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Diagnosing borreliosis

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Abstract:	Borrelia species fall into two groups, the Borrelia burgdorferi sensu lato (Bbsl) complex the cause of Lyme borreliosis (LB; also known as Lyme disease LD) and the relapsing fever group. Both groups exhibit inter- and intra-species diversity and thus, have variations in both clinical presentation and diagnostic approaches. A further layer of complexity is derived from the fact that ticks may carry multiple infectious agents and are able to transmit them to the host during blood feeding, with potential overlapping clinical manifestations. Besides this, pathogens like Borrelia have developed strategies to evade the host immune system, which allows them to persist within the host, including humans. Diagnostics can be applied at different times during the clinical course and utilise sample types, each with their own advantages and limitations. These differing methods should always be considered in conjunction with potential exposure and compatible clinical features. Throughout this review, we aim to explore different approaches providing the reader with an overview of methods appropriate for various situations. This review will cover human pathogenic members of Bbsl and relapsing fever borreliae, including newly recognised B. miyamotoi spirochetes.

Diagnosing Borreliosis

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Introductory Remarks:

Borrelia species fall into two groups, the *Borrelia burgdorferi* sensu lato (Bbsl) complex the cause of Lyme borreliosis (LB; also known as Lyme disease LD) and the relapsing fever group. Both groups exhibit inter- and intra-species diversity and thus, have variations in both clinical presentation and diagnostic approaches. A further layer of complexity is derived from the fact that ticks may carry multiple infectious agents and are able to transmit them to the host during blood feeding, with potential overlapping clinical manifestations. Besides this, pathogens like *Borrelia* have developed strategies to evade the host immune system, which allows them to persist within the host, including humans.

Diagnostics can be applied at different times during the clinical course and utilise sample types, each with their own advantages and limitations. These differing methods should always be considered in conjunction with potential exposure and compatible clinical features. Throughout this review, we aim to explore different approaches providing the reader with an overview of methods appropriate for various situations. This review will cover human pathogenic members of Bbsl and relapsing fever borreliae, including newly recognised *B. miyamotoi* spirochetes.

Detection of Borrelia in the arthropod vector:

Various methods can be applied to detect the presence of *Borrelia* in vectors. Widely used approaches that demonstrate significant sensitivity, specificity and reliability include: multiple formats of PCRs, mostly nested PCR that target different genomic loci, selection of which depends on the sample origin (template); reverse-line blotting

(RLB), based on hybridization of amplified selected *Borrelia* genes with spirochete-specific probes; multilocus sequences analysis (MLSA) and multilocus sequence typing (MLST), based on the sequence analysis of amplified fragments of spirochete genome or microscopy with stained spirochetes in tick midgut or salivary glands (Aguero-Rosenfeld, et al. 2005, Margos, et al. 2011). The most recently applied techniques include next generation sequencing (NGS) and proteomic approaches. Cultivation of *Borrelia* in commercial BSK (Barbour-Stoenner-Kelly) or home-made MKP (modified Kelly- Pettenkoffer) media, that for a long time considered to be a gold standard in LB diagnostics, is still widely used, but is rather time consuming and challenging. The culture negative cases do not necessarily mean the absence of spirochetes in a sample. The failure to culture the spirochetes might be caused by multiple vector-, spirochete,- media- or cultivation conditions related factors (Cerar, et al., 2008, Ružić-Sabljić, et al. 2014, Rudenko, et al., 2016). Nowadays, the priority of all used techniques is re-directed form simple detection of pathogen in either environmental sample or clinical sample, to simultaneous detection and identification of spirochete species (or possible co-infection agents). Considering the high possibility of the presence of multiple pathogens in tick vectors, the other question is whether to use singleplex or multiplex formats for their detection/identification. Fluidic microarrays allow the assessment of multiple tick-borne pathogens simultaneously (Vayssier-Taussat, et al. 2013).

Use of proteomic methods to detect presence of some relapsing fever *Borrelia* in the hemolymph of ticks provides additional options for borrelial detection in vectors (Fotso Fotso, et al. 2014).

