteins are variable and commonly used in interspecies population studies.[87]

5-3-Real-time PCR and Melting Temperature Analysis

Real-time PCR combined with determination of the melting temperature of the amplified target DNA. Facilitates identification of Borrelia. The melting temperature of a DNA fragment is well defined by its nucleotide sequence, its length and GC content.[88,89] The correct protocol is used to detect bacteria on the surface of seeds. Several Borrelia genes (hbb, p66, recA, ospA, etc.) have been used to identify Borrelia species.[90-92] Although most Borrelia species with Lyme disease can be distinguished by differences in the hbb gene, it is not possible to distinguish between *Borrelia spirmani* and *Borrelia valaisiana.*[93]

5-4- Flagellin Based Typing

The highly conserved flagellin gene is located on the chromosome and some parts are quite different between different *Borrelia bergdorferi sensurat* species. Its easy access is commonly used for diagnostic purposes, and its diversity is used to identify Borrelia species.[94,95] Jaulhace et al., describe an oligonucleotide typing method using a PCR fragment from the flagellin gene of *Borrelia burgdorferi sensu lato*, representing seven different Borrelia species: *Borrelia garinii, Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia japonica*, *Borrelia andersoni*, *Borrelia valaisiana*, and *Borrelia bissettii.*[96] Technically, this technique is necessary and primarily used to confirm Borrelia in clinical specimens. This method is technically demanding and is mainly used to confirm Borrelia in clinical samples.

6- Multilocus Sequence Typing

As multilocus sequence typing (MLST) was defined by Urwin and Maiden, this definition evolves slowly and has evolved into the amplification, sequencing, and analysis of internal parts of housekeeping genes located throughout the genome to prevent bias. MLST is a molecular typing tool used for population studies, epidemiological surveys, phylogenetic analysis, and evolutionary studies.[97,98] *Borrelia spirochet*, clpA (Clp protease subunit A), clpX (Clp protease subunit X), nifS (aminotransferase), pepX (dipeptidyl aminopeptidase), pyrG (CTP synthase), recG (DNA recombinase), rplB (50S ribosomal protein) and uvrA (exonuclease ABC).[99] The Borrelia MLST scheme is available from the MLST Network (http://www.mlst.net/). This method distinguished *Borrelia bavariensis* from other his strains of *Borrelia garini* in terms of some epidemiological data (birds were chosen as hosts for *Borrelia garini* and small mammals for *Borrelia bavariensis*).[66] In conclusion, MLST is an alternative and exciting route to Borrelia taxonomy, phylogenetic and ecological studies of spirochets.

7- Diagnosis

Diagnosis of Lyme disease is usually based on a combination of clinical examination, evaluation of the patient for possible tick bites, and laboratory testing.[100]

7-1-Clinical Symptoms

The disease includes a wide range of clinical manifestations affecting the skin, nervous system, musculoskeletal system, heart and eyes (Table-2). Because

of its variable clinical manifestations, Lyme is often considered in the differential diagnosis.[101,102]

Table-2. Main Clinical Manifestations of Lyme Boeerliosis[103]

Organ system	Clinical feature
Skin	Erythema Migrans
	Erythema Migrans Multiple Lesions
	Borrelial lymphocytoma
	Acrodermatitis Chronica Atrophicans
Nervous	System Meningitis
	Meningoencephalitis
	Meningo-Radiculoneuritis
	Encephalomyelitis
	Cerebral Vasculitis
	Pheripheral Neuropathy
Musculoskeletal	Arthritis
	Myositis
Heart	Carditis
Eye	Conjunctivitis, Endophtalmitis, Anophthalmitis

7-2- Lab Diagnosis

Except in cases where Erythema migrans manifests clinically, a microbiological diagnostic assay is usually required to confirm a Lyme disease diagnosis (Figure-1).[102,104]

