Systematic review

Unconventional diagnostic tests for Lyme borreliosis: a systematic review

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Background: Lyme borreliosis (LB) diagnosis currently relies mainly on serological tests and sometimes PCR or culture. However, other biological assays are being developed to try to improve Borrelia-infection diagnosis and/or monitoring.

Objectives: To analyse available data on these unconventional LB diagnostic assays through a systematic literature review.

Methods: We searched PubMed and Cochrane Library databases according to the PRISMA-DTA method and the Cochrane Handbook for Systematic Reviews of Interventions. We analysed controlled and uncontrolled studies (published 1983–2018) on biological tests for adults to diagnose LB according to the European Study Group for Lyme Borreliosis or the Infectious Diseases Society of America definitions, or identify strongly suspected LB. Two independent readers evaluated study eligibility and extracted data from relevant study reports; a third reader analysed full texts of papers to resolve disagreements. The quality of each included study was assessed with the QUADAS-2 evaluation scale.

Results: Forty studies were included: two meta-analyses, 25 prospective controlled studies, five retrospective uncontrolled studies, six retrospective controlled studies and two case reports. These biological tests assessed can be classified as: (i) proven to be effective at diagnosing LB and already in use (CXCL-13 for neuroborreliosis), but not enough to be standardized; (ii) not yet used routinely, requiring further clinical evaluation (CCL-19, Ospa and interferon-γ); (iii) uncertain LB diagnostic efficacy because of controversial results and/or poor methodological quality of studies evaluating them (lymphocyte transformation test, interferon-γ, ELISPOT); (iv) unacceptably low sensitivity and/or specificity (CD57+ natural killer cells and rapid diagnostic tests); and (v) possible only for research purposes (microscopy and xenodiagnoses).

Discussion: QUADAS-2 quality assessment demonstrated high risk of bias in 25/40 studies and uncertainty regarding applicability for 32/40, showing that in addition to PCR and serology, several other LB diagnostic assays have been developed but their sensitivities and specificities are heterogeneous and/or under-evaluated or unassessed. More studies are warranted to evaluate their performance parameters.

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Introduction

Lyme borreliosis (LB) is the most commonly reported vector-borne disease transmitted by ticks in the USA and western Europe [1–3]. It is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. The greater variety of human pathogenic genospecies in Europe (mainly *Borrelia afzelii*, *Borrelia garinii*, *Borrelia burgdorferi* sensu stricto) than the USA explains the wider range of clinical pictures [4]. The most common clinical manifestations in Europe are erythema migrans (EM) and Lyme neuroborreliosis [4]. The early localized stage of EM is pathognomonic and does not require further exploration [5]. The early (<6 months) and late (>6 months) disseminated LB stages mainly affect skin, nervous system and joints, and rarely heart and eyes [4]. Some subjective symptoms (asthenia, polyalgia, cognitive complaints) may also be present at all stages and may persist after well-conducted treatment, with the latter being called post-treatment Lyme disease syndrome (PTLDS) [4,6–8]. Reliable diagnostic tests are needed to dissociate active from inactive LB, and from other diseases that share some similar clinical manifestations.

Diagnosis of LB relies mainly on serological tests and PCR. Their sensitivities and specificities at disease onset may be less reliable and depend on the anatomical site sampled [9]. A recent meta-analysis [10] showed that LB serological tests had heterogeneous sensitivities, depending on the disease stage: 50% (95% CI 40–61%) for localized EM, 77% (95% CI 67–85%) for Lyme neuroborreliosis, 97% (95% CI 94–99%) for acrodermatitis chronica atrophicans (ACA), 96% (95% CI 93–100%) for Lyme arthritis (LA), and 73% (95% CI 53–87%) for unspecified LB. Specificity was ~95% for investigations with healthy controls and 80% for other diseases that share some similar clinical manifestations.

The development of active infection biomarkers would greatly advance LB diagnosis and monitoring. AR and AG extracted data from retained reports. They knew the authors’ names, institutions, publication journal and results when they applied the eligibility criteria. When they disagreed about a study’s relevance, a third expert (BJ) was asked to analyse the full text. The quality of each included study was assessed with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) evaluation scale (Figs. 1 and 2, and see Supplementary Table S4) [33].

