Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease

Supplement Materials

Literature Search Strategy

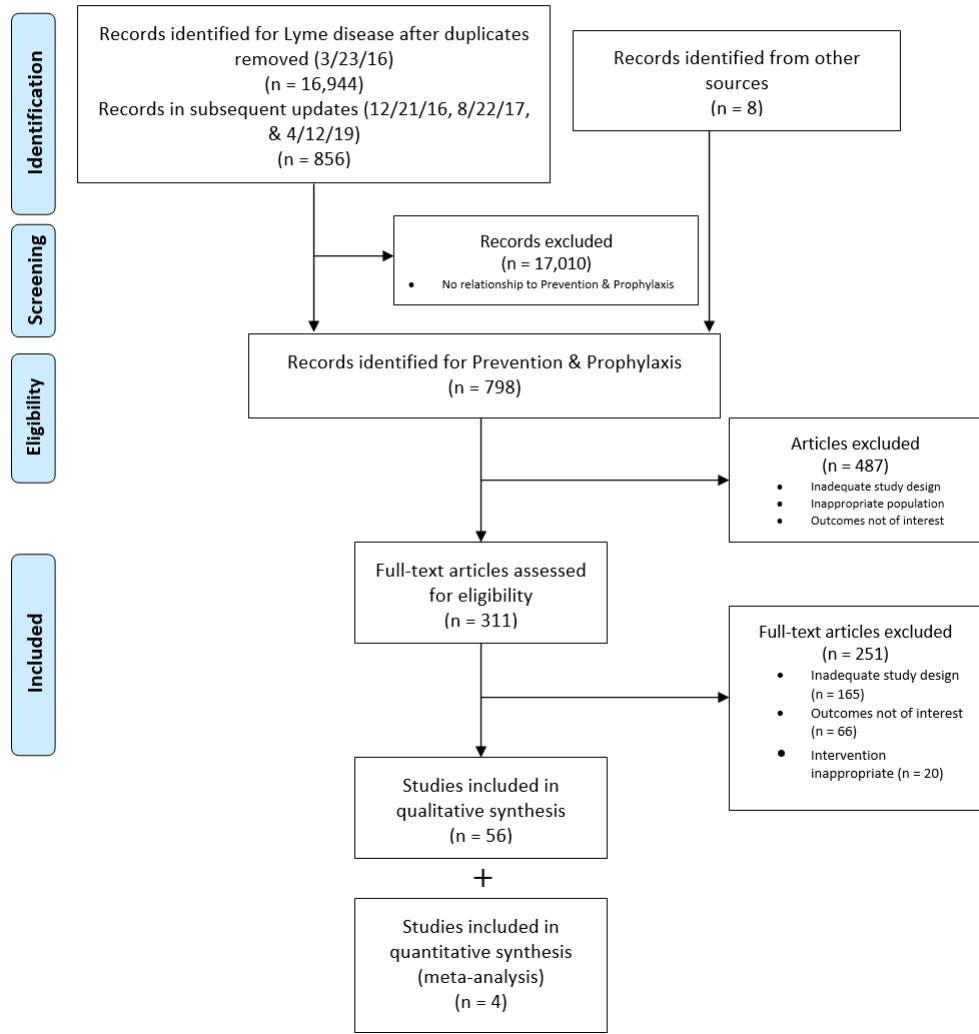1. borrelial lymphocytoma$.mp.
2. acrodermatitis chronicum atrophicans.mp.
3. STARI.mp.
4. southern tick-associated rash illness.mp.
5. exp Borrelia Infections/
6. post lyme disease syndrome.mp.
7. erythema migrans.mp.
8. exp Borrelia/
9. borrel$.mp.
10. lyme disease$.mp.
11. neuroborreliosis.mp.
12. neuro$.mp.
13. acrodermatitis.mp.
14. exp Acrodermatitis/
15. carditis.mp.
16. arthrit$.mp.
17. meningitis.mp.
18. exp Meningitis/
19. radiculoneuropath$.mp.
20. cranial neuropath$.mp.
21. exp Cranial Nerve Diseases/
22. lyme.mp.
23. (11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21) and 22
24. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 23
25. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 22 or 23
26. limit 25 to humans
27. limit 25 to animals
28. 26 or 27
29. 25 not 28
30. 26 or 29
31. remove duplicates from 30

PRISMA Flow Diagram
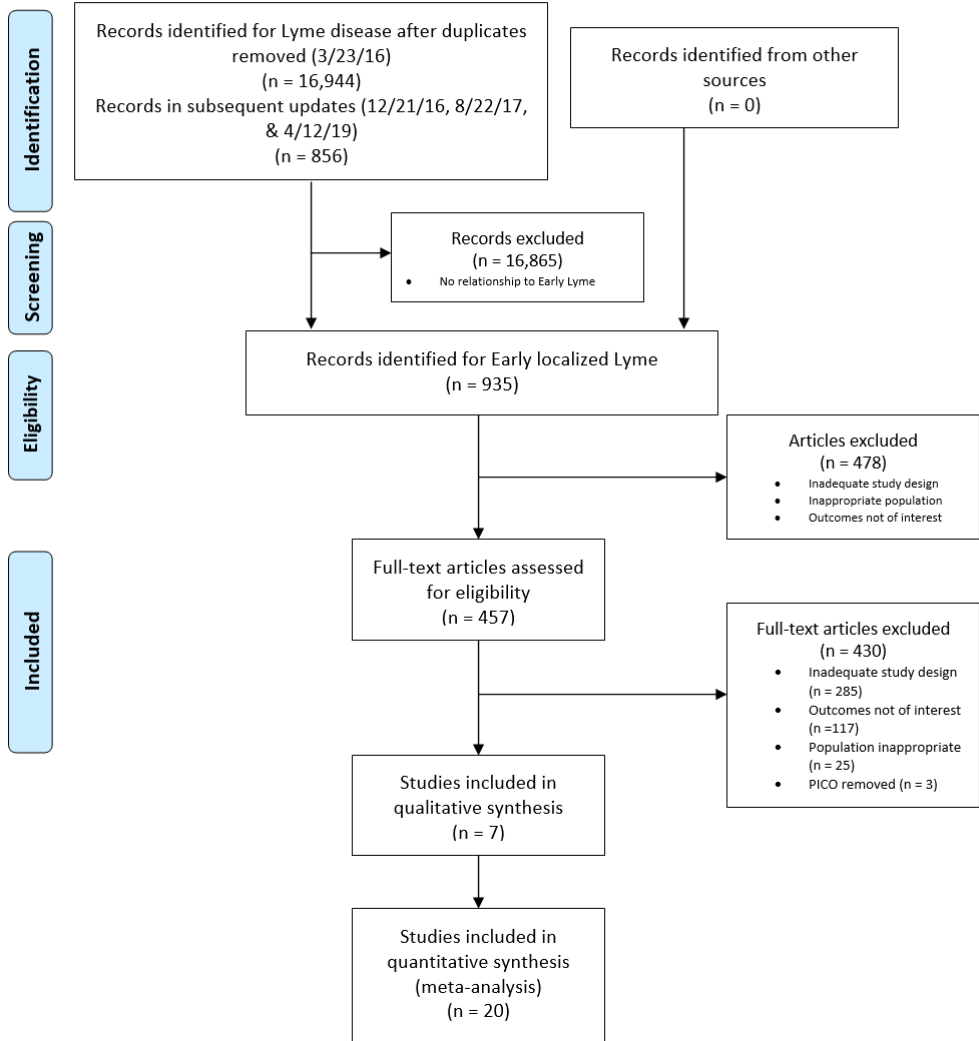**Diagnostic Testing of Lyme disease**

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Records excluded
(n = 14,945)
- No relationship to Diagnostic testing

Screening

Records identified for Diagnostic Testing
(n = 2,855)

Articles excluded
(n = 1,337)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Eligibility

Full-text articles assessed for eligibility
(n = 1,518)

Full-text articles excluded
(n = 1,299)
- Inadequate study design
- Outcomes not of interest

Included

Studies included in qualitative synthesis
(n = 219)

Studies included in quantitative synthesis (meta-analysis)
(n = 0)

3

**PRISMA Flow Diagram
Tick bites prevention and
prophylaxis of Lyme disease**

*(Diagnostic references for this section
are included in the PRISMA Flow
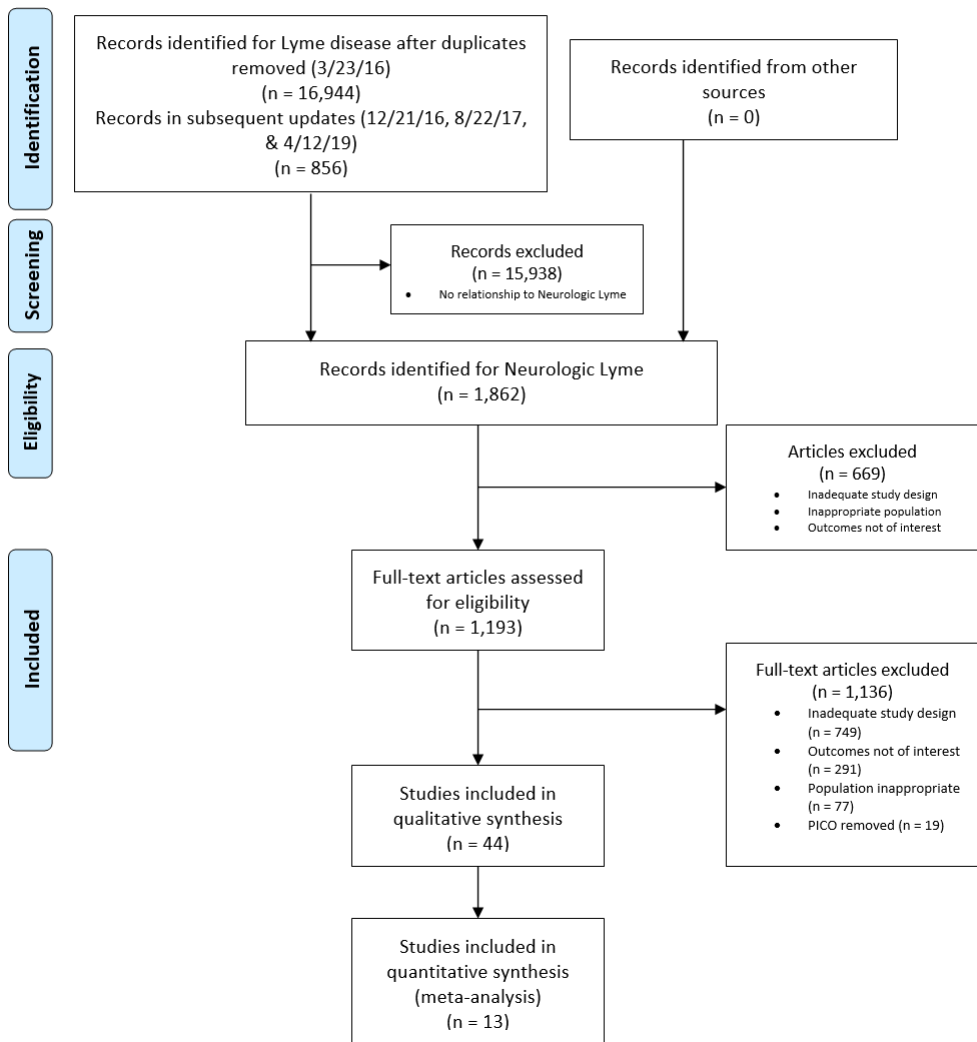Diagram for Diagnostic Testing)*

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 8)

Screening

Records excluded
(n = 17,010)
- No relationship to Prevention & Prophylaxis

Eligibility

Records identified for Prevention & Prophylaxis
(n = 798)

Articles excluded
(n = 487)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed for eligibility
(n = 311)

Full-text articles excluded
(n = 251)
- Inadequate study design (n = 165)
- Outcomes not of interest (n = 66)
- Intervention inappropriate (n = 20)

Included

Studies included in qualitative synthesis
(n = 56)

+

Studies included in quantitative synthesis (meta-analysis)
(n = 4)

(Diagnostic references for this section are included in the PRISMA Flow Diagram for Diagnostic Testing)

PRISMA Flow Diagram
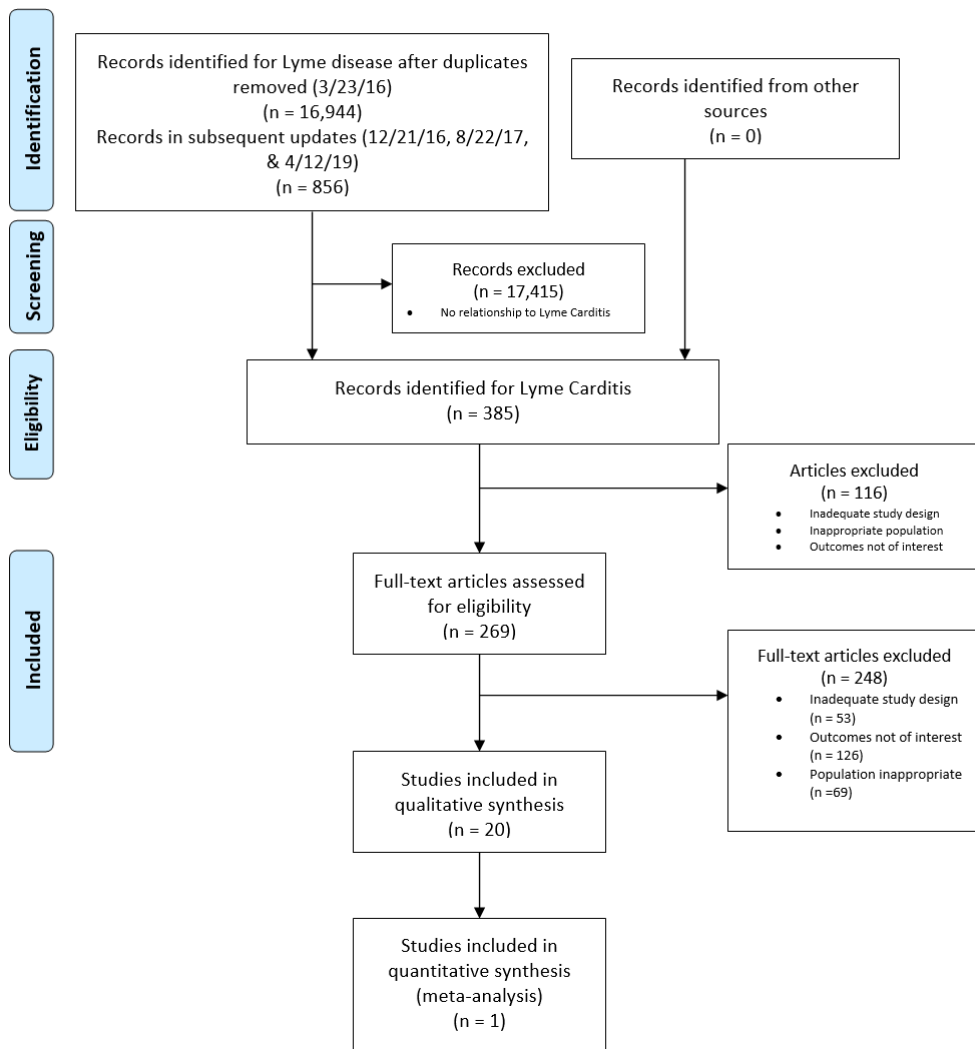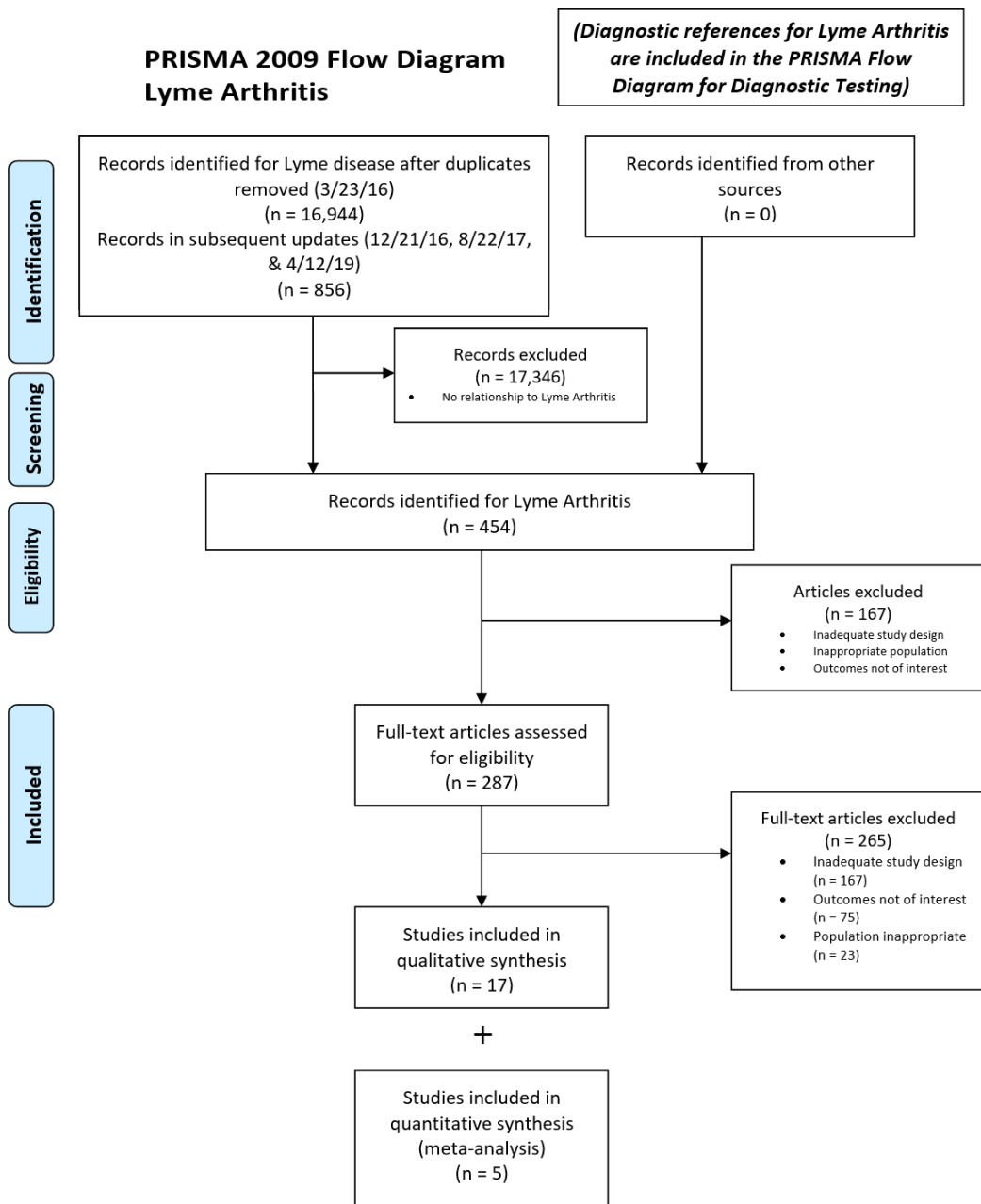**Early localized Lyme disease**
(erythema migrans)

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Screening

Records excluded
(n = 16,865)
• No relationship to Early Lyme

Records identified for Early localized Lyme
(n = 935)

Eligibility

Articles excluded
(n = 478)
• Inadequate study design
• Inappropriate population
• Outcomes not of interest

Full-text articles assessed for eligibility
(n = 457)

Included

Full-text articles excluded
(n = 430)
• Inadequate study design (n = 285)
• Outcomes not of interest (n =117)
• Population inappropriate (n = 25)
• PICO removed (n = 3)

Studies included in qualitative synthesis
(n = 7)

Studies included in quantitative synthesis (meta-analysis)
(n = 20)