The CDC and the European Union Concerted Action on Lyme Borreliosis (EUCALB) have developed a case definition of Lyme disease for surveillance purposes. This purpose includes either physician-diagnosed Erythema migrans along with solitary lesions with diameters of at least 5 cm or at least one late joint, neurologic, or cardiac manifestation along with laboratory confirmation (EUCALB, CDC). Although this definition is not meant to be completely specific or sensitive for a clinical diagnosis, it can be used as a starting point for a differential diagnosis and emphasizes the importance of laboratory testing, particularly for extra cutaneous Lyme Borreliosis. Clinicians have seen significant advancements in laboratory tests over the past few years.[103] *Borrelia burgdorferi* culture isolation Using skin biopsy or cutaneous lavage specimens from Erythema migrans lesions and blood from patients with the early-disseminated disease, the Lyme disease standard for diagnosis is still clinical specimens.[101,102] Patients with early Lyme Borreliosis have had positive culture rates of nearly 90% for secondary Erythema migrans lesions, 50% for primary Erythema migrans lesions, and 48% for large volumes of blood or plasma specimens.[103] The majority of assays amplify specific *Borrelia burgdorferi* using PCR. Nucleic acid sequences taken from blood, CSF, joint fluid, or tissue biopsy samples. Assays for conventional and nested PCR have been developed, and detection techniques range from Southern hybridization and gel electrophoresis to real-time PCR. Both chromosomal targets and plasmid targets have been utilized, and each has its own advantages. Plasmid targets like ospA, ospC, and vlsE are present in multiple copies within each bacterium, making them more sensitive than single-copy chromosomal targets like fla, recA, rpoB, 16S and 23S ribosomal DNA, and rDNA intergenic spacers. Nowadays, it is simple to select the best DNA sequences for amplification because several specific sequences are available in databases.[105] Before serum antibodies appear and without the delay of culture isolation, PCR can be used to confirm Erythema migrans lesions.[102,103] Skin biopsy samples from patients with Erythema migrans have the highest PCR detection rate of Borrelia, with a median sensitivity of around 70%, and joint samples from patients with Lyme arthritis (LA) have a median sensitivity of up to 80%.[105,106] A negative PCR test result cannot exclude Lyme disease. Since the number of spirochetes in infected tissues or bodily fluids of patients is typically very low, it is essential to use the right methods for collecting, transporting, and preparing DNA from clinical samples for PCR results that are both reliable and consistent.[105] The production of false positive results because of contamination is one of the limitations of methods for amplification of nucleic acids. In assays designed for maximum sensitivity, which are necessary for diagnosing infections caused by *Borrelia burgdorferi,* amplicon contamination is extremely problematic.[107] Real-time PCR enables monitoring of the exponential phase, whereas analysis can only identify the plateau phase. In a PCR, only those few cycles in which the amount of DNA grows logarithmically from just above the background to the plateau provide quantitative information. Frequently simply 4 to 5 cycles out of 40 will fall in this log-direct piece of the bend. The log-linear region can be easily identified in every reaction because real-time PCR monitors the entire PCR.[108] There are a number of reports that apply this principle to qualitative diagnosis; however, only one application was reported for a quantitative clinical diagnosis of a pathogen.[108] It reports the development of a constant PCR measure for the quantitative recognition of *Borrelia burgdorferi* that addresses an exact and effortless demonstrative instrument for this bacterium. In addition, its application in the mouse Lyme disease model demonstrates that there

are two different inbred strains of mice that are known to exhibit different disease susceptibilities: antibiotic-treated mice and untreated mice.[109]

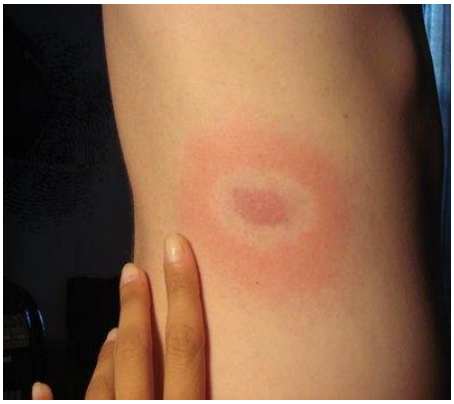

Figure-1. Erythema migrans is an expanding rash that is the initial sign of about 80% of Lyme infections

8- Compare of Clinical Diagnosis Tests Accuracy

8-1- Enzyme Immunoassays (EIA)