We included studies published from 1983 to 2018, conducted on adult humans, with control groups or not, randomized or not and in which LB cases were defined according to the European Study Group for Lyme Borreliosis (ESGBOR) or the Infectious Diseases Society of America (IDSA) criteria [4,34], or cases reported as strongly clinically suspected LB but without microbiological documentation. The latter choice was made to avoid excluding studies describing diagnostic tools for ambiguous LB cases with no microbiological documentation. Meta-analyses and case-series were included; reports with only abstracts available, congress communications and letters were excluded, as were studies evaluating serological tests and PCR. When Cochrane Handbook for Systematic Reviews of Interventions were closely respected, studies already included in those meta-analyses were excluded and all subsequently published studies were included. No language restriction was applied.

First, we conducted a free search to obtain an overview of all the available diagnostic tests using the following terms: ‘Lyme borreliosis’ or ‘Lyme disease’ and ‘diagnostic tests’ or ‘diagnosis’. Then, the following MeSH terms were used: ‘Lyme borreliosis’ or ‘Lyme disease’, and ‘diagnostic tests’, ‘CXCL-13’, ‘apolipoprotein B-100’, ‘CCL-19’, ‘lymphocyte transformation test’, ‘interferon’, ‘CD57’, ‘xenodiagnosis’, ‘membrane protein OspA’, ‘rapid diagnostic test’, ‘focus-floating microscopy’, ‘dark-field microscopy’, ‘silver-stain microscopy’ and ‘microscopy’. We could not conduct a meta-analysis for any of the tests detailed below because study quality was too heterogeneous and too few publications reported test performances (sensitivity/specificity).

Results

Forty studies were included: two meta-analyses, 25 prospective controlled studies, five prospective uncontrolled studies, six retrospective controlled studies and two case reports (Fig. 3).

Tests exploring inflammatory or autoimmune responses

CXCL-13

The C-X-C motif chemokine ligand-13 (CXCL-13), produced by antigen-presenting cells, selectively attracts B lymphocytes [35–37]. During Lyme neuroborreliosis, CXCL-13 released into the CSF by resident mononuclear cells [38] triggers B-cell migration into the CSF, resulting in the characteristic B-cell-enriched CSF pleocytosis [39].

Database searches identified 117 reports. After removing duplicates, 52 publications were screened. Nine full-text articles were
assessed for eligibility. Four studies were finally retained: two meta-analyses [40,41], one retrospective controlled study [42] and one prospective uncontrolled study [43]. QUADAS-2 quality assessment demonstrated low risk of bias and low applicability concerns for both meta-analyses [40,41], unclear risk of bias and uncertainty regarding applicability for the retrospective controlled study [42], and high risk of bias and high concerns regarding applicability for the prospective uncontrolled study [43].

As demonstrated previously for Lyme neuroborreliosis, the CSF CXCL-13 concentration is high [40–44]. CXCL-13 is detectable from days to weeks before antibodies in CSF and its level rapidly declines after antibiotics, suggesting its potential role as a therapeutic marker [40–43,45,46]. For the meta-analyses, including 961 Lyme neuroborreliosis patients and 3282 controls, the pooled CSF CXCL-13 sensitivity range was 89%–97% and its pooled specificity was 96% (95% CI 92%–98%) [40,41]. The specific antibody index recommended to diagnose definite Lyme neuroborreliosis had 86% (95% CI 63%–95%) sensitivity and 94% (95% CI 85%–97%) specificity [47,48].

Optimal cut-off values were determined to be 162 pg/mL in one meta-analysis [41] and 131 pg/mL using the Luminex platform versus 259 pg/mL for ELISA in a retrospective, controlled study [42]. Cut-off determination is a major issue for CXCL-13 use to diagnose Lyme neuroborreliosis, because no official recommendation has yet been advanced. In addition, few available kits are marked European Community-in vitro diagnosis (European conformity authorization). Each laboratory has to determine its own threshold, which might lead to different interpretations.
Moreover, the CSF CXCL-13 level is elevated in other central nervous system disorders, like neurosyphilis, cryptococcosis, trypanosomiasis, viral meningitides, meningoencephalitides and central nervous system lymphoma [41,42]. Attention should also be paid to those pathologies when interpreting the results. CXCL-13 seems to be a good add-on marker, with high sensitivity and specificity to diagnose untreated, acute Lyme neuroborreliosis. It could be useful for patients with early typical clinical symptoms, CSF pleocytosis and a negative specific antibody index, and for cases of re-infection. It remains to be evaluated for late Lyme neuroborreliosis.