5

**PRISMA Flow Diagram
Neurologic Lyme disease**

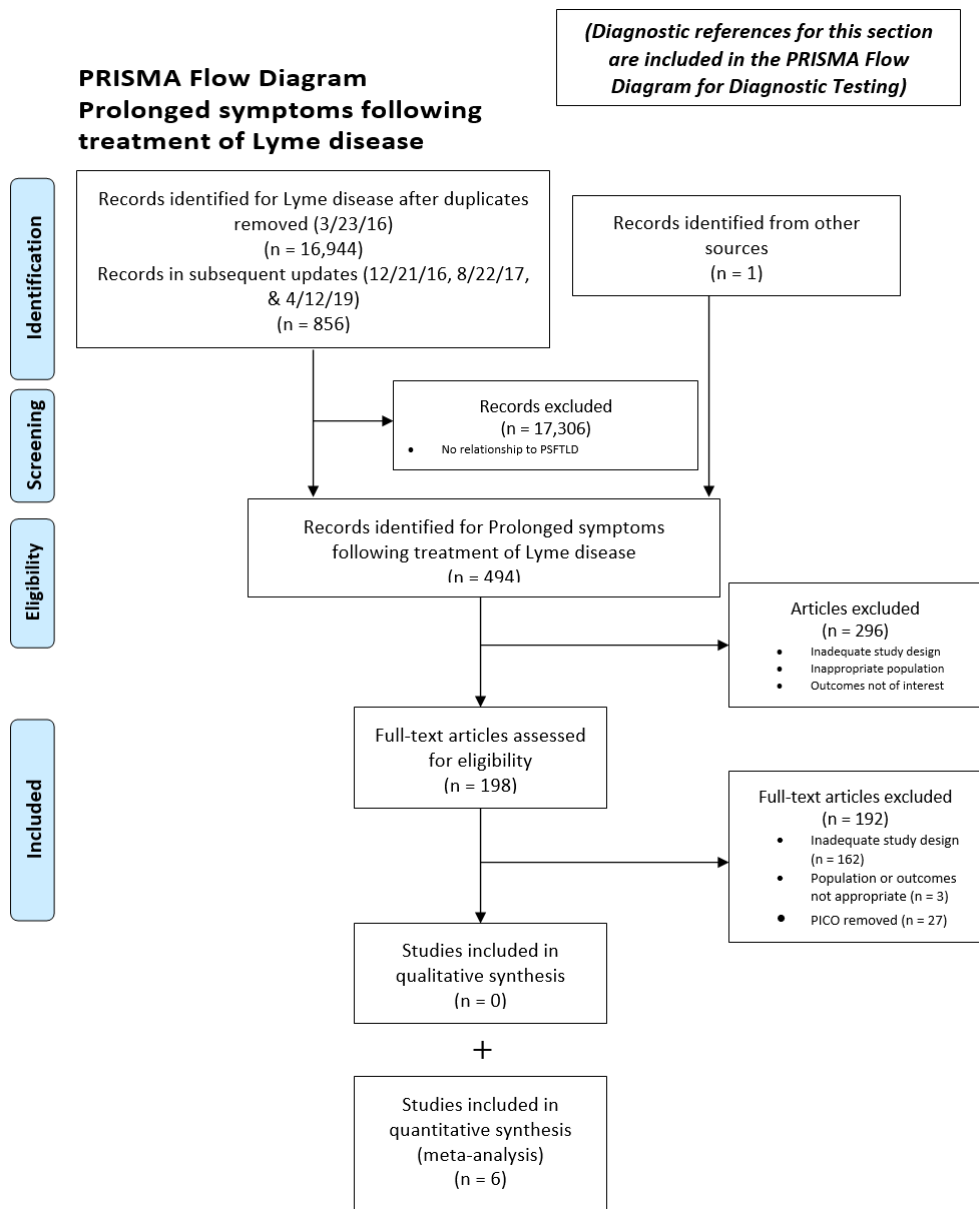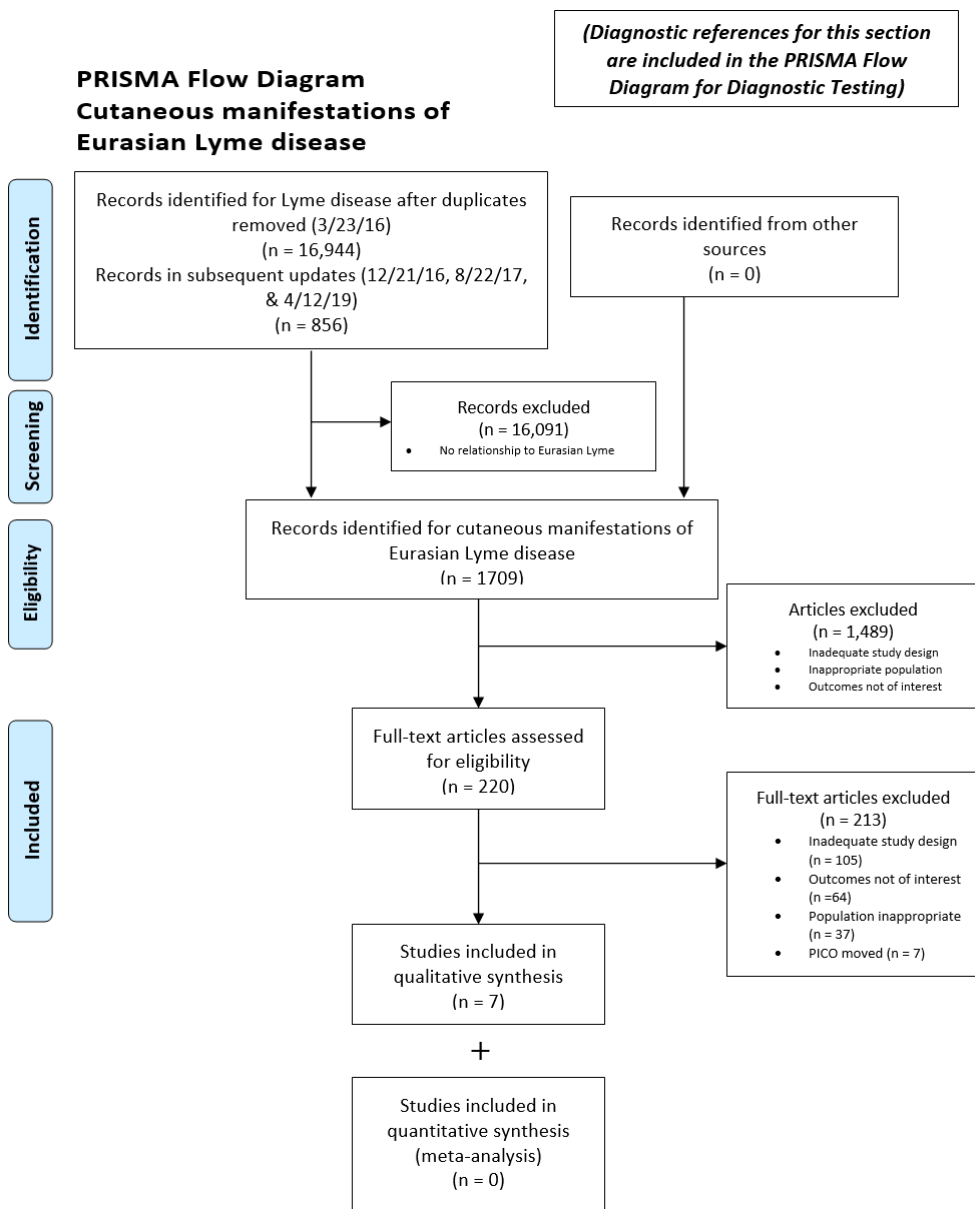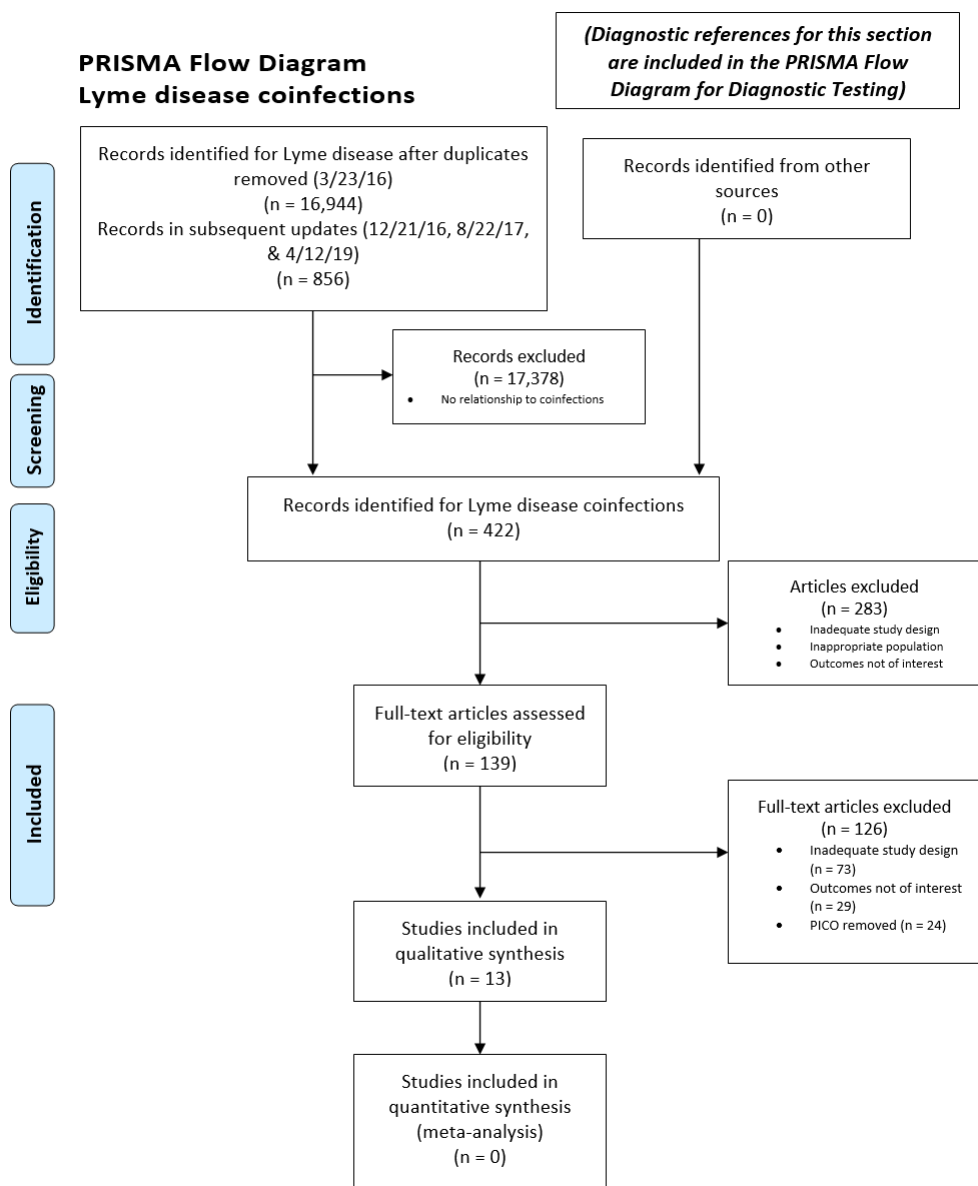*(Diagnostic references for this section are included in the PRISMA Flow Diagram for Diagnostic Testing)*

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Screening

Records excluded
(n = 15,938)
- No relationship to Neurologic Lyme

Eligibility

Records identified for Neurologic Lyme
(n = 1,862)

Articles excluded
(n = 669)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed for eligibility
(n = 1,193)

Full-text articles excluded
(n = 1,136)
- Inadequate study design (n = 749)
- Outcomes not of interest (n = 291)
- Population inappropriate (n = 77)
- PICO removed (n = 19)

Included

Studies included in qualitative synthesis
(n = 44)

Studies included in quantitative synthesis (meta-analysis)
(n = 13)

6

**PRISMA Flow Diagram
Lyme Carditis**

(Diagnostic references for this section
are included in the PRISMA Flow
Diagram for Diagnostic Testing)

Identification

Records identified for Lyme disease after duplicates
removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17,
& 4/12/19)
(n = 856)

Records identified from other
sources
(n = 0)

Screening

Records excluded
(n = 17,415)
- No relationship to Lyme Carditis

Eligibility

Records identified for Lyme Carditis
(n = 385)

Articles excluded
(n = 116)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed
for eligibility
(n = 269)

Full-text articles excluded
(n = 248)
- Inadequate study design
(n = 53)
- Outcomes not of interest
(n = 126)
- Population inappropriate
(n =69)

Included

Studies included in
qualitative synthesis
(n = 20)

Studies included in
quantitative synthesis
(meta-analysis)
(n = 1)

PRISMA 2009 Flow Diagram
Lyme Arthritis

(Diagnostic references for Lyme Arthritis are included in the PRISMA Flow Diagram for Diagnostic Testing)

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Records excluded
(n = 17,346)
- No relationship to Lyme Arthritis

Screening

Records identified for Lyme Arthritis
(n = 454)

Eligibility

Articles excluded
(n = 167)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed for eligibility
(n = 287)

Full-text articles excluded
(n = 265)
- Inadequate study design (n = 167)
- Outcomes not of interest (n = 75)
- Population inappropriate (n = 23)

Included

Studies included in qualitative synthesis
(n = 17)

+

Studies included in quantitative synthesis (meta-analysis)
(n = 5)

9

**PRISMA Flow Diagram
Prolonged symptoms following
treatment of Lyme disease**

*(Diagnostic references for this section
are included in the PRISMA Flow
Diagram for Diagnostic Testing)*

Identification

Records identified for Lyme disease after duplicates
removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17,
& 4/12/19)
(n = 856)

Records identified from other
sources
(n = 1)

Screening

Records excluded
(n = 17,306)
- No relationship to PSFTLD

Eligibility

Records identified for Prolonged symptoms
following treatment of Lyme disease
(n = 494)

Articles excluded
(n = 296)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Included

Full-text articles assessed
for eligibility
(n = 198)

Full-text articles excluded
(n = 192)
- Inadequate study design
  (n = 162)
- Population or outcomes
  not appropriate (n = 3)
- PICO removed (n = 27)

Studies included in
qualitative synthesis
(n = 0)

+

Studies included in
quantitative synthesis
(meta-analysis)
(n = 6)

**PRISMA Flow Diagram
Cutaneous manifestations of
Eurasian Lyme disease**

(Diagnostic references for this section are included in the PRISMA Flow Diagram for Diagnostic Testing)

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Screening

Records excluded
(n = 16,091)
- No relationship to Eurasian Lyme

Eligibility

Records identified for cutaneous manifestations of Eurasian Lyme disease
(n = 1709)

Articles excluded
(n = 1,489)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed for eligibility
(n = 220)

Included

Full-text articles excluded
(n = 213)
- Inadequate study design (n = 105)
- Outcomes not of interest (n =64)
- Population inappropriate (n = 37)
- PICO moved (n = 7)

Studies included in qualitative synthesis
(n = 7)

+

Studies included in quantitative synthesis (meta-analysis)
(n = 0)

PRISMA Flow Diagram
Lyme disease coinfections

(Diagnostic references for this section are included in the PRISMA Flow Diagram for Diagnostic Testing)

Identification

Records identified for Lyme disease after duplicates removed (3/23/16)
(n = 16,944)
Records in subsequent updates (12/21/16, 8/22/17, & 4/12/19)
(n = 856)

Records identified from other sources
(n = 0)

Screening

Records excluded
(n = 17,378)
- No relationship to coinfections

Records identified for Lyme disease coinfections
(n = 422)

Eligibility

Articles excluded
(n = 283)
- Inadequate study design
- Inappropriate population
- Outcomes not of interest

Full-text articles assessed for eligibility
(n = 139)

Included

Full-text articles excluded
(n = 126)
- Inadequate study design (n = 73)
- Outcomes not of interest (n = 29)
- PICO removed (n = 24)

Studies included in qualitative synthesis
(n = 13)

Studies included in quantitative synthesis (meta-analysis)
(n = 0)

Tick bites prevention and prophylaxis of Lyme disease

I. Which measures should be used to prevent tick bites and tick-borne infections?

A) Personal protective measures

Protective clothing, tick checks, bathing, drying clothing, and limiting pet exposure

Bibliography: 1. Stjernberg, et al. *Scand J Infect Dis.* 2005;37(5):361-4; 2. Ley, et al. *Am J Epidemiol.* 1995 Nov 1;142(9 Suppl):S39-47; 3. Klein, et al. *Clin Pediatr.* 1996 Jul;35(7):359-63; 4. Orloski, et al. *Am J Epidemiol.* 1998 Feb 15;147(4):391-7; 5. Vazquez, et al. *Emerg Infect Dis.* 2008 Feb;14(2):210-6 ; 6. Connally, et al. *Am J Prev Med*. 2009 Sep;37(3):201-6; 7. Carroll, et al. *J Med Entomol.* 2003 Sep;40(5):732-6; 8. Nelson, et al. *Ticks Tick Borne Dis*. 2016 Jul;7(5):958-963.

Study; Location	Study Design	Risk of bias*	Tick Type	Population Characteristics	Interventions and Comparisons	Outcomes	Results and Conclusions
Stjernberg, 2005 Sweden	Randomized study with cross-over design	Unclear risk of bias	*I. ricinus*	10 participants (5M, 5F), exposed by walking in tick endemic areas.	1. The participants wore alternately **light clothing or dark clothing** before every new exposure (6 exposures for each clothing type per participant; 3.5 minutes per exposure). 2. Differences in tick detection were tested by placing random N of ticks (unknown by both the exposed participant and the searchers) on the participant wearing light vs. dark clothing	**1. Mean N of adult and nymphal ticks collected** from each type of clothing after exposure; **2. Differences in tick detection** (% of ticks detected on each type of clothing)	1. Participants had **significantly more ticks on light than on dark clothing**. Mean N of ticks was 54.7 (SD 18.1) vs. 33.9 (SD 9.2) for light vs. dark groups, P = 0.003 2. There was **no difference in tick detection** on light (91%) vs. dark (93%) clothing Thus, **dark clothing seemed to attract fewer ticks with no disadvantage with regard to tick detection**
Ley, 1995 CA, USA	Case-control study of risk factors for incident Lyme disease	6	NA	101 cases with Lyme disease (EM). Each case was age-, sex-, and location-matched with a control. Subjects were interviewed by telephone using a questionnaire on activities during the	Questionnaire evaluated: location of home; presence of wildlife around the house; hours of outdoor work and outdoor leisure activities; knowledge about Lyme disease; **pet ownership;** and **personal protective measures such as protective clothing** (e.g. light-colored clothing, long pants	**Odds ratios of acquiring Lyme disease** (identified as a case of Erythema migrans) for each risk factor.	The only activity associated with Lyme disease was the use for more than 5 hours per week of wide maintained trails (OR = 11.33,95% CI 1.33-123.5); this association occurred only in persons with other outdoor leisure activities. **No other behaviors or activities were identified as risk factors for**

				month prior to the case onset of EM.	and sleeves, and tucking socks into pants), **tick checks,** and **tick removal methods**		**acquisition of Lyme disease in California**.
Klein, 1996 CT, DE, MD, NJ, PA states; USA	Case-control study of risk factors for incident Lyme disease in children	6	NA	44 pediatric cases with LD from the Lyme Clinic population were age- and sex-matched 1:1 to controls from the same neighborhood. Site visits were performed to assess environmental variables; parents were interviewed using a questionnaire. Controls were tested for Lyme disease (EIA and Western immunoblot).	Questionnaire evaluated: the amount of time children spent outdoors, play activities and household chores engaged in by the child, the animals identified on the families' property, **owning pets**, **frequency of bathing**, etc., and the use of **personal protective measures** such as tick checks and **protective clothing (long pants and shirts, socks)**	**Odds ratios of acquiring Lyme disease** were calculated	Significant associations with Lyme disease were found for deer ticks in the home environment, ground cover containing moist humus, and leaf litter in the yard. **There was no correlation with Lyme disease for the use of any anti-tick measures or for any childhood activities.**
Orloski, 1998 NJ, USA	Case-control study of risk factors for incident Lyme disease	6	NA	51 cases with documented EM in 1993 in Hunterdon County, NJ were age-matched with 51 controls. Subjects were interviewed by telephone using a questionnaire on activities during the summer of 1993 and on other possible risk factors. Blood samples were tested for Lyme disease (EIA and Western immunoblot).	Questionnaire evaluated: clinical details of the illness (cases only); the characteristics of residential property; the frequency of observing deer on the residential property; outdoor activities; **cat ownership**; and **personal protective measures**, such as use of repellent; use of **protective clothing** (long pants; tucking pants into socks, light-colored clothing), and **tick checks.**	**Odds ratios of acquiring Lyme disease** (identified as a case of erythema migrans); univariate and multivariate (for variables with $p \leq 0.10$) conditional logistic regressions were performed	Rural residence; clearing peri-residential brush during spring and summer months; and the presence of rock walls, woods, deer, or a bird feeder on residential property were associated with incident Lyme disease. **Higher proportion of controls than of cases performed regular tick checks, but the difference was not significant. None of the other personal protective measures showed a statistically significant effect on incident Lyme disease.** Cat ownership also had no effect on the incidence of Lyme disease.
Vazquez, 2008 CT, USA	Case-control study of risk factors for incident	7	NA	709 cases with LD reported to Connecticut's Dep. of Public Health and classified as having	Questionnaire evaluated: clinical features of LD (cases); demographics; occupational (forestry or landscaping) and	**Odds ratios of acquiring Lyme disease** (adjusted for possible confounders, i.e. sex, race, receipt of Lyme	Definite and possible **case-patients were less likely than controls to report using protective clothing outdoors** (OR 0.6, effectiveness 40%,