These methods provide invaluable research tools and facilitate epidemiological studies, but their clinical relevance is debatable. Detection of a pathogen in the vector does not imply that it has been successfully transmitted to the host upon which the tick has fed. Transmission dynamics are complex and multi factorial and beyond the scope of this review. Home use diagnostic kits are available and allow individuals to test collected ticks for the presence of Lyme borreliae. The reliability of these tests has been highly debated. Tick bites are frequently unnoticed and might only demonstrate that you have been in a risk environment, but do not necessarily correlate with any infectious consequences. That is why use of such tests is of limited value for diagnosis, but can be useful for epidemiological studies.

Recommendation:

Tick testing as supportive data for identification of LB endemic regions; correct selection of PCR target based on the final goal of tests and sample nature; re-analysis of tested sample targeting different genomic loci; consider the presence of co-infection with multiple pathogens as highly possible.

Clinical diagnosis of Lyme borreliosis and supportive diagnostic strategies

A reliable clinical diagnosis of LB is only evident to the non-expert physician when a typical erythema migrans (EM) is present (Stanek and Strle 2003). Since the large majority of LB symptoms have minimal diagnostic value because of their lack of specificity, diagnosis of LB might be challenging for general practicioners in patients without EM (Strle and Stanek 2009). Generally there exists a tendency towards overdiagnosis of chronic Lyme disease (Czupryna, et al. 2016,Koedel, et al.

2015,Sigal 1996). Although different diagnostic approaches (mentioned later) have been explored, to date the only recommended supportive tests used are serological confirmation. Serological results alone are insufficient to distinguish whether the patient suffers from an acute or re-infection that needs treatment, or is only seropositive because of a past infection. This might be especially problematic for individuals that are frequently exposed to ticks and therefore have at high risk of re-infection. However even in low risk areas, the positive predictive value of serological tests can be very low (Lantos, et al. 2015), meaning that clinical manifestations still remain crucially important criteria for a reliable diagnosis of the disease. Factors that need to be integrated for a reliable diagnosis are therefore the occurrence of compatible symptoms, serological results and risk of tick exposure. Figure 1 provides a diagnostic overview for LB.

To date only serological tests are recommended to support the diagnosis of Lyme borreliosis in the absence of EM

In cases where EM is clearly evident, serological tests are not needed and treatment should start immediately (Stanek, et al. 2012). In patients who do not develop EM, serological tests are recommended to support the diagnosis (Aguero-Rosenfeld, et al. 2005). Initial problems with the specificity and sensitivity of serological tests have resulted in controversial statements on their efficacy to support diagnosis of acute LB. Recently, serological tests have been optimized switching from a single *Borrelia* strain cell extract to a use of combination of more precisely chosen recombinant antigens or synthetic peptides (Fang Ting, et al. 2000,Goettner, et al. 2005). Previously a two-tier test approach, in which the presence of antibodies is first tested

by a highly sensitive ELISA and, in case of a positive result, further confirmed by a highly specific immunoblot, was recommended (Branda, et al. 2010,Koedel, et al. 2015). Noteworthy, the reported accuracy of ELISAs and immunoblots varies throughout Europe and a recent study revealed no overall benefit of two-tiered tests over single tests (Leeflang, et al. 2016). Only early stage patients (symptoms <6 weeks) might still be seronegative, as they have not developed antibodies yet. Therefore, diagnosis of LB should be re-evaluated in seronegative late stage patients (Stanek, et al. 2012). Low antibody titers have been observed after antibiotic treatment indicating that the induced B-cell immune response is probably not very long-lived and robust. Especially patients where *Borrelia* took longer to disseminate seem to develop long-lived antibody titers less efficiently (Aguero-Rosenfeld, et al. 1996,Elsner, et al. 2015,Hammers-Berggren, et al. 1994,Nowakowski, et al. 2003). Recent mouse studies have shown that *Borrelia* have a direct effect on the mouse B-cell response (Elsner, et al. 2015,Elsner, et al. 2015,Hastey, et al. 2012,Hastey, et al. 2014). However, the underlying mechanism in humans requires further investigation. Showing the induction of strain specific immunity (not non-crossprotective), mouse and human studies together (Khatchikian, et al. 2014) may explain reinfection of LB. Consequently, previous *Borrelia* infections must be taken into account when considering serological testing (Nadelman and Wormser 2007).