CCL-19

In their recent prospective controlled study, Aucott et al. described the T-cell chemokine CCL-19 as a potential immunological risk factor of PTLD [49]. Seventy-six patients with physician-documented EM were followed for 1 year post-antibiotics; 11/76 (14.5%) developed PTLD-compatible symptoms [49]. Persistently high 1-year CCL-19 levels were only observed in patients with PTLD.

QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability in this study [49].

CCL-19 could be of potential interest to screen patients at risk of PTLD. More studies are warranted.

Apolipoprotein B-100

We identified four papers through database searches. After removing duplicates, two prospective controlled studies were screened, their full texts were assessed for eligibility and, finally, they were included for the review [50,51]. QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability in these two studies [50,51]. Crowley et al. identified Apolipoprotein B-100 (ApoB-100) as a target of T-cell responses in 14/37 (38%) individuals with LA [50]. Moreover, 5/37 (13%) individuals with LA had autoantibodies directed against ApoB-100, an autoantigen involved in refractory LA [50,52,53].

To date, no study has used ApoB-100 as a diagnostic tool for LB. It cannot be used in routine practice.

Tests exploring cellular immunity

Lymphocyte-transformation test

A lymphocyte-transformation test evaluates the lymphoproliferative response of peripheral blood mononuclear cells to B. burgdorferi antigens. The results are expressed as a stimulation index (SI), with SI >10 considered positive and SI <10 considered negative [54].

Among 38 publications identified through database searches, 24 were screened, after removing duplicates, and 14 full texts were assessed for eligibility. Ten studies were finally retained for the review: eight prospective controlled studies [54–61], one prospective uncontrolled study [62] and one retrospective controlled study [63]. QUADAS-2 quality assessment demonstrated low risk of bias and uncertainty regarding applicability for two prospective controlled studies [54,61], unclear risk of bias and uncertainty regarding applicability for six prospective studies [55–60], and high risk of bias and high applicability concerns for the two others [62,63].
Only four provided sensitivities and specificities for any stage with combined respective ranges of 45%–89.4% and 33%–98.7% [54,59–61]. Only one study reported data on late LB with 45% (95% CI 30%–60%) sensitivity and 95% (95% CI 87%–99%) specificity [54]. Among the ten studies retained, for early LB, mean SIs were >10 in two [60,61]; <10 in one [58] and not mentioned in seven [54–57,59,62,63]; whereas for late LB, mean SIs were >10 in five [54,55,57,60,61], <10 in two [58,63] and not mentioned in three [56,59,62]. For controls (healthy participants or other diseases), mean SIs were <10 in five studies [54,55,57,58,63], >10 in one [61], and not mentioned in four [56,59,60,62]. Exposed healthy participants (forest workers, Borrelia laboratory workers) had high seroprevalence, with mean SI >10 in one report, <10 in two [54,59] and not given in seven [55–58,61–63]. Fig. 4 summarizes these findings.

After antimicrobials, SI decreased in three studies [56,59,60] and was not reported in the other seven. Zoschke et al. noted the late immunostimulation assay [59,62]. Second, LB was defined with lymphocyte-transformation test [45] that used lymphocyte-transformation tests were standardized only in two investigations [54,56,58,59,61,62]. For controls (healthy participants or other diseases), mean SIs were <10 in five studies [54,55,57,58,63], >10 in one [61], and not mentioned in four [56,59,60,62]. Exposed healthy participants (forest workers, Borrelia laboratory workers) had high seroprevalence, with mean SI >10 in one report, <10 in two [54,59] and not given in seven [55–58,61–63]. Fig. 4 summarizes these findings.

After antimicrobials, SI decreased in three studies [56,59,60] and was not reported in the other seven. Zoschke et al. noted the late persistence of lymphocyte proliferation after antibiotics [61]. Concordance with serological tests was found in four studies, two each performed on late LB or at all stages [55,57,60,63], and discordance in the six others conducted at all stages combined [54,56,58,59,61,62].

Those ten studies had numerous biases. First, lymphocyte-transformation tests were standardized only in two investigations that used lymphocyte-transformation test—memory lymphocyte immunostimulation assay [59,62]. Second, LB was defined with criteria other than those of ESGBOR or IDSA in six [45,54–56,58,59,62]. Third, the international SI cut-off to interpret results was not respected in four [59,60,62,63] and not mentioned in five [55–58,61], meaning that nine studies do not enable any conclusion to be drawn. Finally, the numbers of patients with SI >10 were not given in six studies [55–57,59,62,63] that reported only mean SIs.