	Lyme disease			definite (66%), possible (15%), or unlikely (19%) LD. Each case was age- and location-matched with 2 controls. Subjects were interviewed by telephone using a questionnaire.	recreational risk factors (camping or other outdoor activities); and **personal protective measures:** use of tick repellents on the skin or clothing while outdoors; spraying one's property with acaricides; use of **protective clothing** such as long pants, long-sleeved shirts, and light-colored clothing; and **tick checks.**	vaccine, and use of other personal protective measures); **Effectiveness** was calculated as [1 - the matched OR]	p<0.0001) and to use tick repellents on their skin or clothing (OR 0.8, effectiveness 20%, p = 0.05). **Checking one's body for ticks** was not effective.
Connally, 2009 CT, USA	Case-control study of risk factors for incident Lyme disease	7	NA	349 cases with Lyme disease (EM) reported to Connecticut's Dep. of Public Health were age- and neighborhood-matched with 1:1 control. Subjects were interviewed by telephone using a questionnaire on disease-prevention measures during the month prior to the case onset of erythema migrans.	**Personal protective measures:** **checking for ticks**; **bathing** within 2 hours after spending time in the yard; wearing repellent or permethrin-treated clothes; landscape features/ modifications, such as fencing, leaf litter cleaning, etc.; and use of **protective clothing** such as long pants and/or light-colored clothes, tucking pants into socks (few cases→ not incl. in final analysis);	**Odds ratios of acquiring Lyme disease** (identified as a case of Erythema migrans) for each activity	**Checking for ticks within 36 hours of spending time in the yard at home was protective** against Lyme disease (OR 0.55; 95% CI 0.32, 0.94). **Bathing within 2 hours after spending time in the yard was also protective** (OR 0.42; 95% CI 0.23, 0.78). No other measures were significantly protective against Lyme disease.
Carroll, 2003	A life-simulating study where tick nymphs were subjected to laundry and drying in a dryer in different settings	NA	*I. scapularis, A. americanum*	Host-seeking tick nymphs who were placed within polyester mesh packets and included into laundry and drying cycles	(1) Automatic washer's laundry cycles: hot vs. warm vs. cold (2) Automatic clothes dryer's settings: high heat vs. no heat for 1 hour (3) Different detergents: "Clout" powdered detergent (Costco); "Tide" powder with a non-chlorine bleach (Proctor and Gamble); "Heavy Duty Ultra" liquid (Rite Aid)	**%% of live, dead, and moribund ticks**	Most nymphs (~90%) of both spp. survived the cold and warm washes, and 95% of *A. americanum* nymphs survived the hot wash. All ticks were killed by the 1h dryer cycle at high heat, but with unheated air some nymphs of both species survived.

| Nelson, 2016 | Study in ticks: ticks were subjected to washing and drying in different settings. "Dryer only" tests were also conducted. | NA | *I. scapularis* | Laboratory-reared, uninfected, unfed nymphs that were 30–60 days post-molt (when they are in their prime and most likely to bite) were placed in muslin cloth bags. 5 bags containing 5 ticks each were placed in each wash/dry cycle.

10-20 ticks were secured in petri dishes with a piece of moist paper towel during each round of testing as controls. | (1) Effects of automatic washer's laundry cycles: hot vs. warm vs. cold
(2) Effects of automatic dryer's settings: low vs. high heat for 20-70 min.
(3) Effects of detergent & dryer sheets versus none: "Tide" original liquid detergent;   "Bounce" dryer sheets
(4) Effects of clothing type: thin clothing (polyester, rayon, nylon) vs. thick clothing (fleece) | **Tick mortality** | All control ticks survived for 20-24 hours. 98% of the ticks subjected to "fluff cycle" in the dryer (no heat) survived.

All nymphal and adult ticks survived washing with cold water, and most ticks survived washing with warm water. Washing with hot water killed the ticks when the temperature of the water exceeded 54°C.

It took 70 minutes to kill all nymphs and adults on low heat in the dryer, versus 50 minutes on high heat.

In "Dryer only" tests, all ticks were killed when dried with dry towels on low heat for 6 min (nymphs) and 7 min (adults). All ticks were killed when dried at high heat for 4 minutes.

Neither use of detergent or dryer sheets nor thickness of clothing impacted tick survival. |

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk".
Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

B) Repellents to prevent tick bites

I. Permethrin-treated clothing

Bibliography: 1. Vaughn, et al. *Am J Prev Med*. 2014 May;46(5):473-80; 2. Wallace, et al. *Vector Borne Zoonotic Dis*. 2016 May;16(5):302-8; 3. Ho-Pun-Cheung et al. *Bull Soc Pathol Exot*. 1999 Dec;92(5):337-40; 4. Faulde, et al. *Parasitol Res*. 2015 Feb;114(2):671-8; 5. Kegel, et al. *Occup Environ Med*. 2014 Feb;71(2):112-7; 6. Evans, et al. *J Med Entomol*. 1990 Sep;27(5):829-34.

Study; Location	Study Design	Risk of bias*	Tick Type	Population Characteristics	Interventions and Comparisons	Outcomes	Results and Conclusions

Vaughn, 2014 NC, USA	Double-blind RCT	Low risk of bias	*A. americanum >90%*	159 outdoor workers whose work uniforms were sent to a facility for permethrin/ sham treatment according to participant allocation. Subjects kept weekly tick bite logs. Subjects were instructed to launder their clothing as usual. They were followed up over two tick seasons (March - September 2011 and 2012).	• Long-lasting permethrin impregnated uniforms (LLPIU) • Control uniforms that received a sham treatment	Incidence of work-related tick bites (ITB) reported on weekly tick bite logs; **Protective effectiveness** ([ITB for sham – ITB for LLPIU]/ITB for sham*100) and 95% CIs were calculated	**The protective effectiveness** of LLPIU for the prevention of work-related tick bites was **0.82 (95% CI 0.66, 0.91) and 0.34 (95% CI -0.67, 0.74) for the 1ˢᵗ and 2ⁿᵈ years** of follow-up (LLPIUs were highly effective for at least 1 year in deterring tick bites in the context of typical tick bite prevention measures employed by outdoor workers). There were **no AEs reported** related to the subjects' uniforms. Five subjects reported **illnesses suspected to be tick-related**, two were confirmed (one case of ehrlichiosis and one case of spotted fever rickettsiosis), both among subjects in the control group.
Wallace, 2016 NC, USA	Double-blind RCT (follow-up Lyme disease-related **results of Vaughn, 2014**)	Low risk of bias	*A. americanum >90%*	This is a follow-up for Vaughn 2014 study. Outdoor workers from that study (N=159) were followed for 2 years for seroconversion to tick-transmitted pathogens. Seroconversions were assessed for any worker with paired sera available (**N = 90**). **Incident infection** was defined as a fourfold increase in IgG titer over a 1-year period.	• Long-lasting permethrin impregnated uniforms (LLPIU) • Control uniforms that received a sham treatment	Antibody titers against *Rickettsia parkeri, Rickettsia rickettsii, Rickettsia amblyommii,* and *Ehrlichia chaffeensis* were measured at baseline (n = 130), after 1 year (n = 82), and after 2 years (n = 73). **Antibody titers against *Borrelia burgdorferi*** (by C6 ELISA) were measured at baseline and after 2 years (**n = 90**).	There were 40 total seroconversions to at least one pathogen, including *R. parkeri* (n = 19), *R. amblyommii* (n = 14), *R. rickettsii* (n = 9), and *E. chaffeensis* (n = 8); 38 of the 40 incident infections were subclinical (there was 1 clinical case of spotted fever rickettsiosis and 1 clinical case of ehrlichiosis). **There were no subjects whose sera were reactive to *B. burgdorferi*** at any point of the study.
Ho-Pun-Cheung, 1999 France	Double-blind RCT	High risk of bias	*D. marginatus (predominantly), I. ricinus, D. reticulatus*	Soldiers wearing permethrin-treated (N=429) vs. untreated (N=424) battle dress uniforms (BDU) on military site. Follow-up lasted 3 months.	• Uniforms impregnated with permethrin cis/trans 25/75 vs.	**N of ticks collected** from permethrin-treated vs. untreated uniforms and **N of subjects on whom ticks were found**.	There was a significant difference in both the intensity (number of ticks per individual, P <0.0001) and prevalence (number of individuals with ticks, P < 0.001) of ticks on individuals between impregnated and non-impregnated uniforms.

					• Non-impregnated uniforms	Subjects were monitored for symptoms and those with attached ticks had blood tested on day 0 and day 90 for anti-borrelial and anti-rickettsial a/b.	
Faulde, 2015 Germany	Retrospective cohort study	5	*I. ricinus*	Soldiers (N=7151) wearing factory-treated, long-lasting permethrin-impregnated BDU (PIBDU) in 2010-2011, the first two years after PIBDU formal introduction vs. non-treated BDU worn in 2009, the year preceding PIBDU introduction.	• Permethrin impregnated polymer-coated BDU (PIBDU) worn in 2010 and 2011 • Non- impregnated BDU (NTBDU)	**Tick bite incidence** estimated via analysis of mandatory tick bite report forms required by the Bundeswehr Medical Service since 2009; **Protective effectiveness.**	In **2009,** tick bite incidence was **8.8 % per exposed person** when wearing NTBDUs only. **In 2010 and 2011,** annual tick bite incidence was **0.035 and 0.078 % per exposed person,** respectively. This corresponded into a **protective effectiveness of 99.6% and 98.6 % of PIBDU** in 2010 and 2011.
Kegel, 2014 Germany and Afghanistan (winter time)	Two separate prospective cohorts studying differences in uptake of permethrin in wearers of PIBDU and NTBDU	8	NA	Soldiers (n=549 in study 1 and 195 in study 2) wearing PIBDU in Afghanistan and Germany.	• Permethrin impregnated battle dress uniforms (PIBDU) vs. • Non- impregnated BDU (NTBDU)	**Permethrin metabolites in urine** samples at different time points.	Subjects of the Afghan and German **control groups had permethrin levels in the range of the German general population.** In contrast, subjects **using impregnated BDU daily had ~200-fold higher exposure levels.** Within this group, subjects located in Afghanistan (possibly due to longer uniform daily wearing time (16 vs. 10 hrs for Germany) and smokers (presumably induced by hand-mouth contact) had significantly higher exposure levels. A longer period of wearing the BDU was associated with lower metabolite levels, possibly due to an increasing number of launderings.
Evans, 1990 MD, USA	RCT; field trial	Unclear risk of bias	*A. americanum, D. variabilis, I. dammini*	Six volunteer test subjects clothed in either untreated, DEET-treated, permethrin-impregnated (PI), or permethrin-sprayed (PS) uniforms were exposed to field populations of ticks	• Permethrin-impregnated (PI) uniforms • Permethrin-sprayed (PS) (Permanone Tick Repellent once/48 hours)	N of ticks collected from the uniforms; **Protection efficiencies =** ([mean number of ticks for untreated — mean number of ticks for repellent-treated]/	**Permethrin,** applied as either a spray (0.5%), or as an impregnant (0.125%/cm2), **was more effective than DEET** in protecting individuals from tick bite. The mean numbers of ticks on **DEET-treated, PI, and PS uniforms were 60, 97, and 98% lower, respectively, than on untreated**

				during a series of 30-min field trials (with 15 min of exposure each time).	• DEET-treated military battle dress uniforms (33% lotion once a day, applied to clothing only) • Untreated uniforms (control)	[mean number of ticks for untreated]) x 100.	uniforms against all encountered life stages of ticks

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk".
Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies.

II. Other repellents

Bibliography: 1. Solberg, et al. *J Med Entomol.* 1995 Nov;32(6):870-5; 2. Staub, et al. *Wilderness Environ Med.* 2002 Spring;13(1):12-20; 3. Gardulf, et al. *J Med Entomol*. 2004 Nov;41(6):1064-7; 4. Carroll, SP. *J Med Entomol*. 2008 Jul;45(4):706-14; 5. Witting-Bissinger, et al., J Med Entomol. 2008 Sep; 45(5):891-8; 6. Carroll, et al. *J Med Entomol*. 2010 Jul;47(4):699-704; 7. Bissinger, et al., Med Vet Entomol. 2011 Jun; 25(2):217-26; 8. Jordan, et al. *J Med Entomol*. 2012 Jan;49(1):101-6; 9. Bissinger, et al. *Exp Appl Acarol*. 2014 Jan;62(1):105-13; 10. Buchel, et al. *Ticks Tick Borne Dis*. 2015 Jun;6(4):494-8.

Study; Location	Study Design	Risk of bias*	Tick Type	Population Characteristics	Interventions and Comparisons	Outcomes	Results and Conclusions
Solberg, 1995 NJ, USA	Randomized field tests in humans with cross-over	Unclear risk of bias	*A. americanum*	11 volunteers were randomized to treatments; the repellents were applied to 1 leg (repellent leg selected at random), and absolute ethanol was applied to the other leg (control). At 0, 2, 4, 5, and 6 h post-application, volunteers walked slowly through the test site for 30 min.	• **DEET 25%** • **AI3-37220 25% (Piperidine)** • **Control: absolute ethanol** applied to the other leg. A crossover experiment was employed, with each experimental unit (volunteer) receiving both treatments.	**Repellency (%) = [(c-t)/(c+t)]*100,** where *c* = mean N of ticks that remained on the control legs or traversed the treated area to the shorts, and *t* is the mean N of ticks that remained on the repellent-treated legs or that traversed the treated area successfully to the shorts during the 5-min test period.	**AI3-37220**, at 0.5 mg/cm2, **provided >90% repellency** against adult and nymphal ticks **over a 6-h test** period and showed **significantly better repellent efficacy than DEET**. **DEET**, at the same concentration, provided **85% repellency at 0 h** and **deteriorated to 55% repellency at 6 h.**
Staub, 2002 Switzerland	Double-blind RCT	Unclear risk of bias	Type of collected ticks not reported (I. ricinus?)	Forestry workers (N=276) and orienteers were randomized to treatment vs. placebo-sprays applied	• "Parapic –Tick – Repellent" **(DEET 15% + EBAAP 15%** (ethyl-	**% effectiveness** = 100 x (T(P) - T(R))/T(P), where T(P) and T(R) were the average **number of**	The average **number of attached ticks per hour** of exposure to wooded areas **differed significantly between the placebo and repellent** groups, 0.17 vs. 0.10 (P < .05).

				to clothes twice a day (8-hr shift). Afterwards, they collected ticks off clothes and filled out 10-day tick logs.	butylacetylaminopropionate) spray) vs. • **Placebo spray**	**acquired ticks per hour** spent in wooded areas for the repellent and placebo groups, respectively	A product **containing DEET (15%) plus EBAAP (15%) was 41% effective against *I. ricinus*** compared to the control group treated with solvent only (**moderate effectiveness**).
Gardulf, 2004 Sweden	Crossover RCT	Unclear risk of bias	*I. ricinus*	111 healthy, outdoor active adult volunteers. Subjects filled out a daily diary on outdoor activities and observed/attached ticks	• **Lemon eucalyptus** extract (Citriodiol) sprayed daily **on legs** vs. • **No repellents** Spray vs. no intervention for 2 wks→ crossover →another 2 wks	**N of observed, attached, or not yet attached ticks** + anatomical location of ticks found (daily diary records)	42 attached ticks were reported during the weeks when the Citriodiol spray was used, and 112 were reported when it was not. The number of ticks below the waist was 13/42 (31%) during the period when the spray was used and 73/112 (65%) when no spray was used (*P* <0.001). The **median number of reported attached ticks per person decreased from 1.5 (range, 0-9) to 0.5 (range, 0-2; *P* < 0.05) during the weeks when Citriodiol was used.**
Carroll, 2008 USA	Laboratory tests on human skin	Unclear risk of bias	*I. scapularis*	Nymphal, laboratory raised, disease-free ticks were used for the study. For the purposes of testing, 50 unused nymphs were placed in small, water-filled trays that prevented easy escape for each test. 10 human subjects participated in efficacy tests of repellency of IR3535 on human skin. Ticks were first tested on untreated arms, then on treated arms.	● **0.001 mL IR3535 lotion** (0.99 g/mL, 10% IR3535) ● **0.0006 mL IR3535 pump spray** (0.95 g/mL, 20% IR3535) ● **0.001 mL IR3535 aerosol** (0.94 g/mL, 20% IR3535)	Repellency was predicated on the ability of a formulation to prevent ticks from walking 3 cm into the treated region of a forearm. Exposures began 15 min after the application of a test material. Subjects were withdrawn when they experienced the first confirmed crossing of the 3 cm limit.	Complete protection time was defined as the time until the first confirmed crossing, or if none occurred, until 15 min after the conclusion of data collection. The use of IR3535 resulted in complete protection times of 9.1 hours (SD: 2.5 hours) for lotion, 12.2 hours (2.8) for pump spray, and 11 hours (2.8) for aerosol against confirmed crossings from *I. scapularis* in laboratory tests. The average subject experienced two or fewer crossings over an average exposure period of approximately 11 hours.

Witting-Bissinger, 2008 NC, USA	Laboratory tests on human skin	Unclear risk of bias	*Dermacentor variabilis*	6 ticks were enclosed in a 12.6-cm^2 arena covered with a lid lined with two layers of cheesecloth with the open-end covered with aluminum screening to prevent the ticks from biting the subject but permitting the ticks to make direct contact with the skin. Repellent trials were replicated six times using 3 volunteers. Controls were replicated four times using the same 3 volunteers.	● **20 μL BioUD lotion** (7.75% 2-undecanone) ● **Control** (no treatment)	Tick distribution was converted to repellency indices (RI) calculated as follows: (# ticks on untreated surface - # ticks on treated surface)/ (# ticks on untreated surface + # ticks on treated surface). -1 designated repellency, +1 indicated attraction, and 0 indicated a neutral response.	BioUD with 7.75% 2-undecanone showed statistically signiÞcant repellency (p=0.003; chi square test) compared with untreated controls against *D. variabilis* 30 min after application. BioUD repelled ticks at least 2.5 hours after application to human skin.
Carroll, 2010 USA	Randomized laboratory study in humans	Unclear risk of bias	*A. americanum*	17 volunteers (70 ticks per leg) were randomized to treatments; repellent was applied in a 5-cm-wide band encircling a volunteer's lower leg. Nymphs were released on each volunteer's ankle, and tick locations were recorded 10 min afterwards. Volunteers were challenged with ticks at each post-application time point (2, 4, 6, 8, 10, and 12 h).	● **DEET 33%** cream, ● **Picaridin 20%** (lotion and spray), and ● **IR3535 10%** (lotion) and **20%** (spray) ● Because there was no carrier common to all the repellent formulations, the control was bare skin	**Proportion of ticks not repelled** (i.e., those that completely crossed the 5-cm-wide band) and the proportion of ticks that dropped off the subject.	For **all formulations** and time points, **significantly fewer (all *P* <0.0001) nymphs crossed the treatment bands** than the untreated control. **Formulations containing ≥20% active ingredient were highly effective**, with <6% of the ticks crossing through the treatment bands during the 12 h (for 20% picaridin lotion and 33% DEET it was ~1%). There was no significant difference in effectiveness between the 20% spray and 20% lotion formulations of picaridin. The proportions of ticks not repelled by 20% picaridin lotion and 33% DEET were significantly lower than that of 20% IR3535 spray. The 10% IR3535 lotion was significantly less effective than the formulations with higher concentrations of repellent.