Despite the described improvement of these tests, we still face the problem of non-standardization and inappropriate application of current serological tests (Ang, et al. 2011,Leeflang, et al. 2016,Markowicz, et al. 2015,Muller, et al. 2012). Different (in-house) assays and result interpretation remain a major problem (Fallon, et al. 2014)

that should be solved in the future by the implementation of a universial and worldwide (or Europe/USA wide) diagnostic standard test, or as a minimum, use of internationally agreed standards and participation in quality control schemes. However, the problem remains (especially amongst high risk groups) to distinguish between an acute and a resolved infection. Future studies should therefore focus on the development of new strategies that would allow a yes or no result.

Noteworthy, serological tests should not be used as a proof of efficacy of the antibiotic treatment, although antibody titers generally decrease after antibiotic treatment, however patients may remain seropositive for years after the infection in the absence of active disease (Aguero-Rosenfeld, et al. 1996,Glatz, et al. 2006,Hammers-Berggren, et al. 1994,Kalish, et al. 2001,Kowalski, et al. 2010,Lomholt, et al. 2000). Instead, the disappearance of symptoms is a more reliable sign of cure.

When neuroborreliosis is suspected, detection of intrathecally produced anti-*Borrelia* antibodies significantly supports the diagnosis. However, results might be negative at early stages and more often in children (Christen, et al. 1993). Measurement of *Borrelia*-specific antibodies in CSF cannot be used to assess the efficiency of treatment (Koedel, et al. 2015).

Since antibiotic treatment is generally considered efficient, differential diagnosis is crucial in case of a chronic course of the disease (Halperin 2015,Halperin 2016,Hjetland, et al. 2015,Markowicz, et al. 2015,Rebman, et al. 2015,Wills, et al.

2016). A chronic course has been observed in patients infected by *Borrelia*, viral and non-viral pathogens, such as Epstein-Barr virus (glandular fever), *Coxiella burnetii* (Q fever), or Ross River virus (epidemic polyarthritis) (Aucott, et al. 2013,Galbraith, et al. 2011,Hickie, et al. 2006,Katz and Jason 2013) and the underlying causes are not clear. In this context, also the general health status and/or the lifestyle of the patient should be considered. In general, immunocompromised or otherwise not completely healthy patients might be at higher risk to develop chronic symptoms after treatment. Patients with hematological malignancies for example seem to suffer more often from disseminated disease and more frequently require retreatment (Maraspin, et al. 2015). In non-immunocompromized cases, where symptoms continue to persist even after appropriate antibiotics treatment, it is currently not recommended to prolong the treatment. Clinical studies have shown that the risk of side effects outweighs any potential therapeutic benefits (Klempner, et al. 2001,Koedel, et al. 2015,Krupp, et al. 2003). In these cases, co-infections with other tick borne diseases or other possible causes of the symptoms should be excluded (Belongia 2002,Berghoff 2012,Godar, et al. 2015,Swanson, et al. 2006) and symptomatic treatment considered (Koedel, et al. 2015). Only in late neuroborreliosis, is prolongation of the antibiotic treatment justifiable in cases of persistent cerebrospinal fluid (CSF) lymphocytic pleocytosis (Koedel, et al. 2015).

In rare cases, *Borrelia* can cause problems with the heart and vascular system and might be considered as underlying cause of stroke-like symptoms in patients which otherwise have no obvious risk for cardiovascular diseases (Allen and Jungbluth 2016,Zajkowska, et al. 2015). Full description of LB clinical manifestations and their

diagnosis have been recently reviewed by Stanek and co-workers (Stanek, et al. 2011).

When encountering a tick bite, correct and early removal of the tick is a good way to reduce probability of infection. In Europe, only about 2% (Wilhelmsson, et al. 2016) and in USA, about 1% (Heymann and Ellis 2012) of patients bitten by a tick develop LB. Detection of spirochete DNA in ticks alone does not necessarily means the succesful pathogen transmission, which is why the value of this test has limited diagnostic value for LB ((ESGBOR) 2013), but is useful for epidemiological studies (Reye, et al. 2010) to define risk areas. In this context, next generation sequencing is a new emerging technique that allows screening of the same tick in parallel for various tick-borne pathogens, with the potential of getting more detailed information about co-infections of ticks and identification of new yet unrecognised pathogens (Michelet, et al. 2014,Vayssier-Taussat, et al. 2013). As transmission of *Borrelia* (and indeed other pathogens) depends on the length of tick attachment, measurement of scutal and coxial indexes can indicate duration of attachment (Crippa, et al. 2002,Gray, et al. 2005,Kahl, et al. 1998,Meiners, et al. 2006,Tijsse-Klasen, et al. 2011). In the absence of an EM and the presence of other LB related symptoms, seroconversion can be used for supportive diagnosis. However, in the absence of symptoms, seroconversion is no indication for antibiotic treatment as a study in a Swiss risk group demonstrated that only 2% of patients who seroconverted developed clinical LB (Fahrer, et al. 1991). Thus, as tick bite is a poor predictor of disease, treatment is advisable only upon appearance of LB symptoms.