At present, no guidelines recommend using lymphocyte-transformation tests because of their lack of specificity [11,13,14,16,64,65]. Studies on lymphocyte-transformation tests yielded heterogeneous results and mixed quality, without clear sensitivity and specificity. They do not permit conclusions to be drawn about the usefulness of lymphocyte-transformation tests to diagnose LB, including for rare seronegative patients.

**Interferon-γ ELISPOT**

Interferon-γ (IFN-γ) release into the blood is assessed to explore T-cell activation after *B. burgdorferi* sensu lato stimulation.

Among 73 publications identified by database searches, 39 were screened, after removing duplicates, and 16 full texts were assessed for eligibility. Eight studies were finally retained for the review: seven prospective controlled studies [66–72] and one retrospective controlled study [73]. All but one of them [73] respected ESGBOR or IDSA clinical and microbiological definitions of LB. A QUADAS-2 quality assessment demonstrated low risk of bias and low applicability concerns for one study [67], low risk of bias and uncertainty regarding applicability for three studies [69,71,72], unclear risk of bias and unclear applicability concerns for one study [70], unclear risk of bias and high applicability concerns for two studies [66,68], and high risk of bias and high applicability concerns for one retrospective controlled study [73]. All eight studies showed that IFN-γ was associated with exposure to *Borrelia*. Circulating IFN-γ was elevated in patients with early LB, late LB or PTLDs and seropositive, asymptomatic participants. It was significantly less elevated in healthy seronegative participants in all studies (p <0.01). Moreover, IFN-γ concentrations did not parallel *Borrelia* activity in six studies [66–68,70,72,73]. Sensitivities during early LB ranged from 36% to 69% in 2/3 studies [69,70] and only one study provided specificity (~82%) [70]. Calister et al. demonstrated that combining positive *Borrelia*-specific C6-peptide—ELISA and IFN-γ results increased sensitivity to 83% [69]. Only one study on late LB reported 84% sensitivity and 96% specificity for the iSpot Lyme [68]; four studies failed to mention any test performance [66,71–73].

Only one study found an IFN-γ decrease after antibiotics [69]. No study demonstrated any concordance between serological test results and circulating IFN-γ.

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Fig. 4. Pooled results from lymphocyte-transformation tests of the included studies for Lyme borreliosis (LB), according to different groups of patients. SI, stimulation index.
The main limitation of IFN-γ ELISPOT appears to be its wide variability. First, the cut-off to interpret results was not given in 6/8 studies. In the two studies indicating a threshold, only two patients with anaplasmosis served as controls [69] or a receiver operating characteristics curve with 105 controls was used [68]. Second, specificity varied markedly according to the control population chosen. Third, among the studies, different Borrelia species were used to assess the performance of the circulating IFN-γ level: two did not mention the species [68,69], three used a B. afzelii strain (ACA-I) [66,71,73], two used a B. garinii strain (lp90) [70,72], and the last used the B. burgdorferi strain B31 combined with antigens specific to each of four species (B. afzelii, B. garinii, B. burgdorferi senso stricto, Borrelia bavariensis) [67]. Pertinently, the B. burgdorferi B31 lysate cross-reacts with other spirochetes [67,71]. Finally, no standardized test has been validated at present: notably, three studies had demonstrated lack of reproducibility for LB [67–69].

Interferon-γ secretion has been also studied in other tissues/biological fluids (skin biopsies, synovial fluid, CSF) to assess its role in the inflammatory response to Borrelia but it was not considered a diagnostic tool [66,71,72,74–78]. At present, IFN-γ ELISPOT cannot be used in routine practice because its role has only been established as a witness of Borrelia exposure. Specificity, cut-offs and reproducibility require more investigations.

Interferon-α

Among 24 articles identified through database searches, 12 were screened, after removing duplicates, and three full texts were assessed for eligibility. Finally, only one prospective comparative study was included [79]. QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability for this study [79]. Jacek et al. [79] demonstrated higher IFN-α activity in patients with PTLDs, which suggests the existence of an immune-related disease process in patients with persistent symptoms, perhaps contributing to ongoing symptoms. The authors also reported that β-lactam therapy did not modulate the activated immune response.

More studies are warranted to assess the reliability of this test.

CD57+ natural killer cells

CD57 is a natural killer cell marker but is also expressed on other cells. The method is based on an immunophenotyping technique.