Bissinger, 2011 NC, USA	Randomized field tests in humans (sock test)	Unclear risk of bias	*A. americanum*	4 volunteers walked randomly over a 4000 m² area at a slow pace for 15 min. Experiment was repeated 3-4x per day, with each repellent being assessed in 3 particpants per day. Treatments were randomized, then reassigned each day; untreated sock on the other leg served as control.	● **BioUD** (7.75% 2-undecanone) ● **DEET** (98.1%) ● **Permethrin** (0.5%) ● **"Carrier"** (control solvent) on the other leg	N of ticks crossing the upper sock barrier and N of ticks on socks; **Repellency (%)** was calculated as: [(control count -treatment count)/ control count] * 100.	Significantly fewer ticks were collected from socks treated with BioUD® or DEET than from socks treated with the carrier and **there was no significant difference in repellency between these two agents.** No difference in the mean number of ticks collected was found between permethrin-treated and corresponding carrier-treated socks.
Jordan, 2012 NJ, USA	Randomized field tests in humans	High risk of bias	*I. scapularis, A. americanum*	"Observers" (3 study authors) with randomly assigned coveralls were slowly walking through forested areas and counting ticks encountered and dropped	Coveralls treated with: • **Nootkatone**, • **Carvacrol**, • **EcoSMART organic insect repellent** (rosemary, cinnamon leaf, and lemongrass oils (all 0.5%) and geraniol (1.0%)) in solution with other ingredients (isopropyl alcohol, isopropyl myristate, and wintergreen oil), • Commercial **permethrin**-based clothing repellent (**Repel Permanone)**, • or left **UNTREATED.**	Tick count every 10 meters and timing of tick drop-off; Encounter rates = average N of ticks per 100 m per day **Repellency (%)** = [(N of ticks counted on untreated sample - N of ticks counted on treated sample)/ (N of ticks on untreated sample) *100.	**One day after** treatment, **nootkatone and carvacrol provided 100% repellency of *I. scapularis*** adults, with nootkatone maintaining complete protection through 3 d, whereas carvacrol showed steadily declining repellency against *I. scapularis* during the 7-d course of the trials. **Nootkatone** was at least as effective against host-seeking *A. americanum* as against *I. scapularis* through 3 d. **Carvacrol p**rovided little protection against *A. americanum* adults. Both natural compounds performed well initially in comparison with the commercial products. **After 7 d, nootkatone was the most effective against both species followed in order of activity by Permanone, EcoSMART, and carvacrol.**
Bissinger, 2014 NC, USA	Randomized field tests in humans (sock test)	Unclear risk of bias	*A. americanum, D. variabilis, I. scapularis,*	5 volunteers who walked through the woods while wearing over-the-calf tube socks with repellent. Treatments were	• **DEET 15%** and • **TT-4302 (5 % geranio**l) and its variant **TT-4228**	N of ticks crossing the upper sock barrier and N of ticks on socks;	In the field (predominant tick *A. americanum*), 2.5 or 3.5 h after treatment, **mean *percentage repellency* was significantly greater for socks treated with TT-4228** (90 and 70%, respectively) **vs. DEET** (55 and 20%).

			and *R. sanguineus*	randomized; untreated sock on the other leg served as control	• **control – untreated sock** on the other leg Each repellent was tested on four different subjects	**Repellency (%)** was calculated as: [(control count -treatment count)/ control count] * 100.	Significantly fewer ticks were recovered from socks treated with TT-4228 vs. their paired untreated controls 2.5 or 3.5 h after treatment; ditto for DEET vs. controls 2.5 h after application. However, no significant difference was found in the number of ticks collected from DEET-and untreated socks 3.5 h after treatment
Buchel, 2015 Germany	Randomized laboratory study in humans	Unclear risk of bias	Nymphs of *I. ricinus* and *I. scapularis*	Repellent and control treatments were randomly assigned to subjects on the first day. 10 volunteers were used for each repellent. Repellents were applied to the forearm with the untreated arm served as control; then, the subjects cycled through each repellent and both Ixodes species.	• **DEET 20%,** or • **Icaridin 10% (=picaridin)**, or • **EBAAP 10%** vs. **control** - the untreated arm	*EPA protocol*: **N (%) of ticks** walking onto or back from treated skin, falling off from treated skin, and remaining on treated skin. **The complete protection time (CPT)** was defined as the time interval between the application of repellent and the 1st confirmed crossing (Kaplan–Meier analysis).	20% DEET resulted in median complete protection times (CPT; Kaplan–Meier median) between 4 and 4.5 h, while 10% EBAAP yielded CPTs of 3.5–4 h. No significant differences were found between the efficacies of two repellents or between the two species tested. The median of the CPT of a 10% Icaridin was 5 h in nymphs of *I. scapularis*, but 8 h in those of *I. ricinus* (P < 0.01). **Based on these studies, EBAAP and Icaridin are efficacious alternatives to DEET**. **No AEs**, i.e. cutaneous itching or flushing, were observed or reported.

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk".

C) Removal of Attached Ticks

Tick removal – only animal studies are available.

Bibliography: 1. Piesman, et al. *J Med Entomol*. 2002 May;39(3):509-12; 2. De Boer, et al. *J Med Entomol.* 1993 Jul;30(4):748-52; 3. Duscher, et al. *Parasitol Res.* 2012 Oct;111(4):1505-11; 4. Needham. *Pediatrics.* 1985 Jun;75(6):997-1002; 5. Stewart, et al. *Wilderness Environ Med.* 1998 Fall;9(3):137-42; 6. Zenner, et al. *Vet Rec.* 2006 Oct 14;159(16):526-9.

Study	Study Design	Risk of bias*	Tick species/ pathogens under study	Interventions studied/ study question	Outcomes, Results and Conclusions
Piesman, 2002	**Lab. animal study (ticks attached to mice or rabbit ears)**: Studied whether crushing the tick during removal with forceps increased the risk of *B. burgdorferi* transmission; what degree of protection from transmission was provided by removal of nymphal *I. scapularis* at specific intervals; and whether commercial devices marketed for tick removal worked on the nymphs.	NA	*Ixodes scapularis/ Borrelia burgdorferi*	**Tick removal methods:** ● Grasping near mouthparts and pulling with forceps using steady gentle pressure; ● Grasping throughout the length of the body with the opisthosoma of the tick crushed or pierced during removal ● "Sham procedure": placing the forceps on the tick but then withdrawing the forceps without effecting tick removal.	Both **removal** via gentle pressure or crushing the tick **caused a significant decrease in transmission vs. the sham control**. There was **no significant difference between the gentle and crushing methods regarding transmission risk**. Transmission of *B. burgdorferi* was significantly impacted by tick removal at 24, 48, 54, and 60 h. **At 66 hours, tick removal offered no protection against transmission.** The commercially available devices (N=12; see table in the article) varied widely in their efficacy for removing nymphal *I. scapularis* (counted as N of successful removals out of 5 attempts each).
De Boer, 1993	Chemical and mechanical methods of removing I. ricinus attached to the skin of **pigs and sheep** were tested experimentally.	NA	*Ixodes ricinus*	● **chemical treatments (gasoline, fingernail polish**, and **methylated spirit)** ● **mechanical removal** of the tick: **(1) pulling straight out** with blunt **forceps** and **(2) rotation of the tick** around its body axis, using a **"Tick Solution" forceps** device	**Chemical treatments failed** to induce self-detachment of the ticks. **Pulling** frequently resulted in the complete removal of the tick, but fragments of the mouthparts that remained in the skin were often quite large. In contrast, if the tick was removed by **rotation** without pulling, the tip of the hypostome usually broke off and remained in the skin, but the remaining parts were small.
Duscher, 2012	**Field study of tick removal devices** with different mechanisms that were **tested on pets**.	NA	*Ixodes ricinus*	**Five commercial devices:** ● "forceps", i. e. Adson forceps (pulling); ● "card", i. e.TickPic (pulling);	The devices were rated according to force required for extraction, the ease of handling, the adverse reaction of the animal, the time needed for removal, and the

	Veterinarians and pet owners removed ticks from various animals by using the different devices and filled in a questionnaire for each case.			"lasso", i. e. Trix® tick remover (rotation); - the Tick Twister® (V-shaped slot, twisting); and - pen-tweezers (twisting)	quality of removal as evaluated by the female *I. ricinus* tick's mouthparts and body injury (amount of squeezing). **Twisting (i.e. rotation as opposed to pulling) of the ticks reduced the force required for extraction, the adverse reaction of the animal and the time needed for removal**. The device with a "**V**"-shaped slot, which allows a grabbing of the mouthparts (*Tick Twister*), delivered the best results according to the condition of the mouthparts and the intactness of tick's body.
Needham, 1985	**Lab. animal study**: American dog ticks or Lone Star ticks were placed on shaven compartments on a back of a female Dorset sheep. For dog ticks, two groups of ticks were used; attached for 72-96 hours, and for 12-15 hours. Common folk methods of tick removal were tested: passive and mechanical/active in *D. variabilis* and only mechanical in *A. americanum*	NA	*Dermacentor variabilis; Amblyomma americanum*	*Dermacentor*: **five common methods of tick removal:** - Petroleum jelly; - Fingernail polish; 70% isopropyl alcohol; - Hot kitchen match; and - Forcible removal with protected fingers or forceps *Amblyomma*: **only mechanical removal methods w. forceps**: - Pulling steadily straight up; - Jerking straight up; - Twisting clockwise; - Pulling parallel to the skin	Mechanical removal was satisfactory in all cases for *D. variabilis* leaving no mouthparts or tick cement behind. However, passive methods failed to induce tick detachment. For *A. americanum*, removal with forceps left no mouth parts behind, but cement remained in the skin in all cases. The authors suggested that the **point of grabbing the mouthpart (as close to skin as possible) is more important than the method of pulling the tick off. Passive methods should not be used due to inefficacy.**
Stewart, 1998	**Lab. animal study (ticks attached to rabbit ears)** evaluated three commercially available tick removal tools against medium-tipped tweezers when used by untrained volunteers.	NA	*Dermacentor variabilis; Amblyomma americanum*	- The Original Ticked Off™ - The Pro-Tick Remedy™ - The Tick Plier™ - The Tick Nipper™ - vs. medium-tipped non-tissue tweezers	**Tick damage occurring from removal and quantity of attachment cement** were compared. No tool removed nymphs without damage and **all tools removed adults of both species successfully.** Nymphal ticks were consistently removed more successfully with commercial tools when compared with tweezers but with more difficulty than adult ticks. American dog ticks proved easier to remove than lone star ticks, whose mouthparts often remained in the skin.

| Zenner, 2006 | **Field animal study (ticks attached to cats and dogs who were brought to clinic)** compared (1) a tick removal device (with a slit for tick prehension and rotation = SR) vs. surgical forceps, in the hands of a trained operator (veterinarian); and (2) three commercial tick-removal devices that use different methods of tick prehension and removal, in the hands of pet owners. | NA | *Ixodes ricinus*; *Dermacentor reticulatus*; *Rhipicephalus sanguineus* | **Tick removal devices:**
• Surgical forceps (Adson forceps) (use apposing jaws and traction (**AT**);
• Pen-Tweezers (use apposing jaws and rotation (**AR**);
• Pro-Tick Remedy (uses a slit and traction (**ST**);
• Crochet O'Tom (uses a slit and rotation (**SR**); | The devices were evaluated according to time required to remove the tick; ease with which the tick was grabbed and held by the device; force needed to extract the tick; reaction of the animal; the outcome of the manipulation (success or failure); and correct or incorrect use of the instrument when tick removal was carried out by the owner.

The **SR device (Crochet O'Tom) appeared to be easier to use and more efficient** for use by both veterinarians and pet owners. |

D) Educational interventions

Bibliography: 1. Daltroy, et al. *Health Educ Behav.* 2007 Jun;34(3):531-42; 2. Malouin et al. *Am J Epidemiol.* 2003 Jun 1;157(11):1039-51; 3. Shadick et al. *Vector Borne Zoonotic Dis.* 2016 Aug;16(8):507-15; 4. Beaujean, et al. *BMC Public Health*. 2016 Nov 16;16(1):1163.

Study; Location	Study Design	Risk of bias*	Population characteristics	Interventions and comparisons	Outcomes	Results and conclusions
Daltroy, 2007 Nantucket Island, MA	An RCT of a Lyme disease (LD) primary prevention program.	High risk of bias	30,164 passengers on ferryboats going to Nantucket Island, MA, during 3 summers of 1997-1999. Boats were randomized to receive experimental or control educational interventions. A questionnaire on occurrence of various tick-borne illnesses (TBI) such as Lyme disease, Ehrlichiosis, and Babesiosis; symptoms consistent with Lyme disease based on the CDC case definition; visits to the doctor; and practice of preventive behaviors while on the Island was mailed to the	**1. Experimental educational intervention**: participants learned **how to prevent Lyme disease**: A <u>15-min communication act</u> conveying the severity and likelihood of acquiring LD and the benefits of tick avoidance and search/removal behaviors; a demonstration of removal of *Ixodes* ticks (modeling); and <u>free education materials</u> **2. Control educational intervention**: participants learned **how to prevent summer injuries as bicyclists and roller-bladders.**	• Rates of self-reported tick-borne illnesses (TBI) • Relative risks (RR) of TBI in exp. vs. ctr. groups were calculated (also assessed separately in short-term visitors (≤ 2 wks), long-term visitors (> 2wks), and Nantucket residents).	Overall, there were lower rates of TBI among participants receiving TBI education compared with control participants (RR =0.79, 95% CI 0.56 to 1.10, p <.17 - non-significant) and a 60% reduction in risk among those receiving TBI education who visited Nantucket Island for more than 2 weeks compared to control participants (RR = 0.41, 95% CI = 0.18 to 0.95, p < .038). The rates for Nantucket residents were also lower in experimental vs. control groups, but not significantly (RR = 0.75, 95% CI = 0.30 to 1.89, p < .54).

			participants at 2 months. Physicians were contacted for details in case of positive responses regarding tick-borne illnesses.			TBI-educated participants were also significantly more likely to take precautions (use repellent, protective clothing, limit time in tick areas) and check themselves for ticks.
Malouin, 2003

Baltimore, MD | An RCT of a targeted educational intervention in an area with endemic Lyme disease. | High risk of bias | 317 subjects recruited by random digit dialing in the Baltimore county who were randomized to receive experimental or control educational materials.

The participants had 3 clinic visits afterwards, completed questionnaires (related to residential, recreational, and occupational tick bite risk; history and knowledge of tick bites and Lyme disease; and knowledge and use of tick bite prevention methods), and submitted serum samples for anti-recombinant tick calreticulin antibody (ARTCA) analysis.
Also, they filled out 10 checklists with questions related to tick exposure and use of the intervention materials included in the previous mailing. | **1. Tick-related educational materials** (literature and tools designed to educate and to enable each individual to examine his or her entire body for ticks, identify and remove ticks found during these body checks, and apply the repellent DEET to the skin and the acaricide permethrin to the clothing); or

2. General health-related educational materials

Educational materials were sent bimonthly through the mail in Apr-Sep 1999. | **ARTCA** (anti-recombinant tick calreticulin antibody) - a biomarker of tick bites.
Linear and logistic regression analyses were used to determine:
(1) whether the educational intervention was associated with a change in knowledge, attitudes, and behaviors (KAB) and (2) whether change in KAB predicted change in ARTCA levels. | Proportions of desired responses increased significantly among intervention subjects versus the comparison group on KAB measures related to examining the body for ticks and insect repellent use; however, this change was not associated with significant change in ARTCA levels. |
| Beaujean, et al., 2016

The Netherlands | A cluster-RCT of a targeted educational intervention among at-risk school children. | High risk of bias | 887 children between the ages of 9 and 13 years were recruited from 25 primary schools. 254 children were randomized to the game group, 328 were randomized to the leaflet group, and 399 were randomized to the control group and completed an initial questionnaire in February-March of 2012; of the children randomized, 199 (78%), 316 (96%), and 372 (93%) were available to | **1. Educational video game**

An online educational video game was developed using the results of a pre-intervention study (Beaujean, et al. *BMC Public Health*. 2013 Dec 9;13:1148). The game was constructed based on determinants of preventive behavior according to Protection Motivation Theory, which comprises both "threat | Primary outcomes were knowledge, perception (perceived susceptibility and importance), and preventive behavior in relation to tick bites and Lyme borreliosis. | Knowledge about ticks and tick bites improved significantly in all three groups. Knowledge improvements were not significantly different between groups. The frequency of checking for ticks increased significantly in the game group, but not in the other two groups, suggesting that the game was effective at improving preventive behavior. |

			complete the second questionnaire in June-July 2012. Children were asked to complete the short questionnaires before and immediately after each intervention.	appraisal" and "coping appraisal". The objective of the video game was to simulate threat appraisal scenarios specific to tick bite prevention and to teach children the appropriate coping appraisals. Children played the game individually on a personal computer. **2. Educational leaflet** A leaflet was distributed to children in this group that contained similar information to that which was contained within the video game. Children were instructed to read the leaflets. **3. Control** Children in this group received no information	Generalized linear mixed models were used to analyze the data.	The game did not outperform the leaflet or control group on all outcome measures.The authors note that educational video games like the one in this study may be of value as a complementary role, in addition to other media, in child-specific public health education programs on ticks and Lyme borreliosis.
Shadick, 2016 MA, USA	A cluster-RCT of a targeted educational intervention among at-risk school children.	High risk of bias	3570 students grade 2-5 from 19 school districts. Districts were randomized to intervention (1562 students) or control (2008 students).	**1. Experimental educational intervention**: short in-class program (Health Belief Model) that covered: (1) awareness and knowledge about LD, (2) benefits of preventive behavior, and (3) confidence in ability to perform preventive behaviors. The program included presentations, videos, games, and tick-and LD-related educational materials.	Students' knowledge, attitudes, and self-reported preventive behaviors were surveyed before implementing the program and 1 year later. Follow-up surveys asked whether	The children in the intervention group increased their overall knowledge of LD more than those in the control group (**overall knowledge score** improvement, mean difference 1.38 vs. 0.36; p < 0.0001). All children in classes receiving the intervention reported an increase in precautionary behavior, positive attitude toward taking precautions, and self-efficacy compared with the wait list controls. 38 (intervention) vs. 34 (control) children reported a new case of LD at follow-up (2%). Only

				2. Control: no intervention (the control students received the educational presentation after the follow-up survey was completed)	students had had **LD in the year since their 1ˢᵗ questionnaire**, and the cases were confirmed via medical records.	two **LD cases** were confirmed by pediatrician's record, **one in the intervention group and one in the controls**.

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk".

II. Which diagnostic tests should be used following a tick bite?

Diagnostic tick testing and Diagnostic testing of asymptomatic patients

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Study; Location	Study Design	Risk of bias *	Tick type	Population characteristics and Diagnostic method	Treatment(s) administered	Lyme disease DS definition	Outcome: The incidence of Lyme disease after the tick bite, % (N of cases per N of patients)
Korenberg, 1996

Russia | Controlled clinical trial and a prospective cohort serving as an epidemiological control | High risk of bias | *I. persulcatus.* | Patients bitten by ticks and referred for first aid. Ticks were tested for *Borrelia* infection (**microscopic examination of tick's gut contents**).

Those bitten by an infected tick (N=358) were divided into experimental (N=261) and control (N=97) groups.

Those bitten by a tick tested negative for *Borrelia* (N=823) served as an epidemiological control. | Tick-test-positive experimental group received oral doxycycline 100 mg BID for 3 to 5 days.