Recommendation:

Clinical diagnosis alone, given a history of potential exposure and presence of EM, can be sufficient, however clinical interpretation should generally be made in conjunction with supporting laboratory findings to reach a reliable diagnosis.

Alternative strategies explored for the diagnosis of Lyme borreliosis but not on the list of recommended tests (ECDC 2016)

Direct detection of *Borrelia* in the peripheral blood, other body fluids or tissues by microscopy or molecular methods can be used as strong additional evidence in the diagnosis of LB, but might have limited significance when used alone (Aguero-Rosenfeld, et al. 2005). The sensitivity of PCR on skin biopsies is significantly higher than some other molecular tools, however, recognition of the EM itself is the best diagnosis for LB (Aguero-Rosenfeld, et al. 2005), nevertheless this provides useful research data regarding strain prevalence, virulence and provides insights into deciphering pathogenesis of LB (Strle, et al. 2013). Cultivation of *Borrelia* from patient samples might be an alternative method to detect viable *Borrelia*, but is both time consuming and challenging (Rudenko, et al. 2016). As such, cultivation is best reserved as a research tool.

Lymphocyte transformation tests (LTT) have been explored for their potential to overcome the diagnostic gap in LB patients without EM but before seroconversion and in re-infected seropositive patients. This assay measures lymphocyte proliferation *in vitro* after stimulation with *B. burgdorferi* specific antigens. Currently,

results are contradictory and consequently LTT is not recommended as a routine diagnostic tool (Dessau, et al. 2014,Mygland, et al. 2010). T-cell ELISPOT is another *in vitro* stimulation assay currently explored and improved (Jin, et al. 2013). More direct methods measuring peripheral blood levels of specific cell subpopulations (CD57+ cells (Marques, et al. 2009) or antigen-reactive cells (Tario, et al. 2015) by flow cytometry, direct measure of CXCL13 levels in the CSF or metabolites within serum (Molins, et al. 2015) are also not at a point yet to be used reliably for clinical diagnosis. CD57 cell counts seem not to be reliable as a validation study found no difference between patients and healthy controls (Marques, et al. 2009). Demonstration of CSF CXCL13 as an activation marker is not specific for LB, its absence is believed to have some value in excluding neuroborreliosis (Rupprecht, et al. 2014) and it might become a valuable supportive tool to estimate treatment efficiency in case of neuroborreliosis (Koedel, et al. 2015,Schmidt, et al. 2011,Senel, et al. 2010). Problems with HLA types and identification of epitopes for antigen-specific T-cell staining are challenges that need to be addressed to validate the potential of *Borrelia* specific T-cell counts in peripheral blood to support diagnosis of LB. Metabolite measurement is a future strategy under investigation but needs further validation.

Generally, the detection of *Borrelia* DNA within ticks as well as other methods discussed above should be considered as valuable research tools providing useful information about the epidemiology of tick-borne diseases in general and LB particularly. As with serological methods, their value is lower when used alone. Combination of diagnostics, clinical and molecular tests provides a more robust and

timely diagnosis of disease. In any case, interpretation of tests results and clinical diagnosis of LB remains controversial and should currently be restricted to experts.

Development and application of new molecular tools allow the detection and differentiation among Lyme borreliosis or relapsing fever spirochetes, clearly separating *B. burgdorferi* sensu lato spirochetes from recently described *B. miyamotoi* (Margos, et al. 2008,Rudenko, et al. 2009,Venczel, et al. 2015). Combination of multilocus PCR with electro spray ionisation and mass spectrometry has recently been investigated for the detection and genotyping of *Borrelia* species in whole blood (Eshoo, et al. 2012).