Twenty-three studies with well-defined whole-cell targets evaluated first-tier serological tests like enzyme-linked immunosorbent assays (ELISA) and other serological assays. There was a blend of FDA-authorized tests and in-house tests. For patients with stage 1 lyme disease, test performance was highly variable and had poor sensitivity, just like the two-tiered tests. The sensitivity got better as the lyme disease progressed further along. More than for the two-tier tests, the overall specificity varied between studies and by test. Fifty-three data lines were used to evaluate the ELISA function during the initial stages of the early lyme disease. To find out where there was variation between studies, these were further categorized according to the type of ELISA. The Immunetics1 C6 *Borrelia burgdorferi* ELISATM kit contained four lines for three studies, and unlicensed C6 ELISAs contained seven lines for four studies. Representing whether the C6 ELISA was authorized made sense of 27% of the heterogeneity among studies and demonstrated the business ELISAs had an inconsequential higher awareness 91(81-100) versus 64(47-80) and comparable particularity 97(94-100) versus 97(95-99) over all phases of lyme sickness. An early lyme disease whole-cell sonication (WCS) ELISA included 10 strains from 6 studies. Three commercial test kits were included. The Lime Stat Test Kit, VIDAS Lime Screen II, and Wampole Bb ELISA test systems span six lines and three studies. The authors did not provide an explanation for the divergent results—these performed differently than the four internal WCS ELISAs. Recombinant proteins and/or chimeric proteins from Osp A-F (primarily A and C) served as the targets for experiments conducted on patients with early lyme disease. The reported sensitivities ranged from 0 to 86% and were based on in-house ELISAs with small sample sizes in each study. An ELISA that utilized PEG-peptide conjugates and reported 100% sensitivity and specificity on a small sample was one of the other assays.[110] A *Borrelia burgdorferi* strain *B31* and *B126* indirect hemagglutination antibody (IHA) test had a low sensitivity of 464 percent and a high specificity of 98-99 percent, which is comparable to other tests for early Lyme disease.[111] Compared to early lyme disease results, it was more specific and sensitive for experiments used in the late lyme disease. The Immunetics1C6 *Borrelia burgdorferi* ELISATM outperformed the in-house C6 ELISAs, the commercial WCS ELISAs (VIDAS Lyme Screen II and Wampole Bb (IgG/IgM) ELISA test system), and the in-house ELISAs employing various recombinant/chimeric Osp targets in a meta-regression controlled for test in the late lyme disease. An evaluation of the reactivity of individuals who had previously been vaccinated with the Osp A vaccine, which was discontinued in 2002, was left out of the meta-analyses. The results showed that a WCS ELISA had a 95% false positive rate and a recombinant Osp A ELISA had a 5% false positive rate.[112]

8-2- Immunoblots Methods

Nine studies compared clinical diagnoses of a variety of lyme diseases to several commercial Western blots. These incorporated the Marblot test strip framework by MarDx1, the Boston Biomedica Inc. (BBI) *B. burgdorferi* western smear test kit1, Immuno Speck Borrelia Spot Smudge Test1 and Viramed Biotech *Borrelia burgdorferi* B31 Virablot 1. The diagnostic sensitivity of a few recombinant targets was examined using a single in-house immunoblot. The MarDx1Lyme sickness Marblot Strip test framework was assessed in four examinations on select lyme Illness gatherings and across ahead of schedule to late lyme Illness gatherings.[113-116] In patients with lyme disease, significant and better tests were performed, according to a meta-regression that controlled for the group. However, whether the investigator evaluated results for IgM, IgG, or both simultaneously did not significantly affect the sensitivity or specificity. In two separate examinations, the BBI western smear of a similar CDC test board was assessed. However, the classification criteria differ slightly. One used the BBI criteria, which stated that IgM needed 2+ bands of 23,39,41, and 83 kDa,