Tick-test-positive control group received no antibiotics and was followed up.

Tick-test-negative untreated group received no antibiotics and was followed up as well. | The development of typical erythema migrans or "other **clinical manifestations" in combination with** a fourfold **seroconversion** at 5--6 weeks as compared with the serum sample analyzed the first week after the tick bite. | 5-6 weeks after tick bite:

Tick-test-positive treated group: **1.2%** (3 of out 261)

Tick-test-positive untreated group: **12.4%** (12 out of 97)

Tick-test-negative untreated group: **0.7%** (6 out of 823) |

Costello, 1989

CT, USA | Randomized double-blind controlled trial | Unclear Risk of Bias | *I.dammini* | 56 subjects (≥5 years old) with *I. dammini* tick bites within the preceding 72 hours.

Ticks were tested for *Borrelia* infection (by a direct **immunofluorescence assay**). | Subjects were randomized into 2 groups: oral penicillin VK 250 mg (N=27) or oral placebo (lactose 333 mg) (N=29), 4 times daily for 10 days.

Patients were followed up for 6-12 months. | **Development of clinical signs of Lyme** disease at 6-12 months, **seroconversion** at 3 weeks and 6 months. | Overall, **29% of** 21 **ticks** suitable for testing **tested positive** for *B. burgdorferi*.

Both <u>tick-test-positive (treated and untreated) and tick-test-negative (ditto)</u> groups had **0%** *B. burgdorferi* transmission. The only patient who developed Lyme disease (placebo group) had a tick that was not suitable for study. |
| Shapiro, 1992

CT, USA | Randomized double-blind controlled trial | Unclear Risk of Bias | *I.dammini* | 387 subjects (children and adults 12 years or older) who had been bitten by a deer tick within the preceding 72 hours.

Ticks were tested for *Borrelia* infection (mostly by **PCR,** with the first 13 ticks – by an indirect **immunofluorescence assay**). | Subjects were randomized into 2 groups: oral amoxicillin 250mg (N=205) vs. oral placebo (N=182) suspension TID for 10 days.

Patients were followed up for 6-12 months | **Symptomatic infection with *B. burgdorferi*** (defined as EM at the site of bite; symptoms of early disseminated Lyme or late Lyme with seroconversion), **or an asymptomatic infection (defined as seroconversion** without signs and symptoms of LD). | Overall, **15% of ticks tested positive** for *B. burgdorferi* by PCR test, and 2 out of 13 **(15%)** were positive by IFA assay.

6-12 months after tick bite:
<u>Tick-test-positive treated group</u>: **0%** (0 of out 30) developed infection

<u>Tick-test-positive untreated group</u>: **4.3%** (1 out of 23) **or 8.3%** developed infection (if including a patient whose tick's PCR was indeterminate).

<u>Tick-test-negative treated and untreated group</u>: **0% -** no other patient developed Lyme disease **or 0.3% in the untreated** group if including the above patient. |

Maiwald, 1998 2 sites in Germany	Prospective cohort	5	mostly *I. ricinus*; 47% of ticks in site 1 were not identifiable	730 patients bitten by ticks and referred to a general practitioner. Ticks were tested for *Borrelia* infection (**PCR**). Patients were followed for 2-6 weeks (site 1) or 8-13 weeks (site 2).	All patients were followed for Lyme disease symptoms and seroconversion. Seroconversion was defined as ≥4-fold titer rise above the cut-off titer in the immunofluorescence assay, a conversion from negative to positive in the IgM enzyme immunoassay, or the unit values of the IgG enzyme immunoassays increased ≥2-fold to a value above the cut-off.	**Seroconversion and/ or "the clinical criteria"** (including non-specific symptoms) <u>Note</u>: the transmission from PCR-positive ticks is likely inflated due to the lax criteria for a Lyme disease case; the transmission from PCR-negative ticks is likely underestimated due to the lack of attrition data (the total N of patients followed up after a PCR-negative tick bite was not given and was likely lower than our estimate).	Overall, **11% of ticks were positive** for *B. burgdorferi*. The total transmission rate was **2.6%** (19 out of 730), and the transmission from PCR- positive ticks was **24.6%** (16 out of 65) *2-6 weeks after bite (Site 1):* Patients bitten by <u>ticks tested positive</u> for *Borrelia* infection: **21.0%** (8 out of 38). Patients bitten by <u>ticks tested negative</u> for Borrelia infection: **0.7%** (2 out of 307). *8-13 weeks after bite (site 2):* Patients bitten by <u>ticks tested positive</u> for *Borrelia* infection: **29.6%** (8 out of 27). Patients bitten by <u>ticks tested negative</u> for Borrelia infection: **0.3%** (1 out of 358).
Fryland, 2011 Sweden	Prospective cohort	8	Ticks not specified	394 subjects ≥18 y.o. who were bitten by a tick and brought it to the primary health center visit. Ticks were screened for *Borrelia* DNA with PCR. Patients were followed for 3 months.	Patients were followed for Lyme disease symptoms and seroconversion (using two ELISA assays). Seroconversion was defined as an at least 2-fold increase in anti-Bb antibodies after 3 months, confirmed using a Strip-Immunoassay.	**Seroconversion** was deemed sufficient for active *B. burgdorferi* infection	Overall, **19% of ticks were positive** for *B. burgdorferi.* Seroconversion at 3 months: Patients bitten by (+) tested ticks: **6.3%** (4 out of 64) Patients bitten by (-) tested ticks: **2.5%** (7 out of 277). The only patient with Lyme-specific symptoms (EM + seroconversion) was bitten by a tick that tested negative for Bb.

| Huegli, 2011 Switzerland | Prospective cohort | 7 | *I. ricinus* | 474 patients bitten by ticks and referred to a physician. Ticks were tested for *Borrelia* infection (**PCR**).

Patients were followed for 8 weeks | All patients were followed for Lyme disease symptoms and seroconversion.

Patients were screened by EIA tests for IgM and IgG, and **seroconversion** at 8 weeks (change from a (-) to a (+) index, from equivocal to (+) or from (-) to equivocal but with an index increase corresponding to 0.236 for IgM and 0.077 for IgG) was confirmed by immunoblot. | **Clinical manifestations of infection with *B. burgdorferi*** (defined as **EM) or seroconversion** ("asymptomatic infection") | Overall, **33% of ticks were positive** for *B. burgdorferi sensu lato* (predominantly *B. afzelii*). EM developed in 5.2% participants overall (14 out of 269). There was a 3.5% seroconversion rate in asymptomatic patients (9 out of 255).

EM at 8 weeks:
Patients bitten by (+) tested ticks: **6.6%** (4 out of 61).
Patients bitten by (-) tested ticks: **3.2%** (4 out of 125).
Symptomatic or asymptomatic (seroconversion) infection at 8 weeks:
Patients bitten by (+) tested ticks: **8.2%** (5 out of 61).
Patients bitten by (-) tested ticks: **5.6%** (7 out of 125). |

Wilhelmsson, 2016 Sweden and Finland	Prospective cohort	8	*I. ricinus*	1896 subjects (1546 completed) ≥18 y.o. who were bitten by a tick and brought it to the primary health center visit. Ticks were tested for *Borrelia* infection (**PCR**). Patients were followed for 3 months.	Patients were followed for Lyme disease symptoms and seroconversion (using two ELISA assays followed by commercial immunoblot). **Seroconversion** was defined as either a change from seronegative to seropositive or an at least 2-fold increase in anti-Bb antibodies after 3 months.	**Seroconversion and/ or clinical manifestations (**EM, borrelial lymphocytoma, neuroborreliosis, or Lyme arthritis; 2 patients had non-specific symptoms).	Out of 1546 patients, 428 **(28%)** were bitten by *B. burgdorferi*-(+) ticks. Clinical Lyme borreliosis developed in 2.1% participants overall (33 out of 1546). There was a 3.0% seroconversion rate in asymptomatic patients (45 out of 1513). Clinical manifestations at 3 months: Patients bitten by ticks tested positive for *Borrelia* infection: **4.0%** (17 out of 428, 1 with non-specific symptoms). Patients bitten by ticks tested negative for *Borrelia* infection: **1.4%** (16 out of 1118, 1 with non-specific symptoms). Symptomatic or asymptomatic (seroconversion) infection at 3 months: Patients bitten by (+) tested ticks: **8.2%** (35 out of 428). Patients bitten by (-) tested ticks: **3.8%** (43 out of 1118).
Sood, 1997 NY, USA	Prospective cohort	6	*I. scapularis*	225 subjects bitten by confirmed *I. scapularis* ticks in the preceding 72 hours (NY state). Ticks were identified, measured for engorgement, and assayed by **PCR** for *B. burgdorferi*. Duration of attachment was determined from the **scutal index** of engorgement.	Sera of 115 subjects were tested at baseline and 4-6 weeks afterwards (EIA + immunoblot). The rest of the patients were followed for Lyme disease symptoms.	Lyme disease (+) case was defined as **EM developed between the two visits, asymptomatic seroconversion (AS), or both**. <u>Note</u>: If subjects with AS were excluded, the incidence rates would be	Overall, **14% of ticks were positive** for *B. burgdorferi* (32 out of 227); however, in the sample subjected to scutal index measurement (with a high scutal index being indicative of prolonged feeding), the prevalence was only 1.8% (2 out of 109)**.** **Overall, the Lyme disease incidence rate was 3.7% in**

						1.8% (2 out of 109) in the tested and 0.9% (2 out of 225) in the total sample, and 6.7% (1 out of 15) for ≥72 h vs. 1.1% (1 out of 94) for <72 h attachment duration. For patients after a tick-test-(+) bite, the rate would be 50% (1 out of 2), and after a tick-test-(-) bite it would be 0.9% (1 out of 107).	**subjects with tested sera** (4 Lyme disease cases out of 109 bites) **or 1.8%** (4 out of 225) in the total sample. The incidence was significantly higher for duration of attachment **≥72 h (20%,** or 3 out of 15) than for **<72 h (1.1%,** or 1 out of 94) (*P* =0.008). *4-6 weeks after bite:* Patients bitten by ticks tested positive for *Borrelia* infection: **100%** (2 out of 2). Patients bitten by ticks tested negative for *Borrelia* infection: **1.9%** (2 out of 107). **PCR test** was 100% positive in predicting *Borrelial* infection after a nymph attachment for ≥72 h (N=2; 1 EM + 1 AS) but was negative when the infection occurred after a bite by an adult female tick attached for either <72 h (N=1; EM) or ≥72 h (N=1; AS).
Briciu, 2016 Romania	Prospective cohort	6	*I. ricinus*	386 patients who presented after a tick bite at the Clinical Hospital of Infectious Diseases. Ticks were identified and tested for *B. burgdorferi sensu lato* infection using **PCR**. **20 out of 38** patients bitten *by B. burgdorferi*-infected ticks and a control group (age, sex, and residence-matched individuals bitten by *B. burgdorferi*-	Patients were followed up for Lyme disease symptoms. Sera were tested at baseline and at 1 year (2-tiered test: ELISA and immunoblot) for seroconversion.	**Symptoms of Lyme disease** (according to European guidelines – Stanek, 2011) **and/ or seroconversion** at one-year follow-up. Note: 18 out of 20 tick-test (+) and 19 out of 20 tick-test (-) patients received antibiotic prophylaxis after the	Overall, 11% of ticks were positive for *B. burgdorferi s.l.* (43 out of 389) (mainly *B. afzelii*, but also *B. garinii, B. burgdorferi sensu stricto, B. spielmanii/ B. valaisiana* and *B. lusitaniae*). **Lyme disease (EM) incidence rate** 1 year after bite: Patients bitten by ticks tested positive for *Borrelia* infection:

				negative ticks, **N=20**) were followed up for 1 year.		bite, which may have biased the results. Also, additional tick bites were recorded during the long follow-up – another source of bias.	**10%** (2 out of 20) – both after prophylaxis with amoxicillin. Patients bitten by ticks tested negative for *Borrelia* infection: **0%** (0 out of 20). **Seroconversion** after 1 year: Patients bitten by ticks tested positive: **10%** (2 out of 20) Patients bitten by ticks tested negative: **15%** (3 out of 20).
Tijsse-Klasen, 2011 Netherlands	Prospective cohort	4	*I. ricinus*	246 participants who had consulted a general practitioner for tick bites were included. Ticks were identified and tested for *Borrelia burgdorferi s.l., Rickettsia spp., Babesia spp. or Ehrlichia/Anaplasma spp* infection using **PCR**.	Patients received an initial questionnaire concerning number of tick bites and duration of tick attachment. Patients were followed up approximately 6 months later and received a questionnaire assessing the presence of potential Lyme disease symptoms.	Potential Lyme disease cases were identified by self-reported symptoms related to the tick bite, including local redness or erythema migrans and systemic symptoms as fever, malaise, palpitations, joint problems, or neurological symptoms	Overall, 16% of ticks were positive for *B. burgdorferi s.l.* ≥6 months after tick bite, 14 out of 193 participants (8.3%) reported reddening at the bite site and 6 participants (4.1%) reported systemic symptoms. No association between symptoms and tick-borne microorganisms was found. Attachment duration ≥24 h was positively associated with reddening at the bite site and systemic symptoms.
Hofhuis, 2013 Netherlands	Prospective cohort	8	*I. ricinus* (94%)	644 patients who consulted a cooperating general practitioner for a recent EM or tick bite were included. 361 cases (55%) consulted their physician for a tick bite, and 283 (43%) consulted their physician at baseline with an EM that was undisputed by their physician	Blood samples were collected at baseline and ticks were removed. Over half (56%) of the cases reported tick removal within 24 hours. Ticks removed from patients at baseline were tested for presence of *Borrelia* by **PCR**. Patients also received a	*Borrelia* infection was defined as development of physician-confirmed clinical Lyme borreliosis such as EM, **and/or** seroconversion for *Borrelia*-specific antibodies confirmed by two-tiered testing via C6 ELISA and IgM/IgG Immunoblots.	*Borrelia burgdorferi* sensu lato DNA was detected in 92 out of 314 ticks collected (29.3%). Seroconversion for *Borrelia*-specific antibodies was observed in 3.2% of tick bite cases. 14 tick bite cases had evidence of early *Borrelia* infection, with EM developing in seven cases. The risk of developing EM after tick

				questionnaire about baseline data. A follow-up questionnaire was sent and blood samples were collected 3 months later.		bites was 2.6% (95%CI: 1.1%–5.0%), and the risk of either EM or seroconversion was 5.1% (95%CI: 2.9%–8.2%). **Participants with *Borrelia*-positive ticks had a significantly higher risk of either EM or seroconversion (odds ratio 4.8, 95%CI: 1.1–20.4).**
Hofhuis, 2017 Netherlands	Pooled analysis of Hofhuis 2013, Wilhelmsson 2016, and Sprong 2013 (Parasit Vectors. 2013 Dec 4;6:338)	NA	*I. ricinus*	3,525 single tick bite reports extracted from three large prospective studies, with 50 reports of Lyme borreliosis during the 2-3 month follow-up period, among 1,973 reports with known outcome.	All study participants were asked to fill out questionnaires at enrollment and at follow-up, including questions on duration of tick attachment in the skin, the number of tick bites, and development of Lyme borreliosis.	Lyme borreliosis within three months after a tick bite was categorized as "physician-confirmed erythema migrans" or "physician-confirmed disseminated Lyme borreliosis" when patient-reported Lyme borreliosis was confirmed by the general practitioner through an additional questionnaire. If no report from a general practitioner was available, researchers used patient-reported EM and looked at antibiotic records.	The overall risk of developing Lyme borreliosis after a tick bite was 2.6% (95%CI 1.4–5.1). The risk increased with degree of tick engorgement, increased duration of tick attachment, and detection of Borrelia burgdorferi s.l. DNA in ticks. Detection of Borrelia DNA in the tick was the strongest and statistically significant predictor for the risk of developing Lyme borreliosis.
Steere, 2003 10 US states	*Post hoc* analysis of a randomized controlled trial	NA	Ticks not specified	269 study participants who participated in a randomized controlled trial (RCT) assessing the efficacy of a Lyme disease vaccine versus placebo who met the criteria for Lyme disease were assessed for incidence of asymptomatic conversion.	As part of the RCT protocol, participants received injections of recombinant outer-surface protein A (OspA) of *B. burgdorferi* in adjuvant or of placebo at study entry and at 1 and 12 months after study entry.	Asymptomatic seroconversion was defined as development of IgG antibody response to *B. burgdorferi*, by whole-cell sonicate Western blotting during the intervals encompassing the 2	30 patients (11%) were classified as having asymptomatic IgG seroconversion to *B. burgdorferi*. 28 of these 30 patients were shown to have a ≥4-fold increases in antibody levels to the VlsE (IR6) peptide between

| | | | | | | summer transmission seasons (2-12 months or 12-20 months). Testing by sonicate ELISA was not done. | pre- and post-summer serum samples, in addition to an increase in the number of bands seen on Western blots; however 6 patients were suspected to have been reinfected.

The authors conclude that infection with *B. burgdorferi* may be asymptomatic, but that asymptomatic infection is rare in the United States. |

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk".

Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

III.Who should receive antibiotic prophylaxis to prevent Lyme disease following presentation with a tick bite?

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Study	Study Design	Risk of bias*	Population characteristics	Interventions and comparisons	Probability of *Borrelia burgdorferi* infection after a tick bite (P:BB) formula	Outcomes	Study conclusions
Magid, 1992	Cost-effectiveness analysis	NA	Patients bitten by Ixodes ticks in areas of endemic Lyme disease	Three alternative strategies: 1. **Treat all**: empirically treat all patients with two weeks of doxycycline; 2. **Follow**: treat only patients in whom erythema migrans develops; 3. **Test**: treat only patients with erythema migrans or a positive serologic test for Lyme disease one month after exposure.	**P:BB = probability of the tick to be *Ixodes*** (ideally =1.0 after tick identification*) **multiplied by prevalence of ticks infected with *B. burgdorferi* in the area † multiplied by probability of the *Borrelia* transmission after the bite by the infected tick** † The prevalence of ticks infected with *B. burgdorferi* in the pacific states, where *I. Pacificus* is found, was estimated as 1-3%; and in New England, Mid-Atlantic states, and upper Midwest it was between 25% of nymphs and 50% of adult ticks (citations from Magid 1992)	Number of Lyme disease cases. Number of major complications ‡ (both from Lyme disease and antibiotics). Number of minor complications (both from Lyme disease and antibiotics). ‡ Erythema migrans was considered minor, and cardiac, neurologic, and rheumatologic complications of late Lyme disease were considered major sequelae.	When **P:BB is ≥0.036**, empirical treatment was found to have fewest N of cases of Lyme disease, fewest N of complications overall, and fewest N of major complications. When **P:BB is ≥0.01 - <0.036**, empirical treatment still has fewest N of major complications, and it incurs relatively few additional minor complications. When **P:BB is <0.01**, "Follow" is the best strategy as the N of excess minor complications incurred by empirical treatment strategy greatly exceeds the N of major disease sequelae averted. Therefore, with the cut-off point of P:BB of 0.01 for the "Treat all" strategy and the average probability of the *Borrelia* transmission after an infected tick's bite of 0.1 (as per Magid 1992), **the empirical therapy is warranted in all areas with the prevalence of infected ticks of 10% or greater** (0.1 x 0.1 = 0.01).