Recommendation:

These tests are valuable research tools providing useful information about the patient's immune response, but interpretation for clinical diagnosis has not been clearly shown and should currently be restricted to specialised laboratories.

Diagnostics within symptomatic animals:

Veterinary infections are less well documented and benefit from laboratory confirmation to ensure correct diagnosis. This is particularly important as EM lesions have not been reported in animals and clinical signs are often common to several pathologies. As for human cases, serology is the primary diagnostic approach used, sometimes supported by use of PCR. Despite the absence of EM, cardiac, neurological signs and lameness have been reported amongst companion animals (Agudelo, et al. 2011,Hovius, et al. 1999,Krupka and Straubinger 2010). Most

veterinary cases have focussed upon lameness in dogs with positive serology, though this does not necessarily establish borrelial causality for this condition. Rapid immunochormatographic tests are often used in veterinary private practice to aid diagnosis, however these assays have not necessarily undergone the rigorous quality control applied to human serodiagnostic tests (Savić, et al. 2010).

Relapsing fever diagnostics:

Clinical diagnosis of relapsing fever infections:

In general, the clinical presentation of relapsing fever borreliosis is significantly distinct from that of LB. The possible exception to this being the appearance of a skin rash that challenges the previously believed "pathognomonic" EM, caused by the borrelial agent carried by *Amblyomma americanum* ticks in the United States, known as STARI (Borchers, et al. 2015,Masters, et al. 2008).

Human infection by recently described *B. miyamotoi* usually results in fever and associated flu-like signs (headache, chills, fatigue, myalgia), occasionally with neurological complications such as meningoencephalitis (Fonville, et al. 2014,Krause, et al. 2015).

Relapsing fever, as its name suggests, results in relapsing febrile episodes interspersed by afebrile periods. This is often accompanied by jaundice, muscle pain, headaches and sometimes involvement of major organs (Borgnolo, et al. 1993). This clinical picture can often be mistaken for other infections such as malaria that tend to overlap geographically in many endemic regions (Lundqvist, et al. 2010).

Laboratory diagnostics for relapsing fever:

Microscopy

Though for LB, microscopy is not suitably sensitive for detection, this has been the diagnostic gold standard for detection of many relapsing fever spirochetes. Darkfield examination of unstained wet-preparations, Giemsa or silver-stained blood or tissue sections, or immunofluroescence methods have been successfully used. Despite its frequent use, even relapsing fever can be difficult to detect using microscopy with some species such as *B. crocidurae* typically producing lower blood burdens than others, like *B. duttonii*. For such cases, a centrifugation step to concentrate the sample can be beneficial (Larsson and Bergström 2008). Furthermore, detection is restricted to times of febrile episodes when spirochetes are present at detectable levels. On a cautionary note, various artefacts can share the size and helical shape of spirochetes when viewed by darkfield microscopy, but tend not to show the typical gyrating spirochete-characteristic movement. Microscopy will not provide information regarding the infecting species.

Recommendation:

Microscopic methods lack both sensitivity and specificity, but can add value when used in conjunction with other methods. Sample concentration can offer distinct benefits.

Cultivation

Cultivation methods for detection of *Borrelia* have been particularly challenging and some members of the genus being particularly refractory to cultivation (Cutler, et al. 1994) whilst others are cultivable, but only in complex medium. Huge advances were made with the formulation of BSK medium with a commercial variant BSK-H supporting the growth of LB strains (Barbour 1984). Relapsing fever strains appear more diverse in their requirements. *Borrelia miyamotoi* for instance appears to prefer MKP medium (Wagemakers, et al. 2014) or high serum concentrations (Margos, et al. 2015). On a cautionary note, these preferences might reflect batch variations of composite ingredients that can vastly influence performance of these “home-made” media (Cutler personal observation). Collectively, cultivation should be considered a low yield procedure, but vital for recovery of much-needed strains for research purposes (Ružić-Sabljić, et al. 2014).

Animal inoculation or xenodiagnosis (allowing infected ticks to feed upon a test animal) has been used for primary recovery of isolates prior to cultivation in axenic medium (Naddaf, et al. 2015,Schwan, et al. 2012). It must be remembered that some species are refractory to growth in most animal models, such as *B. recurrentis*.