Among ten articles identified through database searches, five were screened, after removing duplicates, and three full texts were evaluated for eligibility. Two prospective controlled studies were finally retained for the review [80,81]. QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability in these two studies [80,81].

Stricker and Winger reported lower CD57+ lymphocyte counts (mean 30 ± 16 cells/μL) in 73 patients with late LB before starting antibiotics; the lymphocyte count increased after treatment (mean 66 ± 39 cells/μL) [80]. That study had several biases: low numbers of patients included, bias of presentation of the results, no repeated test on the same patients to follow CD57-expression kinetics, no validated control groups, and no clear case definition and treatment. Marques et al. found no significant difference (p 0.68) among nine patients with PTLDs, the 12 cured of LB and nine healthy volunteers [81]. The authors did not report CD57 results before antibiotics (all were already treated or healthy), and very few patients and controls were tested. CD57-marker specificity for LB is probably low, because low CD57 levels were also found in patients with numerous autoimmune diseases, infections or cancers [82].

CD57 measurement does not seem useful, even after antibiotics or for persistent symptoms. To our knowledge, no study has provided this test’s performance (sensitivity/specificity).

Microbiological tests

Xenodiagnosis

Xenodiagnosis, an experimental test used in animal models of LB, uses uninfected ticks to detect low-level infection, even after antibiotics.

Our database searches identified 22 papers, among which 11 were screened, after removing duplicates, and five full texts were assessed for eligibility. Only one prospective, controlled study was included [83]. QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability in this study [83].

This study investigates the feasibility of xenodiagnosis in humans: 2/26 patients treated for LB had positive xenodiagnoses post-treatment (one control EM participant and one PTLDs). Xenodiagnosis was positive only by DNA detection after PCR of tick cultures or the tick itself. No spirochetes were detected. Bockenstedt and Radolf questioned whether Borrelia-DNA detection was sufficient to confirm the diagnosis [84].

Currently available xenodiagnosis performance is insufficient to assess its diagnostic usefulness.

Outer surface protein A detection

Outer surface protein A (OspA), a Borrelia burgdorferi lipoprotein that binds to its receptor TROSPA in the tick midgut, is required to infect the tick.

The two identified studies were included in this review [85,86]. QUADAS-2 quality assessment demonstrated low risk of bias and uncertainty regarding applicability in one study [85] and unclear risk of bias and uncertainty regarding applicability in the other study [86].

In their prospective, uncontrolled study on only three patients, Cheung et al. described a liquid chromatography–tandem mass-spectroscopy method to directly detect OspA in sera from EM patients [85]. That technique detected low OspA levels in the three patients’ sera. Although the proof-of-concept merits further examination, neither sensitivity nor specificity was reported. In a prospective controlled study on 140 patients, Magni et al. described a nanotrap technology-based method to detect the OspA C-terminus domain in urine samples [86]. Although the authors reported excellent sensitivity (100%) and specificity (100%) before antibiotics, those results must be confirmed by other studies.

Upcoming investigations on this technique should be of interest.

Direct microscopy of human tissues

Database searches identified 81 studies. After removing duplicates, 29 publications were identified, on ‘dark-field microscopy’, 18 on ‘silver staining’ and 2 on ‘focus-floating microscopy’. All papers were screened. Nine full texts were assessed for eligibility and seven were retained: two prospective, controlled studies [87,88], two prospective, uncontrolled studies [89,90], one retrospective, controlled study [91] and two case reports [92,93]. QUADAS-2 quality assessment demonstrated low risk of bias and unclear applicability concerns for one study [87], unclear risk of bias and uncertainty regarding applicability for two studies [88,91], and unclear risk of bias and high applicability concerns in four studies [89,90,92,93].
Electron microscopy, silver staining with light microscopy and focus-floating microscopy enabled spirochete detection in various samples [87,91–94]. Nevertheless, it has limited clinical utility because of the apparent scarcity of Borrelia in mammalian tissues and its 41% sensitivity [90], and the time required for analysis [95,96]. False-positive results have also been reported [88].

Laane et al. published an uncontrolled study describing a modified dark-field microscopy technique, called LM-method, which identified structures claimed to be Borrelia in 21/32 (66%) patient blood samples among those with non-specific symptoms [97]. Using the same methodology and including a control group, Aase et al.’s findings refuted Laane et al.’s results, i.e. the LM-method was positive for 85% of the 41 healthy controls and was unable to detect Borrelia in the 21 positive controls with five B. afzelii per 100 red cells [98]. The LM-method should not be used for LB diagnosis.