Diuk-Wasser, 2012	Geographic survey of vector ticks for infection with *B. burgdorferi*.	NA	Populations of *I. scapularis* nymphs collected from 37 states east of the 100th meridian were drawn from randomly selected sites using a spatially stratified random design. 304 sites were sampled between 2004 and 2007, with 30 being repeatedly sampled in 2 to 4 years, resulting in a total of 348 site-year samples.	Risk factors for presence and density of infected nymphs were used to model a predictive surface of human risk.	**Annual mean density** of infected nymphs per 1,000 m². Derived from multiplying density of nymphs per 1,000 m² by the infection prevalence at each site-year. The estimated annual mean density derived by 1) calculating the mean number of I. scapularis nymphs collected per visit; 2) plotting the mean number of nymphs per visit by time; 3) calculating the area under phenology curve; and 4) dividing area by the number of days between first and last sampling visit at a site for a given year	% of nymphal blacklegged ticks infected with *B. burgdorferi*.	*I. scapularis* nymphs from 92 of the sites (5,328 nymphs) were tested for the presence of *B. burgdorferi* DNA. Overall infection prevalence was 20%. Infected nymphs were found in 92.3% of the sites where a threshold of 14 nymphs per 1,000 m² were collected. Discontinuous Lyme disease risk foci were identified in the Northeast and upper Midwest; transitional zone included sites with uninfected *I. scapularis* populations. Lower elevation, low vapor pressure deficit, and low seasonal extremes in minimum temperature associated with the presence of infected nymphs. The authors concluded that presence of any number of infected nymphs may be considered sufficient to recommend post-exposure prophylaxis.
Piesman, 1999.	Geographic survey of vector ticks for infection with *B. burgdorferi*.	NA	Populations of adult I. scapularis and I. pacificus, the two principal vectors of Lyme disease spirochetes in the US, collected from 17 sites in 12 states	Female ticks were fed on experimental rabbits; ticks and rabbits were subsequently examined for infection with *Borrelia burgdorferi*.	Addresses the **prevalence of ticks infected with *B. burgdorferi* in the area** part from the formula provided in Magid et al. 1992.	% of adult blacklegged and western blacklegged ticks infected with *B. burgdorferi*.	A total of 165/226 (**73%**) of northeastern I.*scapularis* ticks (CT, NY, NJ, and MD) were infected; 29/51 (**57%**) of I.*scapularis* ticks from the midwestern states of MI, WI, and MN were infected; 0/284 (**0%**) of I.*scapularis* ticks from southeastern states of SC, GA, FL, and MS contained spirochetes; 2/57 (**4%**) of the *I. pacificus* from CA were infected.

Nelder, 2016	A systematic review	NA	Studies (N=78) evaluating prevalence of pathogens in *I. scapularis* only	A systematic and comprehensive compilation of studies describing the variety and the prevalence of the human pathogens in blacklegged ticks throughout the US and Canada.	Addresses the **prevalence of** *I. scapularis* **ticks infected with B. burgdorferi in the area** part from the formula provided in Magid et al. 1992.	Prevalence of the human pathogens in blacklegged ticks throughout the US and Canada.	Blacklegged ticks harbored 91 distinct taxa, including species of *Anaplasma, Babesia, Bartonella, Borrelia, Ehrlichia, Rickettsia, Theileria*, and Flavivirus. Organism richness was highest in the Northeast (esp. CT, NY) and Upper Midwest US (WI). The manuscript **provides maps and data for the prevalence of the pathogens, including** *Borrelia*.
Nadelman, 2001; NY, USA	Randomized double-blind controlled trial	Low risk of bias	482 subjects (≥12 years old) who had been bitten by *I. scapularis* within the preceding 72 hours (NY state).	Patients were randomized into treatment (single dose of oral doxycycline 200mg) (N=235) vs. control (single dose of oral placebo) (N=247) groups and followed up for 6 weeks. **Ticks were identified, and the duration of tick attachment** was estimated for a subset of ticks using the **scutal index.**	Addresses the **probability of the** *Borrelia* **transmission after a tick bite** (covers a combined probability (P) of ticks infected with *B. burgdorferi* in the area x P of the *Borrelia* transmission after the bite by the infected tick).	**Erythema migrans incidence** for untreated patients with nymphal ticks attached for ≥72 vs. <72 hours and for untreated patients bitten by nymphal vs. adult ticks.	**EM incidence after 6 weeks of follow up**: Untreated patients with nymphal tick attached for **<72 hours: 0%** (0 out of 48) Untreated patients with nymphal tick attached for **≥72 hours: 25%** (3 out of 12); *P = 0.006* Untreated patients bitten by **nymphal** ticks: **5.6%** (8 out of 142); Untreated patients bitten by **adult** ticks: **0%** (0 out of 97); *P = 0.02*
Shapiro, 1992; CT, USA	Randomized double-blind controlled trial	Unclear Risk of Bias	387 subjects (≥12 years old) who had been bitten by *I. scapularis* within the preceding 72 hours (CT state).	Patients were randomized into 2 groups: oral amoxicillin 250mg (N=205) vs. oral placebo (N=182) suspension TID for 10 days and followed up for 6-12 months.	Addresses the **prevalence of infected ticks in the area** and the **probability of** *Borrelia* **transmission after the bite by the infected tick** from the formula provided in **Magid et al. 1992.**	**Transmission rate = Lyme disease incidence** (clinical and/or seroconversion) **in untreated patients bitten by infected ticks**; **Prevalence (%) of ticks tested positive** for *B. burgdorferi*	The overall **prevalence of** *B. burgdorferi* in ticks was **15%**. **Transmission rate (incidence of Lyme disease after 6-12 months)**: Tick-test-positive untreated group: **4.3%** (1 out of 23) **or 8.3%** (if including another Lyme(+) patient whose tick's PCR was indeterminate).

				Ticks were identified and tested for *Borrelia* infection (PCR).			
Costello, 1989; CT, USA	Randomized double-blind controlled trial	Unclear Risk of Bias	56 subjects (≥5 years old) with *I. scapularis a* tick bites within the preceding 72 hours (CT state).	Subjects were randomized into 2 groups: oral penicillin VK 250 mg (N=27) vs. oral placebo (lactose 333 mg) (N=29), 4 times daily for 10 days and followed up for 6-12 months. **Ticks were tested** for *Borrelia* infection (IFA)	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after the bite by the infected tick** from the formula provided in Magid et al. 1992..	**Transmission rate = Lyme disease incidence** (clinical cases) **in untreated patients bitten by infected ticks;** **Prevalence (%) of ticks tested positive** for *B. burgdorferi*	The overall **prevalence of *B. burgdorferi*** in ticks was **29%.** **Transmission rate (incidence of Lyme disease after 6-12 months):** The tick-test-positive group had **0%** *B. burgdorferi* transmission. The only untreated patient who developed Lyme disease had a tick that was not suitable for testing.
Maiwald, 1998; Germany	Prospective cohort study	5	730 patients bitten by ticks and referred to a general practitioner (Germany).	Patients were followed for 2-6 weeks (site 1) or 8-13 weeks (site 2) for Lyme disease symptoms and seroconversion. **Ticks were tested** for *Borrelia* infection (**PCR**).	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after the bite by the infected tick** from the formula provided in Magid et al. 1992..	**Transmission rate = Lyme disease incidence** (clinical and/or seroconversion) in **patients bitten by infected ticks;** **Prevalence (%) of ticks tested positive** for *B. burgdorferi*.	The overall **prevalence of *B. burgdorferi sensu lato*** in ticks was **11%.** **Transmission rates from PCR- positive ticks:** *Overall:* **24.6%** (16 out of 65). *2-6 weeks after bite (Site 1):* **21.0%** (8 out of 38). *8-13 weeks after bite (site 2):* **29.6%** (8 out of 27).
Fryland, 2011 Sweden	Prospective cohort study	8	394 subjects ≥18 y.o. who were bitten by a tick and brought it to the primary health center visit.	Patients were followed for Lyme disease symptoms and seroconversion (3 months). **Ticks were tested** for *Borrelia* infection (**PCR**).	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after the bite by the infected tick** from the formula	**Prevalence (%) of ticks tested positive** for *B. burgdorferi*. **Transmission rate = Lyme disease incidence** (seroconversion) **in patients bitten by infected ticks.**	The overall **prevalence of *B. burgdorferi sensu lato*** in ticks was **19%.** **Transmission rates from PCR- positive ticks at 3 months (seroconversion only):6.3%** (4 out of 64). The only patient with Lyme-specific symptoms (EM + seroconversion) was

					provided in Magid et al. 1992..		bitten by a tick that tested negative for Bb.
Huegli, 2011 Switzerland	Prospective cohort study	7	474 patients bitten by ticks and referred to a physician.	Patients were followed for Lyme disease symptoms and seroconversion (8 weeks). **Ticks were tested** for *Borrelia* infection (**PCR**).	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after the bite by the infected tick** from the formula provided in Magid et al. 1992..	**Prevalence (%) of ticks tested positive** for *B. burgdorferi*. **Transmission rate = Lyme disease incidence** (clinical and/or seroconversion) **in patients bitten by infected ticks.**	The overall **prevalence of *B. burgdorferi sensu lato* in ticks was 33%** (predominantly *B. afzelii*). EM developed in 5.2% participants overall (14 out of 269). There was a 3.5% seroconversion rate in asymptomatic patients (9 out of 255). **Transmission rates from PCR- positive ticks at 8 weeks** (**EM**): **6.6%** (4 out of 61). **Transmission rates from PCR- positive ticks at 8 weeks (*symptomatic or asymptomatic (seroconversion) infection)*: 8.2%** (5 out of 61).
Wilhelmsson, 2016 Sweden and Finland	Prospective cohort study	8	1896 subjects (1546 completed) ≥18 y.o. who were bitten by a tick and brought it to the primary health center visit	Patients were followed for Lyme disease symptoms and seroconversion (3 months). **Ticks were identified, and the duration of tick attachment** was estimated using the <u>scutal and coxal indices.</u> **Ticks were tested** for *Borrelia* infection (**PCR**).	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after the bite by the infected tick** from the formula provided in Magid et al. 1992..	**Prevalence (%) of ticks tested positive** for *B. burgdorferi*. **Transmission rate = Lyme disease incidence** (clinical and/or seroconversion) **in patients bitten by infected ticks.**	Out of 1546 patients, 428 (**28%**) were bitten **by *B. burgdorferi*-(+) ticks** **Transmission rates from PCR- positive ticks at 3 months** (**symptomatic LB**):**4.0%** (17 out of 428, 1 with non-specific symptoms). **Transmission rates from PCR- positive ticks at 3 months *(symptomatic or asymptomatic (seroconversion) infection)*: 8.2%** (35 out of 428). Nymphs and adult female ticks removed by participants who later seroconverted had significantly longer duration of tick feeding (median 46 h) than the ticks removed by those who did not seroconvert (median 29 h), P = 0.0003.
Sood, 1997; NY, USA	Prospective cohort study	6	225 subjects bitten by confirmed *I. scapularis* ticks in the preceding 72 hours (NY state).	Sera of 115 subjects were tested at baseline and 4-6 weeks afterwards (EIA + immunoblot). The rest of the patients were	Addresses the **prevalence of infected ticks in the area** and the **probability of the *Borrelia* transmission after a tick bite** depending on	**Transmission rate = Lyme disease incidence** (defined as EM, asymptomatic seroconversion (AS), or both).	The overall **prevalence of *B. burgdorferi* in ticks was 14% (32 out of 227);** however, in the sample subjected to scutal index measurement, the prevalence was only 1.8% (2 out of 109)**.**

| | | | | followed for Lyme disease symptoms.

Ticks were identified, measured for engorgement, and assayed by PCR for *B. burgdorferi*.
Duration of attachment was determined from the <u>scutal index</u> of engorgement. | attachment duration and the life stage of a tick. | **Prevalence (%) of ticks tested positive** for *B. burgdorferi*.

<u>Note</u>: If subjects with AS were excluded, the rates would be 1.8% (2 out of 109) in the tested and 0.9% (2 out of 225) in the total sample, and 6.7% (1 out of 15) for ≥72 h vs. 1.1% (1 out of 94) for <72 h attachment duration. For patients after a tick-test-(+) bite, the rate would be 50% (1 out of 2), and after a tick-test-(-) bite it would be 0.9% (1 out of 107). | The duration of attachment by history had a poor correlation with that obtained by scutal index measurement (<50% of subjects were able to estimate the duration of attachment within a 24-h range).
Overall, the Lyme disease incidence rate was 3.7% in subjects with tested sera (4 Lyme disease cases out of 109 bites) **or 1.8%** (4 out of 225) **of the total sample**. The incidence was significantly higher for duration of attachment **≥72 h (20%,** or 3 out of 15) than for **<72 h (1.1%,** or 1 out of 94) (*P* =0.008).
The difference between the rates for **adult vs. nymphal** ticks was **not significant** (4% vs. 3.4%).

4-6 weeks after bite:
Patients bitten by <u>ticks tested positive</u> for *Borrelia* infection: **100%** (2 out of 2).
Patients bitten by <u>ticks tested negative</u> for Borrelia infection: **1.9%** (2 out of 107).

The study concluded that "**tick identification and measurement of engorgement can be used to identify a small, high-risk subset of persons who may benefit from antibiotic prophylaxis.**" |
| Falco, 1996;

NY, USA | Cross-sectional study and animal study | NA | Feeding times were calculated for 744 *I. scapularis* ticks submitted by bite victims between 1985 and 1989 in Westchester | **Determined mean scutal index** (*the ratio between tick abdominal length and scutum width*) values for 0, 24, 48, 72, and 96 h attachment | The duration of attachment (as determined by scutal index) influences the **probability of *Borrelia* transmission after a tick bite** and depends on the | **Mean scutal indices values** for adult and nymphal ticks.

Attachment duration of nymphal vs. adult ticks collected from humans. | In animal studies, there was a significant effect of attachment time on scutal index (significant differences in scutal indices at 24 vs. 48, 48 vs. 72, and 72 vs. 96 hours), except for the comparison of the ticks measured at 0 vs. 24 hours. |

			County, New York	intervals (animal studies); **Feeding times were calculated for nymphal** (N=444) **and female** (N=300) **ticks** collected from humans in an endemic area.	stage of the tick (nymph vs. adult)		There was a **significant difference between nymphal (mean = 34.7 hours) and female (mean = 28.7 hours) attachment times** of ticks found on humans, with 73% of the females but only 54% of nymphs removed within 24 hours, meaning that adult ticks are found and removed sooner than nymphs.
Yeh, 1995; RI, PA; USA	Cross-sectional study and animal study	NA	Feeding times were calculated for *I. scapularis* ticks submitted by bite victims in selected RI and PA communities	**Determined three engorgement indices** (the ratios between total body length and width as well as the length and width of the scutum) values for 0, 12, 24, 36, 48, 60, and 72 h after tick attachment (animal studies); **Feeding times were calculated for nymphal and adult ticks** collected from humans in an endemic area.	The duration of attachment (as determined by scutal index) influences the **probability of *Borrelia* transmission after a tick bite** and depends on the stage of the tick (nymph vs. adult)	**Mean values for engorgement indices** for adult and nymphal ticks. **Attachment duration of nymphal vs. adult ticks** collected from humans.	In animal studies, there was no detectable change in the mean engorgement indices at 0 vs. 24 h of attachment, but indices for nymphs attached for 36, 48, and 60 h were significantly different from those attached for ≤24 h and from each other. For adult ticks, the indices of the ticks attached for ≤36 h were significantly different from those attached for ≥48 h. More than 60% of tick-bite victims removed **adult ticks** by 36 h of attachment, but only 10% found and removed the smaller **nymphal ticks** within the first 24 h of tick feeding.
Schwameis, et al. 2017 Germany and Austria	Randomized, double-blind, placebo-controlled trial	Low risk of bias	Adults between 18–79 years of age who presented within 72 hours after noticing a tick bite, who either left the tick attached or collected it. Excluded: People who had documented Lyme disease in the	**Topical 10% Azithromycin:** administered twice per day on the tick bite site for 3 consecutive days. Each dispensed drop was supposed to cover an approximate area of 1 cm in diameter and treated surface was to	Primary efficacy endpoint was **treatment failure at 8 weeks.** "Treatment failure was defined as seroconversion (IgM, IgG, or both), appearance of erythema migrans throughout the study participants seronegative at baseline (IgM, IgG, or both), or both	Proportion of participants (intention-to-treat population) who were bitten by infected ticks: ● Azithromycin: 17% ● Placebo: 17% ● Total: 17% Proportion of participants (ITT population) reporting treatment failure-	"Topical azithromycin was well tolerated and had a good safety profile. Inclusion of asymptomatic seroconversion into the primary efficacy analysis led to no prevention effect with topical azithromycin. Topical treatment with 10% azithromycin did not lead to prevention of erythema migrans, seroconversion, or both, 8 weeks after a tick bite when compared with placebo. A subgroup analysis in this study suggested

					seroconversion and erythema migrans throughout the study." Serum samples were assessed for presence of *B. burgdorferi* by ELISA, confirmed by Western Blot. **Ticks were collected and tested for presence of *B. burgdorferi* sensu lato by PCR.**	seroconversion, EM, or both-**at 8 weeks**: ● Azithromycin: 2.2% ● Placebo: 2.2% Proportion of participants (ITT population) reporting EM **at 30 days**: ● Azithromycin: 0.4% ● Placebo: 1.6% Proportion of participants (ITT population) reporting treatment failure-seroconversion, EM, or both-at 8 weeks who were bitten by infected ticks: ● Azithromycin: 3/11, 27.3% ● Placebo: 9/11, 81.8% Proportion of participants (ITT population) reporting EM **at 30 days** who were bitten by infected ticks: ● Azithromycin: 0/2, 0% ● Placebo: 7/8, 87.5%	that topical azithromycin reduces erythema migrans after bites of infected ticks." The results of the study suggest that Azithromycin was more effective in preventing erythema migrans by 30 days among the group of participants who had been confirmed to have been bitten by infected ticks.

(Continued from previous row; left columns:)

previous 12 months, who were seropositive in the previous 2 years, or who had a history of tick bite in the past 60 days.

be left uncovered for 30 minutes until dry.

Placebo: "Azithromycin and placebo preparations were identical in appearance (colorless ethanol-based gel formulations), odor, and feel, and provided in identical packaging that administered gel in individual drops."