Recommendation:

Cultivation is low yield, time consuming and expensive and thus poorly suited to support diagnosis. Nevertheless, it still has a vital role for recovery of isolates for research purposes.

Serological diagnosis:

For the relapsing fever group, specific serology can be undertaken using GlpQ protein as antigen. GlpQ is absent from LB species, thus facilitating it's specificity for diagnostic purposes (Fritz, et al. 2013). Alternatively, BipA can also serve as a differential antigen present in relapsing fever spirochetes, but absent from the LB group (Lopez, et al. 2010). As acutely presenting patients may not yet have had sufficient time for seroconversion, serology is best reserved for retrospective diagnosis.

PCR

PCR provides a valuable diagnostic approach in acutely ill patients (Mediannikov, et al. 2014). This overcomes the poor sensitivity of microscopy and can either be used to diagnose relapsing fever borreliosis, or to further characterise the infecting spirochete. The absence of *GlpQ* in LB species makes it a specific target for detection of relapsing fever spirochetes (Takano, et al. 2014). Other assays can either speciate specific relapsing fever borreliae or be designed to detect a single member of the relapsing fever clade such as *B. miyamotoi* (Elbir, et al. 2013,Reiter, et al. 2015). The limitation of this approach is having an appropriate sample that is likely to contain spirochetal DNA. Blood collected during febrile episodes and CSF samples have given good results (Gugliotta, et al. 2013). Furthermore, in highly relapsing fever endemic areas, it is possible to have positive PCR results unrelated to current clinical pathology (Cutler, et al. 2010).

Recommendation:

PCR can provide useful supporting information, but multiple available assays must be properly standardised, and are hampered by sample timing, type and quality.

Next Generation Sequencing

NGS offers huge potential and data has only recently been forthcoming limiting comprehensive appraisal at this stage. With the exception of dermato-borreliosis, here the challenge is which diagnostic sample type to investigate for LB in the absence of focal lesions. Sensitivity can be further improved, especially amongst high levels of host DNA. Care should be taken to avoid bias when using target enhancement strategies to amplify low copy number targets. Data analysis represents an additional computational challenge. NGS methods combined with bioinformatics tools might overcome the limitations of culture-connected techniques or of some molecular protocols. However, the extreme diversity of spirochetes from *B. burgdorferi* sensu lato complex reduce the usefulness of NGS as it doesn't differentiate between the pathogenic to human spirochete strains from those that were never connected with human LB. Additionally this offers a means of assessing rank abundance, evolving genomic profiles such as those corresponding to vector adaptations (Gatzmann, et al. 2015) and fluctuations over time providing valuable insights into host-microbial interactions (Strandh and Råberg 2015).

To date enrichment techniques can only partially overcome sensitivity problems caused by the giant excess of host DNA (vector, endosymbiont and other microbial DNA) compared to the low proportion of target DNA (borrelial DNA in ticks is <0.01% of total DNA within field-collected nymphal ticks) (Carpi, et al. 2015). This can impact

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upon successful detection with only about a third of infected ticks revealing positive *Borrelia* NGS data (Carpi, et al. 2015).

Recommendations:

NGS offers huge potential and data has only recently been forthcoming limiting comprehensive appraisal at this stage. Sensitivity can be further improved, especially amongst high levels of host DNA. Care should be taken to avoid bias when using target enhancement strategies to amplify low copy number targets. Data analysis represents an additional computational challenge.

Fact sheets and resources

Several excellent fact sheets have been produced by ECDC to provide information on LB and tick-borne relapsing fever. Furthermore, more specific resources can be obtained from European study group for Lyme borreliosis (ESGBOR; www.escmid.org/research_projects/study_groups/esgbor/).

Knowledge gaps and future perspectives

The poor sensitivity of direct detection methods coupled with the poor predictive value of indirect serological methods, particularly in less typical clinical presentations, presents a significant diagnostic challenge. Serology is further challenged by the requirement for sufficient time in order for the host to produce antibody responses to enable detection. Detection of the host response to infections provides a particularly attractive prospect for LB where organism loads are typically low. Indeed, levels of CXCL13 have shown promise for neuroborreliosis, but require

further validation (Schmidt, et al. 2011,Senel, et al. 2010). It is possible that signature biomarker profiles might have value, but whether this would vary too much between individuals or indeed with differing genetic variants of borreliae awaits investigation. Another diagnostic approach under exploration is based on targeted proteomics. By selected reaction monitoring mass spectrometry, specific *Borrelia* proteins can be detected and quantified in skin biopsies (Schnell, et al. 2015). The powerful new emerging technologies provide insights into our understanding of the dynamic interactions of borreliae with their vector, host and other organisms, with the possibility of disclosing opportunities for future intervention.