while IgG needed 3+ bands of 20,23,31,34,35,39, and 83 kDa. In contrast to the CDC criteria (IgG required 5+ bands 18, 23, 28, 30, 39, 41, 45, 58, 66, and 83 to 93 kDa, and IgM 2+ bands 23, 39, and 41 kDa),[117,118] its formulation for positive samples is different. The sensitivity of the CDC criteria for IgM and IgG blots was 77% and 93%, respectively, whereas the specificity of the BBI criteria for IgM and IgG was 93% and 100%. Although the difference was not significant, these two criteria differed in terms of specificity, with a gain in sensitivity and a slight loss in specificity. In a single study, the immunodot Borrelia Dot Blot IgG/IgM test from General Biometric Inc. was at stage 1 sensitivity increased by 50% (95% CI 19, 87), stage 2 sensitivity increased by 70% (35, 93), and stage 3 sensitivity increased by 100% (63, 100).[113] In a small study, the Viramed Biotech Borrelia burgdorferi B31 IgG/IgM Virablot was found to be as sensitive and specific as the other immunoblots.[117] In the published study, one in-house recombinant immunoblot failed, with sensitivities ranging from 7 to 60% for various targets (data not shown).

8-3. PCR Tests for Direct Detection

Six studies investigated the isolation of *Borrelia burgdorferi* by culture and PCR in a variety of human samples from cases of early and disseminated lyme disease.[120-125] In this gathering, there were no Meta-dissects accessible of studies since there were insufficient lines of information inside every location strategy. The Barbour-Stoner-Kelly (BSK) medium, which has been modified by some authors to increase its sensitivity, is the most widely used medium.[126] The sensitivity of this method was 27%, 71%, and 94% in three studies that attempted to isolate *Borrelia burgdorferi* from patients with early Lyme disease (stage 1).[50-52] Concerning the latter sensitivity, it has been suggested that laboratory contamination may be the explanation for the reported extremely high sensitivity.[127] From early lyme disease biopsy samples, two studies reported sensitivities ranging from 62% to 81%.[122,123] While both sample sizes were very small. Phillips et al., evaluated an aMPMo medium for detecting *Borrelia burgdorferi* in the blood of lyme disease patients who had previously been treated for the disease but had since relapsed.[128] In these patients, they reported a sensitivity of 91.5 %; however, two studies failed to replicate these findings and both demonstrated that the BSK-H culture was superior.[129,130] Information on the use of PCR to identify *Borrelia burgdorferi* in early lyme disease was gathered from three studies (eight lines of data).[122,124,125] Blood and tissue biopsies were used as samples, and various primers were the focus of each PCR. Eshoo et al., determined *Borrelia burgdorferi* genotype, utilized blood tests and multi-loci PCR with eight distinct loci, the awareness was 62% and the particularity was 100%.[131] Serum samples and biopsy samples with 40.6 and 42.6% sensitivity were subjected to a nested PCR test by Liveris et al.,[122] also demonstrated a qPCR with a sensitivity of 33.8% on plasma samples. Two nested PCR sets focusing on the Osp A quality were explored in neurological lyme disease. Samples taken from the cerebral spinal fluid in both early and late cases; in all direct diagnostic studies, sensitivity was low even in most cases lower than the two-tier test regime, assays, or immunoblots reported for early lyme disease and they reported a sensitivity of 37.5–50% in acute cases and 12.5-25% in late cases.[125]

Conclusions

Molecular biology could be one of the options for the identification and treatment of Lyme disease. The existence of many scientific resources and modern technologies, precise and effective instructions, suitable laboratories and human resources in the field of molecular biology makes it a suitable field for diagnosis. According to some weak and false results in some methods, new research studies are needed. Finally, molecular biology currently provides the best methods and tests for Lyme disease diagnosis.

Acknowledgment

None.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Centers for Disease Control and Prevention: CDC; Sodium dodecyl sulfate-polyacrylamide gel electrophoresis: SDS-PAGE; Outer surface proteins: OSPs; Restriction fragment length polymorphism: RFLP; Pulsed-field gel electrophoresis: PFGE; multilocus sequence typing: MLST; Lyme arthritis: LA; European Union Concerted Action on Lyme Borreliosis; EUCALB; Whole-cell sonication: WCS; Enzyme-linked immunosorbent assays: ELISA; Indirect hemagglutination antibody: IHA; Barbour-Stoner-Kelly: BSK.

Authors' contributions

All authors read and approved the final manuscript. All

authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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