Briciu, 2016 Romania	Prospective cohort	6	386 patients who presented after a tick bite at the Clinical Hospital of Infectious Diseases. Ticks were identified and tested for *B. burgdorferi sensu lato* infection using **PCR**.	Amoxicillin	Patients were followed up for Lyme disease symptoms. Sera were tested at baseline and at 1 year (2-tiered test: ELISA and immunoblot) for seroconversion.	**Symptoms of Lyme disease** (according to European guidelines – Stanek, 2011) **and/ or seroconversion** at one-year follow-up. Note: 18 out of 20 tick-test (+) and 19 out of 20 tick-test (-) patients	Overall, 11% of ticks were positive for *B. burgdorferi s.l.* (43 out of 389) (mainly *B. afzelii*, but *also B. garinii, B. burgdorferi sensu stricto, B. spielmanii/ B. valaisiana and B. lusitaniae*). **Lyme disease (EM) incidence rate** 1 year after bite: Patients bitten by ticks tested positive for *Borrelia* infection: **10%** (2

			20 out of 38 patients bitten *by B. burgdorferi*-infected ticks and a control group (age, sex, and residence-matched individuals bitten by *B. burgdorferi*-negative ticks, **N=20**) were followed up for 1 year		received antibiotic prophylaxis after the bite, which may have biased the results. Also, additional tick bites were recorded during the long follow-up – another source of bias.	out of 20) – both after prophylaxis with amoxicillin. Patients bitten by ticks tested negative for *Borrelia* infection: **0%** (0 out of 20). **Seroconversion** after 1 year: Patients bitten by ticks tested positive: **10%** (2 out of 20) Patients bitten by ticks tested negative: **15%** (3 out of 20).
Tijsse-Klasen, 2011 Netherlands	Prospective cohort	4	246 participants who had consulted a general practitioner for tick bites were included. Ticks were identified and tested for *Borrelia burgdorferi s.l., Rickettsia spp., Babesia spp.* or *Ehrlichia/Anaplasma spp* infection using **PCR**.	NA	Patients received an initial questionnaire concerning number of tick bites and duration of tick attachment. Patients were followed up approximately 6 months later and received a questionnaire assessing the presence of Lyme disease symptoms.	Possible symptoms related to the tick bite included local redness or erythema migrans and systemic symptoms as fever, malaise, palpitations, joint problems, or neurological symptoms	Overall, 16% of ticks were positive for *B. burgdorferi s.l.* ≥6 months after tick bite, 14 out of 193 participants (8.3%) reported reddening at the bite site and 6 participants (4.1%) reported systemic symptoms. No association between symptoms and tick-borne microorganisms was found. Attachment duration ≥24 h was positively associated with reddening at the bite site and systemic symptoms.
Hofhuis, 2013 Netherlands	Prospective cohort	8	644 patients who consulted a cooperating general practitioner for a recent EM or tick bite were included. 361 cases (55%) consulted their physician for a tick bite, and 283 (43%) consulted their physician at baseline with an EM that was	Among the 361 cases that consulted their physician for a tick bite, 34 (9%) had received antibiotics at baseline.	Blood samples were collected at baseline and ticks were removed. Over half (56%) of the cases reported tick removal within 24 hours. Ticks removed from patients at baseline were tested for presence of *Borrelia* by PCR. Patients also received a questionnaire about baseline data.	*Borrelia* infection was defined as development of physician-confirmed clinical Lyme borreliosis such as EM, or seroconversion for *Borrelia*-specific antibodies confirmed by two-tiered testing via C6 ELISA and IgM/IgG Immunoblots.	*Borrelia burgdorferi* sensu lato DNA was detected in 92 out of 314 ticks collected (29.3%). Seroconversion for *Borrelia*-specific antibodies was observed in 3.2% of tick bite cases. Fourteen tick bite cases had evidence of early *Borrelia* infection, of which EM developed among seven cases. The risk of developing EM after tick bites was 2.6% (95%CI: 1.1%–5.0%), and the risk of either EM or seroconversion was 5.1% (95%CI: 2.9%–8.2%). **Participants**

			undisputed by their physician		A follow-up questionnaire was sent and blood samples were collected 3 months later.		**with *Borrelia*-positive ticks had a significantly higher risk of either EM or seroconversion (odds ratio 4.8, 95%CI: 1.1–20.4).**
Hofhuis, 2017 Netherlands	Pooled analysis of Hofhuis 2013, Wilhelmsson 2016, and Sprong 2013 (Parasit Vectors. 2013 Dec 4;6:338)	NA	3,525 single tick bite reports extracted from three large prospective studies, with 50 reports of Lyme borreliosis during the follow-up period, among 1,973 reports with known outcome.	ND	All study participants were asked to fill out questionnaires at enrollment and at follow-up, including questions on duration of tick attachment in the skin, the number of tick bites, and development of Lyme borreliosis.	Lyme borreliosis within three months after a tick bite was categorized as "physician-confirmed erythema migrans" or "physician-confirmed disseminated Lyme borreliosis" when patient-reported Lyme borreliosis was confirmed by the general practitioner through an additional questionnaire.	The overall risk of developing Lyme borreliosis after a tick bite was 2.6% (95%CI 1.4–5.1). The risk increased with degree of tick engorgement, increased duration of tick attachment, and detection of *Borrelia burgdorferi* s.l. DNA in ticks. Detection of Borrelia DNA in the tick was the strongest and statistically significant predictor for the risk of developing Lyme borreliosis.

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk". Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

IV. What is the preferred antibiotic regimen for the chemoprophylaxis of Lyme disease following a high-risk tick bite?

PROVHYLACTIC ANTIBIOTIC THERAPY vs. NO TREATMENT

In patients following a high-risk tick bite, should prophylactic antibiotic therapy be used over none?

P: In patients following a high-risk tick bite

I: Prophylactic antibiotic therapy

C: No treatment

Bibliography: 1. Agre, et al. Am J Dis Child. 1993 Sep;147(9):945-7 (PEDIATRIC); 2. Costello, et al. J Infect Dis. 1989 Jan;159(1):136-9; 3. Nadelman, et al. N Engl J Med. 2001 Jul 12;345(2):79-84; 4. Shapiro, et al. N Engl J Med. 1992 Dec 17;327(25):1769-73.

Certainty assessment							№ of events/№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Antibiotic prophylaxis	Placebo	Relative (95% CI)	Absolute (95% CI)		
Clinical evidence of Lyme disease after treatment *												
4	RCT [1-4]	not serious	not serious	not serious	not serious [a]	none	3/543 (0.6%)	16/539 (3.0%)	**RR 0.27 (0.10 to 0.75)**	**22 fewer per 1,000 (from 7 fewer to 27 fewer)**	⊕⊕⊕⊕ HIGH	CRITICAL
Seroconversion post-treatment												
3	RCT [1,2,4]	not serious	not serious	serious [b]	serious [c]	none	0/281 (0.0%)	5/263 (1.9%)	NA [e]	19 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Dermatologic Adverse Events												
4	RCT [1-4]	not serious	not serious	not serious	serious [c]	none	3/321 (0.6%)	1/301 (0.2%)	RR 1.75 (0.29 to 10.63)	2 more per 1,000 (from 2 fewer to 21 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Total Adverse Events												

1	RCT [3]	not serious	not serious	not serious	not serious [d]	none	47/156 (30.1%)	17/153 (11.1%)	**RR 2.71 (1.63 to 4.51)**	**190 more per 1,000 (from 70 more to 390 more)**	⊕⊕◯◯ HIGH	IMPORTANT
Adverse Events- Diarrhea											
1	RCT [3]	not serious	not serious	not serious	serious [c]	none	6/156 (3.8%)	6/153 (3.9%)	RR 0.98 (0.32 to 2.97)	1 fewer per 1,000 (from 27 fewer to 77 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Serious Adverse Events											
2	RCT [3,4]	not serious	not serious	not serious	serious [c]	none	0/361 (0.0%)	0/335 (0.0%)	NA [e]	0 per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT

* Clinical evidence is defined as erythema migrans and/or flu-like symptoms or febrile illness accompanied by seroconversion.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Not rated down for imprecision since OIS criteria met (OIS is 481 in each arm, with an effect of antibiotics from 3 to 0.6%: 80%, power: 0.8, alpha: 0.05).
b. Surrogate for clinical evidence of Lyme disease.
c. 95% CI is wide and crossing the null value, and few numbers of events reported.
d. Not downgraded for fragility (fragility index = 14).
e. Due to zero events, unable to estimate relative risk.

PROPHYLACTIC ANTIBIOTIC THERAPY with DOXYCYCLINE vs. NO TREATMENT

In patients following a high-risk tick bite, should prophylactic antibiotic therapy with Doxycycline be used over none?

P: In patients following a high-risk tick bite

I: Prophylactic antibiotic therapy with Doxycycline

C: No treatment

Bibliography: 1. Nadelman, et al. N Engl J Med. 2001 Jul 12;345(2):79-84.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline prophylaxis	Placebo	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Clinical evidence of Lyme disease after treatment *												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	3/235 (1.3%)	11/247 (4.5%)	RR 0.29 (0.08 to 1.01)	**32 fewer per 1,000 (from 2 fewer to 61 fewer)**	⊕⊕⊕◯ MODERATE	CRITICAL
Seroconversion post-treatment												
1	RCT [1]	not serious	not serious	serious [b]	serious [c]	none	0/30 (0.0%)	4/90 (4.4%)	NA [d]	44 fewer per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT
Dermatologic Adverse Events												
1	RCT [1]	not serious	not serious	not serious	serious [c]	none	0/156 (0.0%)	0/153 (0.0%)	NA [d]	0 per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT
Total Adverse Events												
1	RCT [1]	not serious	not serious	not serious	not serious [e]	none	47/156 (30.1%)	17/153 (11.1%)	**RR 2.71 (1.63 to 4.51)**	**190 more per 1,000 (from 70 more to 390 more)**	⊕⊕⊕⊕ HIGH	IMPORTANT
Adverse Events-Diarrhea												
1	RCT [1]	not serious	not serious	not serious	serious [c]	none	6/156 (3.8%)	6/153 (3.9%)	RR 0.98 (0.32 to 2.97)	1 fewer per 1,000 (from 27 fewer to 77 more)	⊕⊕⊕◯ MODERATE	IMPORTANT

Serious Adverse Events											
1	RCT [1]	not serious	not serious	not serious	serious [c]	none	0/156 (0.0%)	0/153 (0.0%)	NA [d]	0 per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT

* Clinical evidence is defined as erythema migrans and/or flu-like symptoms or febrile illness accompanied by seroconversion.

CI: Confidence interval; **RR:** Risk ratio

Explanations
a. Fragility due to low event rate.
b. Surrogate for clinical evidence of Lyme disease.

c. Due to low event rate.
d. Due to zero event, unable to estimate relative risk.
e. Not rated down for imprecision due to fragility due to the large effect size (fragility index = 14).

PROPHYLACTIC ANTIBIOTIC THERAPY with a β-lactam vs. NO TREATMENT

In patients following a high-risk tick bite, should prophylactic antibiotic therapy with a β-lactam be used over none?

P: In patients following a high-risk tick bite

I: Prophylactic antibiotic therapy with a β-lactam

C: No treatment

Bibliography: 1. Agre, et al. Am J Dis Child. 1993 Sep;147(9):945-7 (PEDIATRIC); 2. Costello, et al. J Infect Dis. 1989 Jan;159(1):136-9; 3. Shapiro, et al. N Engl J Med. 1992 Dec 17;327(25):1769-73.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	β-lactam antibiotic prophylaxis	Placebo	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Clinical evidence of Lyme disease after treatment												
3	RCT [1-3]	not serious	not serious	not serious	serious [b]	none	0/278 (0.0%)	5/292 (1.7%)	NA [c]	17 fewer per 1,000	⊕⊕⊕◯ MODERATE	CRITICAL
Seroconversion post-treatment												
3	RCT [1-3]	not serious	not serious	serious [a]	serious [b]	none	0/251 (0.0%)	5/263 (1.9%)	NA [c]	19 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Dermatologic Adverse Events												
3	RCT [1-3]	not serious	not serious	not serious	serious [b]	none	3/291 (1.0%)	1/301 (0.3%)	RR 1.99 (0.33 to 12.08)	3 more per 1,000 (from 2 fewer to 37 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Serious Adverse Events												
1	RCT [3]	not serious	not serious	not serious	serious [b]	none	0/205 (0.0%)	0/182 (0.0%)	NA [c]	0 per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT

* Clinical evidence is defined as erythema migrans and/or flu-like symptoms or febrile illness accompanied by seroconversion.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Surrogate for clinical evidence of Lyme disease.

b. Due to low event rate.

c. Due to zero events, unable to estimate relative risk.

Early localized Lyme disease (erythema migrans)

V. What is the preferred diagnostic testing strategy for erythema migrans?

Serologic testing, skin or blood PCR, direct microscopic detection, spirochetal culture of blood, empiric therapy, spirochetal culture of skin biopsy

I. **Systematic reviews and meta-analyses (serology)**

Bibliography: 1. Waddell et al. *PLoS One*. 2016 Dec 21;11(12):e0168613. doi: 10.1371/journal.pone.0168613. eCollection 2016; 2. Leeflang et al. *BMC Infect Dis*. 2016 Mar 25;16:140. doi: 10.1186/s12879-016-1468-4.

Name	Study Design	Search dates; Inclusion/ Exclusion criteria	Limitations	N of studies included	Results (Number of studies = N; Sensitivity=Sn, %; Specificity=Sp, %; (95% CI))
Waddell, 2016	A systematic review and meta-analysis	**1995 – Sep. 2013**; Included **all North American diagnostic test studies that compared results of one test using a validated test panel, results of clinical diagnosis, or a gold standard test result or investigated inter-test agreement**. No studies were excluded based on their quality assessment. The **included tests were evaluated in the context of clinical diagnosis or compared with one another**. The Early/acute stage was defined as disease duration of <30 days and included erythema migrans (EM). Meta-analysis was conducted using hierarchical logistic	1. Did not include studies originating from outside of the US and Canada; 2. Broad inclusion criteria: many studies with risks of bias in the selection, performance (inadequate blinding), reporting, and funding domains. 3. Included studies evaluating in-house tests; however, heterogeneity analyses on the impact of the non-commercial tests were performed, where applicable.	**48**; all originating from **the US**; study quality was evaluated with QUADAS-2 tool (**8** deemed to have **low** and **40 - unclear risk of bias**)	1. **Two-tier test vs. clinical diagnosis** (total N=13; 9 with at least one in-house test): **Stage 1 (Early acute)** (N=10): **Sn 46.3%** (39.1; 53.7); **Sp 99.3%** (98.3; 99.7); **Convalescent Lyme (treated at stage 1)** (N=3): **Sn 58.2%** (46.4; 69.2); **Sp 99.1%** (97.8; 99.6); "At the early stage of LD **the two-tier testing method was good for ruling in LD if the patient tested positive, but had very poor predictive value for ruling out LD**, which is why it is recommended to retest after 30 days. However, for **convalescent patients treated at stage 1 LD sensitivity remained low even after 30 days**." 2. **EIA (1st tier tests, including ELISA) vs. clinical diagnosis** (total N=24; mix of FDA-licensed and in-house tests): "Similar to the two-tiered tests, [**EIA] test performance for patients with stage 1 LD was highly variable and had poor sensitivity**." **Stage 1 (Early acute)** (N=15): **Sn 54.0%** (42.9; 64.8); **Sp 96.8%** (95.0; 98.0); **Convalescent Lyme (treated at stage 1)** (N=5): **Sn 77.8%** (69.5; 84.3); **Sp 98.8%** (98.4; 99.1); 3. **Immunoblots vs. clinical DS** (total N = 9; 1 in-house): results generally reported per individual test and across all stages. **The CDC-recommended WB algorithm had equivalent or superior specificity over**

		regression and bivariate models that account for the correlation between sensitivity and specificity.		other proposed test algorithms (N=2). For **Early stage and Western Blots (Marblot/ GenBio)**, **Sn was 60.6%** (42.6; 76.0), and **Sp 98.8%** (91.9; 98.7); 4. **Direct detection of *B. burgdorferi* by bacterial isolation or PCR vs. clinical diagnosis** (total N=7): meta-analysis was not possible. **These methods were not as sensitive or timely as the serological methods (2-tier protocol).** *B. burgdorferi* isolation **from blood** using BSK medium in **early LB stages** (N=3): **Sn** were reported as **27%, 71%, 94%,** with the latter result disputed; *B. burgdorferi* isolation from tissues **(EM biopsies)** (small samples; N=2) had reported **Sn of 62-81%.** PCR was used in **early LB** (N=3). **Multi-loci PCR of blood** had **Sn 62%** and **Sp 100%** (N=1); a **nested PCR** had **Sn of 40.6%** in **serum** and **42.6%** in **biopsy samples**, and a **qPCR of plasma** had **Sn of 33.8%** (N=1). **"Across the direct detection studies sensitivity was low and in most cases lower than the two-tier test regime, assays or immunoblots reported for early LD."** 5. **Inter-test comparisons** (total N=22), including 2-tier vs. other tests (N=8): reported results as positive and negative agreements (how well the compared tests agreed to classify samples as positive/negative). **The C6 ELISAs, particularly the commercial assays, had promising sensitivity, specificity and agreement of results with two-tier protocols.** **Overall, in early Lyme disease serological tests had poor and highly variable sensitivity, with the two-tier method associated with higher specificity** (*see Tables below*).

| Leeflang, 2016 | A systematic review and meta-analysis | ? – Feb. 2014; the oldest included study was published in 1987. Included **all European studies evaluating the diagnostic accuracy of serologic assays for LB** against a reference standard for clinical criteria (sometimes combined with positive serology).

Meta-analysis was performed using Hierarchical Summary ROC (HSROC) model, a hierarchical meta-regression method incorporating both sensitivity and specificity while taking into account the correlation between the two. | 1. Evaluated only serologic tests;
2. Only European studies;
3. Studies that reported "possible" or "suspected" Lyme patients were included with these patients counted as "cases";
4. Included studies had high levels of heterogeneity and bias.
5. Indirect fluorescent antibody assays were not evaluated because of the rare use in practice;
6. The included studies did not represent the tests in true clinical settings | **75**; all originating from **Europe**; study quality was evaluated with QUADAS-2 tool; no studies had low bias risk in all four QUADAS-2 domains. | **Sensitivity** was highly heterogeneous, with summary estimates:
1. Erythema migrans: overall (ELISA or immunoblot) Sn 50 % (95 % CI 40 %; 61 %); **Sp 95%** (92; 97); ELISA had a higher accuracy than immunoblot, mostly due to higher sensitivity. Commercial tests did not significantly differ from in-house ones. IgG were less sensitive than IgM. **Two-tiered tests** (N=2) **had Sn range from 12% to 64%** and **Sp 67-96%** (meta-analysis not performed).
Two-tiered algorithms or antibody indices **did not outperform single test** approaches.
See Tables below. |

Table I.1 (duplicated from Table 8, Waddell et al., 2016)

Summary of the sensitivity and specificity of different testing options for early Lyme disease (stage 1) patients.

Description	Studies (lines)	Sn estimate	Sp estimate
Two-tier testing [*]	**10 (19)**	**46.3 (39.1–53.7)**	**99.3 (98.3–99.7)**
Cambridge[FDA] and inhouse IB	1 (1)	69.2[‡]	100[‡]
Vidas[FDA/HC] or Wampole[FDA] and Marblot [FDA/HC]	2 (3)	32–41[‡]	99.5–100[‡]
Vidas[FDA/HC] or Wampole[FDA] and Virablot[FDA]	2 (5)	34.4 (27.7, 41.6)	100.0 (97.5, 100.0)
Vidas[FDA/HC] or Wampole[FDA] and Immunetics C6 Lyme [FDA/HC]	1 (1)	61[‡]	99.5[‡]
Zeus ELISA [FDA/HC] and Zeus AtheNA[FDA]	1 (1)	45.7[‡]	99.6[‡]

Zeus ELISA and Marblot [FDA/HC]	1 (1)	39.2[‡]	99.6[‡]
Immunetics C6 and Marblot [FDA/HC]	2 (2)	37.6–76.9[‡]	99.5–100[‡]
Liason and Marblot [FDA/HC]	1 (1)	61.5[‡]	100[‡]
First tier EIAs[*]	**16 (48)**	**54.0 (42.9, 64.8)**	**96.8 (95.0, 98.0)**
ELISA- C6 target	7 (11)	57.1 (46.7, 66.9)	97.5 (96.2, 98.5)
Commercial [FDA/HC] 3 (4)		65.6 (61.2, 69.7)	98.7 (98.3, 99.0)[¥]
In house 3 (6)		48.4 (37.1, 59.8)	96.1 (93.5, 97.8)[¥]
ELISA- WCS	6 (10)	77.5 (59.5, 89.0)	87.8, (73.9, 94.8)
Commercial [FDA/HC] 3 (6)		65.0 (47.3, 79.4)	94.5 (89.7, 97.3)
In house 3 (4)		94.0 (54.0,100)	61.0 (53.0,69.0)
Liason System *Borrelia Burgdorferi* (diasorin)[FDA/HC]	1 (1)	64.4[‡]	98.0[‡]
ELISA–Osp A-F targets in house 6 (22)		33.3 (19.3, 51.1)	97.5 (94.8, 98.9)
PEG peptide–ELISA in house 1 (1)		100[‡]	100[‡]
IHA (B126 or B31) in house 1 (2)		46–48[‡]	98–99[‡]
BAT (B297 or 50772) in house 1 (1)		72[‡]	99[‡]
Western blots (Marblot/ GenBio)[*]	**4 (8)**	**60.6 (42.7, 76.0)**	**96.8 (91.9, 98.7)**
Direct Detection			
Culture biopsies	2 (2)	61.8–80.8[‡]	NA
Culture blood	3 (3)	26.9–94[‡]	NA
PCR biopsies	1 (1)	42.6[‡]	NA
PCR blood (serum/plasma)	2 (3)	33.8–62[‡]	NA

Sn estimate/ Sp estimate are from the meta-analysis bivariate model unless otherwise noted.

* Summary sensitivity and specificity across all tests on early LD.

‡ Value or range of values for sensitivity and specificity as reported by the author.

Sn = sensitivity, Sp = specificity, ELISA = enzyme-linked immunosorbent assay.

H Based on I^2, a measure of between study heterogeneity, the heterogeneity in this group of studies was <60%, thus considered to be homogenous.

FDA = Food and Drug Administration approved, HC = Health Canada approved, NC = non-commercial.