Concluding remarks

During these brief guidelines, we have attempted to highlight the strengths and limitations of various diagnostic methods used to diagnose borrelial infection. No single approach is suitably robust for this purpose, thus making interpretation challenging. Furthermore, laboratory diagnostics need to be viewed in conjunction with potential exposure and compatible clinical features.

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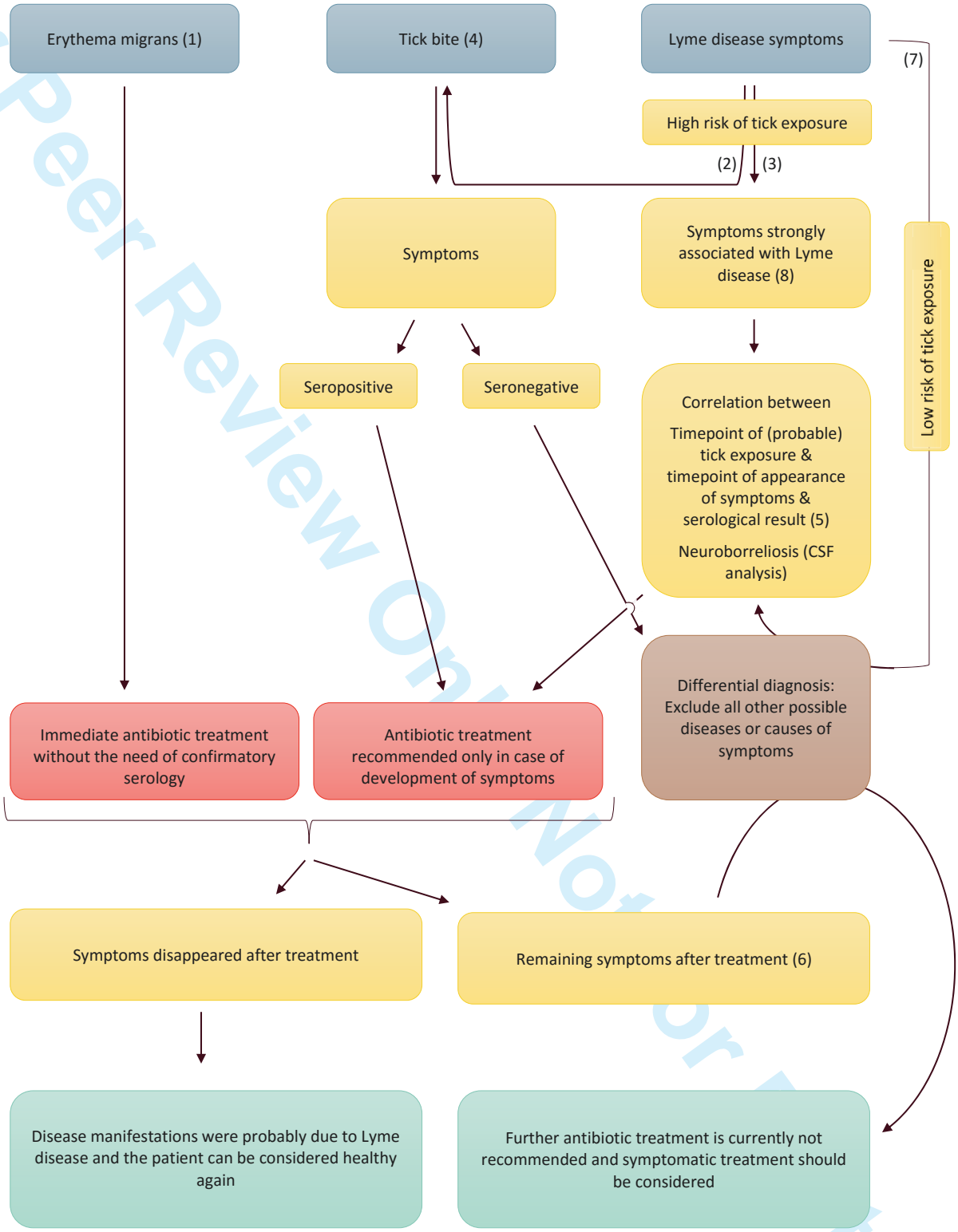