At present, Borrelia detection by microscopy can only be used for research purposes. Inclusion of positive/negative controls and the microscopy reader’s expertise are necessary.

Rapid diagnosis tests

Among the 32 articles identified by database searches, 16 were screened, after removing duplicates, and two full texts were assessed for eligibility. Two retrospective, controlled studies were included in the review [99,100]. QUADAS-2 quality assessment demonstrated unclear risk of bias and uncertainty regarding applicability in these two studies [99,100].

Two rapid diagnosis test (RDT) categories are available: immunochromatography-based methods and microfluidic-based point-of-care tests.

Immunochromatography-based methods are commercially available at pharmacies throughout Europe for patient self-use. These RDTs require a drop of blood and can be performed with minimal training. Only one study assessed RDT performances and no regulations are available [99]. Smit et al. used two commercially available RDTs with sensitivities of 26% and 32%, and specificities of 85% and 88%, values that are much lower than those of laboratory-based diagnostic tools [99]. Their results do not support RDT use for diagnostic strategies. Neither European nor American guidelines recommend using RDTs.

A microfluidic-based point-of-care test that can be performed in 15 minutes is not yet commercially available [100]. This test’s performance was comparable to that of the laboratory-based C6-peptide ELISA with 84% sensitivity and 92% specificity. However, it was shown to be significantly less sensitive than a recombinant (VlsE+DbpA+OspC) ELISA [100].

Rapid diagnosis tests for LB remain experimental, requiring further investigation.

Discussion

Summary of evidence

In addition to PCR and serology, several other LB diagnostic assays have been developed but their sensitivities and specificities are heterogeneous and/or under-evaluated or unassessed.

Study strengths and weaknesses

Included studies

We included studies defining LB cases according to ESGBOR or IDSA criteria [4,34] and avoided excluding studies describing diagnostic tools for ambiguous cases lacking microbiological documentation. That choice represents a strength of our study as it attempts to identify which test might perform well at diagnosing such ambiguous cases.

QUADAS-2 quality assessment demonstrated high risk of bias in 25/40 studies and uncertainty regarding applicability for 32/40 (cf. Figs. 1 and 2, and see Supplementary material, Table S4) [30,33]. Most authors failed to report the sensitivities or specificities of the evaluated diagnostic tests, indicating that the studies on alternative diagnostic tests were not methodologically well-conducted.

Review process

Two databases were screened, without search filters or language restriction. Consensus was reached for the selection of included studies. We report all the QUADAS-2 domains to evaluate each included study (see Supplementary material, Table S4, and Figs. 1 and 2). For all the included studies, Tables S1 to S3 (see Supplementary material) report: study design, patient-inclusion criteria, stage, controls, other tests; main results including reported sensitivities and/or specificities and limitations. A meta-analysis could not be undertaken because the heterogeneity among included studies was too extensive and there were small numbers of studies for some tests.

Conclusion

Studies on unconventional biological LB diagnostic strategies can be classified as follows: (i) tests proven to diagnose LB accurately and already used routinely (e.g. CXCL-13 for Lyme neuroborreliosis), which, nonetheless, remain to be standardized; (ii) tests not yet used in routine practice requiring clinical evaluation (e.g. CCL-19, OspA and IFN-γ); (iii) tests with uncertain abilities to diagnose LB because of controversial results and/or poor methodological quality of the studies (e.g. lymphocyte-transformation tests, IFN-γ ELISPOT); (iv) tests with unacceptably low sensitivity and/or specificity (e.g. CD57+ natural killer cells and RDTs); and (v) tests possibly only for research purposes at present (e.g. microscopy and xenodiagnoses).

For early LB, CSF CXCL-13 is the only test whose performances allow its development for routine practice. For late LB, satisfactory performance has not yet been demonstrated for any test. Interferon-γ was only associated with Borrelia exposure. CCL-19, ApoB-100 and IFN-α might be of potential interest to screen patients at risk for PFTLDs.

In addition to PCR and serology, several other diagnostic assays have been developed for LB but their performances are heterogeneous, under-evaluated and/or unassessed. More studies are warranted to evaluate their sensitivities, specificities and reproducibilities. Identification of biomarkers for active Borrelia infection would represent a major advancement in LB diagnosis and monitoring.

Transparency declaration

The authors declare no conflict of interest related to this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2019.06.033.

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