Figure 1. No diagnostic tests currently provide a yes or no result for acute Lyme borreliosis (LB) exist, thus clinical signs still remain the major factor for deciding whether antibiotic treatment is necessary. In case of unclear symptoms, the risk of tick exposure and serological tests should be considered to support the diagnosis. Represented in blue are three possible scenarios for which LB should be considered: the patient presents with the characteristic skin manifestation erythema migrans (EM) or a recent tick bite. A third possibility is that the patient's symptoms might be compatable with LB. As can be readily deduced from this schematic representation (yellow: clinical decision), erythema migrans is the least complicated case and should be treated (red) immediately without need for further testing. The situation gets more complicated if the patient cannot remember a tick bite (which can occur in up to 2/3 of cases (Hofhuis, et al. 2013)) and/or has nonspecific symptoms. Green: Final outcome. **(1)** EM: Incubation time between 3 days and 1 month. Red skin lesion that might in some cases be associated with slight itching or burning and that expands around the site of the tick bite. EM can be distinguished from a simple tick bite induced irritation of the skin by the fact that it has a minimum diameter of 5cm. Erythema migrans is often associated with non-specific symptoms like fatigue, headache, fever or malaise and can occur at different locations on the same patient (multiple erythema migrans) (Godar , et al. 2015) **(2)** In case a patient presents with symptoms that have been associated with, but are not clearly specific for Lyme disease, an assessment of the risk of prior tick exposure should be done. For this purpose the following questions might be considered: Does the patient pay attention to ticks? Did the patient maybe notice in the recent past an itching and scratched something small off from his body? Does the patient have pets which often have ticks? How much time does the patient spend outdoors in the

green? Has the patient recently been on holidays in a risk area? Season or weather conditions supporting high activity of ticks (might also be interesting to exclude other possble infections)? **(3)** Try to estimate based on the symptoms (early or late stage) the timepoint of infection and check if the season and/or weather conditions have been such that at the possible timepoint of infection ticks might have been active. Ticks are active during wet not too hot seasons of the year. For more information on factors affecting tick activity please refer to reference: (Medlock, et al. 2013). **(4)** If a patient shows up with a tick bite, appropriate and early removal of the tick can prevent transmission of Lyme disease, however since the transmission efficiency and kinetics depends on the *Borrelia* strain (Crippa , et al. 2002), an early tramsmission cannot reliably be excluded (Kahl , et al. 1998) and the patient should be monitored for the development of symptoms and treatment considered only if such appear. In case the tick has been damaged or removed late, a short-term prophylactic antibiotics (oral or cutaneous) treatment might be considered (Warshafsky, et al. 2010) (Piesman and Hojgaard 2012) (Piesman, et al. 2014). However due to the small time period during which this method is efficacious and due to the high number of patients that need to be treated for a successful outcome (Hofhuis , et al. 2013) controversial opinions exist on this procedure. **(5)** Please consider here the fact that patients are not necessarily protected after a first course of Lyme disease and re-infection can occur (Shapiro 2015) (Nadelman and Wormser 2007) (Khatchikian , et al. 2014). In this case the interpretation of serological results might be complicated. **(6)** In case of persistent fluelike symptoms after appropriate treatment of erythema migrans, consider coinfections with other tick borne pathogens (Godar , et al. 2015). Make sure that treatment has been done in the correct way otherwise consider retreatment with appropriate method. In case of a

post treatment chronic course of Lyme disease other possible reasons for the symptoms should be excluded. **(7)** Make sure that the symptoms have only occurred after potential exposure to a tick bite and that they did not already exist before the tick exposure. In case of nonspecific disease manifestations, ask the patient if he might recall symptoms similar to erythema migrans in the past. **(8)** To have a better overview of the symptoms that are frequently associated with Lyme disease consult for example (Stanek , et al. 2012, Koedel , et al. 2015).