Vidas = Vidas Lyme Screen, Wampole = Wampole Bb (IgG/IgM) ELISA test system, Marblot = MarDx Lyme Disease (IgG and IgM) Marblot Strip Test System, Virablot = ViraMed Biotech *Borrelia* B31 (IgG or IgM) Virablot, Immunetics C6 = Immunetics® C6 *B. burgdorferi* ELISA™, Cambridge = Cambridge, Human Lyme EIA for detection of antibodies, IB = immunoblot, Zeus ELISA = Zeus Lyme IgG or IgM ELISA Test system, Zeus AtheNa = Zeus AtheNA Muti-Lyte test system, Liason = Liason *Borrelia* IgG /IgM assay model 310870 (CLIA)
IHA = indirect hemagglutination antibody test, Osp = Outer surface protein.
One study (1 line of data) was excluded from the analyses (Liang 1999, PMID: 10565920) because there was no specificity reported in the paper.

Table I.2 (adapted from Table 3, Leeflang et al., 2016)

Summary estimates of sensitivity and specificity for all case-definitions, derived from a hierarchical summary ROC model.

Case definition	Assay	Design	N (studies); N(2×2 tables); N(cases); N(controls)	Sensitivity (95 % CI)	Specificity (95 % CI)	Heterogeneity	Quality and Study Design
Erythema migrans	In-house ELISA	Case-control, Healthy controls	6, 10, 451, 658	0•41 (0•25 to 0•60)	0•97 (0•95 to 0•98)	IgG lower sensitivity than IgM. Other sources of heterogeneity were not found.	Study quality did not influence the accuracy
	In-house IB		3, 3, 182, 380	0•52 (0•38 to 0•65)	0•98 (0•94 to 0•99)		
	Commercial ELISA		13, 32, 874, 2509	0•54 (0•44 to 0•65)	0•93 (0•90 to 0•95)		
	Commercial IB		3, 5, 161, 289	0•58 (0•49 to 0•67)	0•86 (0•75 to 0•93)		
	Two-tiered tests		2, 7, 125, 190	range 0•12 to 0•64	range 0•67 to 0•96		

ELISA = Enzyme Immuno Assay, *IB* = Immunoblot, *AI* = Antibody Index, *CSF* = Cerebrospinal Fluid

Table I.3 (adapted from Table 5, Leeflang et al., 2016)

Generation of antigens

	Antigen	Sensitivity (95 % CI)	Specificity (95 % CI)
Erythema migrans	Whole cell	0.515 (0.328 to 0.699)	0.957 (0.899 to 0.983)
	Purified	0.579 (0.466 to 0.685)	0.950 (0.895 to 0.977)
	Recombinant	0.551 (0.330 to 0.753)	0.947 (0.881 to 0.977)

95 % CI = 95 % confidence interval.

Bibliography: 1. Branda, et al. Clin Infect Dis. 2017 Apr 15;64(8):1074-1080; 2. Bradshaw, et al. J Clin Microbiol. 2017 Oct;55(10):3046-3056; 3. Molins, et al. J Clin Microbiol. 2017 Jun;55(6):1698-1706; 4. Pomelova, et al. Biomed Res Int. 2018 Jun 28;2018:5291926; 5. Wormser, et al. Diagn Microbiol Infect Dis. 2018 Jul;91(3):217-219; 6. Zwerink, et al. Ticks Tick Borne Dis. 2018 Mar;9(3):594-597.

Study; Location	Study Design	Population Characteristics	Diagnosis Method, % Positive	Study Conclusions

Branda, et al., 2017
 	
Nantucket, MA and Wakefield, RI
	Case-control study	Serum samples were collected from patients in hyper-endemic regions who had erythema migrans (EM) diagnosed by a physician (confirmed by culture in 62%). "Samples were collected during the summer tick-transmission seasons in 2012 or 2015. Acute-phase serum samples (N=55) were obtained at initial presentation; convalescent-phase samples (N=47) were obtained after the completion of standard oral antimicrobial regimens, 3–6 weeks after study entry."
		
		Two groups of control patients: (1) patients who had been referred with possible Lyme (N=50), but were diagnosed with other illnesses; (2) healthy, asymptomatic blood donors (N=1,227).
		
		Samples were analyzed using the C6 *B. burgdorferi* EIA (Immunetics), and the Liaison *B. burgdorferi* CLIA (DiaSorin), as well as 1 of 2 polyvalent Whole Cell Sonicate (WCS) EIA's. "Western Blots were performed
			First-Tier Tests:
			● *WCS EIA*
			  ○ Sensitivity Acute Phase EM: 49%
			  ○ Sensitivity Convalescent Phase EM: 77%
			  ○ Specificity: All controls- 97.4%; Other Illnesses- 78%; Asymptomatic- 98.2%
			● *C6 EIA*
			  ○ Sensitivity Acute Phase EM: 65%
			  ○ Sensitivity Convalescent Phase EM: 81%
			  ○ Specificity: All controls- 98.4%; Other Illnesses- 98%; Asymptomatic- 98.5%
			● *VlsE CLIA*
			  ○ Sensitivity Acute Phase EM: 55%
			  ○ Sensitivity Convalescent Phase EM: 72%
			  ○ Specificity: All controls- 98.1%; Other Illnesses- 96%; Asymptomatic- 98.2%
			
			Two-Tiered Testing with Western Blots:
			● *WCS EIA→Western Blot*
			  ○ Sensitivity Acute Phase EM: 25%
			  ○ Sensitivity Convalescent Phase EM: 55%
			  ○ Specificity: All controls- 99.5%; Other Illnesses- 100%; Asymptomatic- 99.4%
			● *C6 EIA→Western Blot*
			  ○ Sensitivity Acute Phase EM: 36%
			  ○ Sensitivity Convalescent Phase EM: 60%
			  ○ Specificity: All controls- 99.7%; Other Illnesses- 100%; Asymptomatic- 99.7%
			● *VlsE CLIA→Western Blot*
			  ○ Sensitivity Acute Phase EM: 35%
			  ○ Sensitivity Convalescent Phase EM: 57%
				The authors concluded that a Modified Two-Tiered Testing (MTTT) protocol involving the WCS EIA followed by a C6 EIA provides similar or greater sensitivity than Two-tiered protocols involving Western Blots, while maintaining a comparable specificity, in patients with acute or convalescent-phase erythema migrans (EM). They noted no significant difference in sensitivity or specificity when the the C6 EIA was replaced by the VlsE CLIA in this MTTT protocol.
				
				"The highest sensitivity in patients with acute EM was obtained using a third MTTT protocol, involving a VlsE CLIA as the first-tier test, and a C6 EIA as the second-tier test. This was expected; the other 2 MTTT protocols used a WCS EIA in the first tier, and this assay was less sensitive as an individual test compared with the VlsE CLIA or C6 EIA in patients with acute EM. Moreover, the MTTT protocol using a VlsE CLIA followed by a

		using *Borrelia* B31 IgM and IgG ViraBlot or ViraStripe test strips (Viramed Biotech AG)."	o Specificity: All controls- 99.7%; Other Illnesses- 100%; Asymptomatic- 99.7% Modified Two-Tiered Testing: ● *WCS EIA→C6 EIA* o Sensitivity Acute Phase EM: 38% o Sensitivity Convalescent Phase EM: 72% o Specificity: All controls- 99.5%; Other Illnesses- 100%; Asymptomatic- 99.4% ● *WCS EIA→VlsE CLIA* o Sensitivity Acute Phase EM: 36% o Sensitivity Convalescent Phase EM: 66% o Specificity: All controls- 99.5%; Other Illnesses- 100%; Asymptomatic- 99.4% ● *VlsE CLIA→C6 EIA* o Sensitivity Acute Phase EM: 54% o Sensitivity Convalescent Phase EM: 72% o Specificity: All controls- 99.3%; Other Illnesses- 98%; Asymptomatic- 99.4%	C6 EIA was similarly specific compared with the other MTTT protocols or with 2-tiered protocols involving Western Blots." The authors conclude that MTTT protocols provide reliable results regarding seropositivity in the early stages of Lyme disease and suggest that any one of the MTTT protocols assessed would be an adequate substitute for conventional Two-Tiered testing methods.
Bradshaw, et al., 2017	Blind laboratory study	279 human sera from the CDC gathered from Lyme disease patients and negative control subjects. 89 of these sera were from physician-diagnosed Lyme-positive patients. The remaining sera included 100 samples from healthy individuals (50 from areas where LD is not endemic and 50 from areas where it is endemic) and 90 sera from patients with fibromyalgia (n= 15), mononucleosis (n= 15), multiple sclerosis (n= 15), periodontitis (n= 15), rheumatoid arthritis (n= 15), and syphilis (n= 15).	Stage 1 Lyme Disease: ● DpbA/C6-OspC Sensitivity (IgG/IgM): 80% ● 2-tier Sensitivity (IgG/IgM): 63% Stages II and III Lyme Disease: ● DpbA/C6-OspC Sensitivity (IgG/IgM): 100% ● 2-tier Sensitivity (IgG/IgM): 100% Negative controls: ● DpbA/C6-OspC Specificity (IgG): 100% ● DpbA/C6-OspC Specificity (IgM): 100% ● 2-tier Specificity (IgG): 98.8% ● 2-tier Specificity (IgM): 100%	The DbpA/C6-OspC ELISA performed better (80% versus 63%) than the 2-tier test method in detecting anti-*Borrelia* antibodies in stage I Lyme patients. The authors conclude that these antigens could be used in a simple 1-tier ELISA that is faster to perform, easier to interpret, and less expensive than the 2-tier test method, in addition to being better at detecting *Borrelia*-specific antibodies.

Molins, et al., 2017	Blind laboratory study	Serum samples from 471 well-characterized Lyme patients and controls from the CDC Lyme Serum Repository (LSR) were used.	bioMérieux Vidas Lyme IgM II (LYM) EIA (1 tier) ● Early Lyme (EM, Acute), Sensitivity: 60% ● Early Lyme (EM, Convalescent), Sensitivity: 79% ● All Lyme, Sensitivity: 71% ● All Negative Controls, Specificity: 87% bioMérieux Vidas Lyme IgG II (LYG) EIA (1 tier) ● Early Lyme (EM, Acute), Sensitivity: 50% ● Early Lyme (EM, Convalescent), Sensitivity: 74% ● All Lyme, Sensitivity: 74% ● All Negative Controls, Specificity: 97% bioMérieux Vidas Lyme IgG II (LYM/LYG) EIA (2 tier) ● Early Lyme (EM, Acute), Sensitivity: 43% ● Early Lyme (EM, Convalescent), Sensitivity: 61% ● All Lyme, Sensitivity: 68% ● All Negative Controls, Specificity: 97% bioMérieux Vidas Lyme combined (LYT) EIA (1 tier) ● Early Lyme (EM, Acute), Sensitivity: 68% ● Early Lyme (EM, Convalescent), Sensitivity: 89% ● All Lyme, Sensitivity: 85% ● All Negative Controls, Specificity: 85% bioMérieux Vidas Lyme combined (LYT) EIA (2 tier) ● Early Lyme (EM, Acute), Sensitivity: 48% ● Early Lyme (EM, Convalescent), Sensitivity: 63% ● All Lyme, Sensitivity: 69% ● All Negative Controls, Specificity: 97% C6 EIA (1 tier) ● Early Lyme (EM, Acute), Sensitivity: 58% ● Early Lyme (EM, Convalescent), Sensitivity: 84% ● All Lyme, Sensitivity: 81% ● All Negative Controls, Specificity: 97%	The overall sensitivities and specificities for STTT were similar between the two testing strategies (LYT versus LYM/LYG), although differences in first-tier test results between the two were observed. A modified two-tiered (MTTT) algorithm that uses the Vidas EIAs (LYT or LYM/LYG) as the first-tier test followed by the C6 EIA as the second-tier test also gave similar sensitivities and specificities when the dissociated or combined assays were tested but resulted in significantly higher overall sensitivities than and specificities similar to those of STTT.

			C6 EIA (2 tier) ● Early Lyme (EM, Acute), Sensitivity: 43% ● Early Lyme (EM, Convalescent), Sensitivity: 63% ● All Lyme, Sensitivity: 68% ● All Negative Controls, Specificity: 99% LYT-C6 (MTTT) ● Early Lyme (EM, Acute), Sensitivity: 50% ● Early Lyme (EM, Convalescent), Sensitivity: 79% ● All Lyme, Sensitivity: 76% ● All Negative Controls, Specificity: 98% LYM/LYG-C6 (MTTT) ● Early Lyme (EM, Acute), Sensitivity: 55% ● Early Lyme (EM, Convalescent), Sensitivity: 76% ● All Lyme, Sensitivity: 77% ● All Negative Controls, Specificity: 99%	
Pomelova, et al., 2018 Perm, Russia	Laboratory study	Serum samples (n= 1089) were collected from 442 from erythema migrans (EM) (N = 327) and EM-free (EMF) patients (n= 115) originating from a highly endemic region of Russia. In some patients, the disease was accompanied by human granulocytic anaplasmosis or tick-borne encephalitis.	The sera were investigated by multiplex phosphorescence analysis (PHOSPHAN) for IgM to *B. burgdorferi* C6, recombinant OspC and VlsE proteins, and IgG to C6 from *B. burgdorferi*, *B. garinii*, and *B. afzelii*. Positive PHOSPHAN reactions were observed in 81.9% and 86.7% of the EM and EMF patients, respectively, as well as in 59 of 65 (90.8%) patients, whose blood contained *B. burgdorferi* DNA. Additional detection of IgG to *B. garinii* C6 or *B. afzelii* C6 had no significant contribution to serologic diagnosis of Ixodid Tick-Borne Borrelioses in either patient group.	Detection of Bb C6 IgM/IgG provided effective serologic confirmation of Ixodid Tick-Borne Borrelioses in both EM and EMF patients early after disease onset. In the EM-free patients, however, this test needed to be supplemented with detection of VlsE IgM in convalescent-phase sera due to delay in development of the antibody responses for C6 IgG. The authors concluded that the multiplex PHOSPHAN is a promising method for detecting IgM and IgG antibody responses to a number of *Borrelia* antigens.

Wormser, et al., 2018	Retrospective analysis	Unpublished data collected in four previously reported studies that evaluated the specificity of whole-cell sonicate-based (WCS) ELISA and the C6 peptide ELISA were analyzed (comprising over 3900 control samples). To determine if WCS ELISA and the C6 peptide ELISA are independent tests, the authors compared the specificity of the C6 peptide ELISA in control serum samples separated into those that were WCS ELISA negative and those that were WCS ELISA reactive (i.e., positive or equivalent).	The specificity of the C6 peptide ELISA was significantly lower when assessed using sera that were falsely reactive by a WCS EIA, compared with control sera that tested negative by the same WCS EIA (p≤0.0002). The reduction in specificity was approximately 16% using sera from healthy controls and 7.2% using sera from disease controls (serum donors with illnesses other than Lyme disease). The overall reduction in specificity of the C6 peptide ELISA in the total group of controls was 11.9%. The C6 peptide ELISA had significantly lower specificity in the WCS ELISA falsely reactive samples compared with the WCS ELISA-negative samples (p< 0.0001). The specificity of the C6 peptide EIA was about 5% higher in the combined group of controls after excluding these samples, but the difference was not statistically significant (p= 0.12).	This study demonstrated that the WCS ELISA and the C6 peptide ELISA for antibody to *B. burgdorferi* are not independent tests. Although the WCS and the C6 peptide ELISAs are not independent tests, the study findings indicate that a two-tier testing algorithm with these ELISAs significantly improves diagnostic specificity compared with either ELISA used alone. When using the 2-ELISA protocol of a WCS ELISA secondary to the C6 peptide ELISA, the C6 peptide ELISA should not be regarded as a confirmatory test but instead as a supplemental test.
Zwerink, et al., 2018 Apeldoorn, the Netherlands	Retrospective cohort study	1454 patients with suspected Lyme borreliosis referred to a tertiary Lyme center were included. Overall, IgG serology was positive in 33.4% (486/1454) of patients. The prevalence of positive IgG serology was higher in patients with disseminated Lyme borreliosis (68.6%) compared to localized Lyme borreliosis (23.8%) (p<0.001). The prevalence of positive IgG serology was above 90% in patients with ACA or Lyme arthritis, and below 50% in patients with LNB or multiple EM.	Two-tiered testing was performed with ELISA and Immunoblot. 486 (33.4%) patients had an indeterminate/positive IgG ELISA with a positive IgG immunoblot, 69 (4.7%) had an indeterminate/positive IgG ELISA with an indeterminate IgG immunoblot, and 899 (61.8%) had a negative IgG ELISA or negative IgG immunoblot. At the recommended lower cut-off value of<6 IU/mL, the negative predictive value (NPV) and positive predictive value (PPV) of the ELISA were 95.6% and 64.5%, respectively. The sensitivity was 95.5% and the specificity was 64.9%. At a cut-off value of 200 IU/mL the PPV was 99.3%. When 500 IU/mL and >1000 IU/mL were chosen as upper cut-off values, the PPVs of the ELISA for a positive IgG immunoblot were 100% and 100%, respectively.	At IgG levels of 200 IU/mL and higher, an ELISA was sufficient to detect antibodies to *Borrelia burgdorferi*. At those IgG levels, a confirmatory immunoblot may be omitted in patients referred to a tertiary Lyme centre.

II. Direct microscopic detection: *B. burgdorferi* cultures (skin, blood); PCR (skin, blood) – observational studies.

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Study and Location	Population / Sample Characteristics	Diagnostic Method details	Study Conclusions
Direct microscopy (Sn range 25% - 93.8%)			
Aberer, 1996; Austria	(1) Skin-punch biopsies of 19 European patients with untreated EM, 21 patients with ACA, and 60 patients with other conditions (morphea, granuloma annulare lichen sclerosus et atrophicus, basal cell carcinoma, perichondritis nodularis helicis, lupus erythematosus, lichen planus, pemphigus vulgaris, and bullous pemphigoid).	(1) **High-power resolution videomicroscopy** and **staining with** the *Borrelia* genus-specific **monoclonal flagellar antibody H972**4 (stained by an avidin biotin-immunoperoxidase method using the H9724 monoclonal Ab in PBS/1% bovine serum albumin as primary antibody 1:50). Negative controls included sections from each investigated biopsy that were not incubated with the primary antibody.	(1) In culture-positive biopsies of EM, "single or aggregated, delicate or heavily stained borrelial structures of various and sometimes bizarre morphology were discovered." *Borrelia* were detected in **25%** of ECM, ACA, and morphea cases. In biopsies of all other investigated dermatoses and in normal skin, spirochetal structures could not be identified with certainty by the H9724 monoclonal antibody. No stained borrelia structures were detected on negative control sections from which the primary antibody was omitted.
Berger, 1983; NY state, USA	18 skin biopsies from patients with ECM (n=14).	**Light microscopy** with silver stain (**Warthin-Starry stain**).	Spirochetes were found in 6 specimens from 4 patients (**Sn 28.6%**). The organisms were more likely to be found in the specimens taken from the periphery than from the center of EM lesions.
De Koning, 1987;	Skin biopsies from patients with ECM (n=10) and lymphadenosis benigna cutis (LABC, a.k.a. borrelial lymphocytoma) (n=7). The	**Light microscop**y with modified Steiner silver stains: **Warthin-Starry** and **Bosma-Steiner** modifications.	"Lyme spirochetes were demonstrated in all skin preparations [**Sn 100%**] from patients with proven Lyme disease by the Bosma-Steiner stain. By contrast, the Warthin-Starry stain showed only undulating

Netherlands	study also evaluated synovial sections from 4 patients with Lyme arthritis. Biopsies of lesions from 35 patients with other conditions (eczema (5), granuloma annulare (10), erythema annulare centrifugum (3), malignant lymphoma (5), traumatic bursitis (5), and synovial sections from patients with rheumatoid arthritis (7)) were negative controls.		structures in the epidermis and subepidermal zone and spirochetes were not seen with the Steiner stain." Spirochetes were demonstrated in none of the specimens from patients in the control group.
Eisendle, 2007 (*see below*); Austria	Archived H&E-stained sections of 309 specimens from patients with cutaneous borreliosis were re-examined and diagnoses confirmed. 109 negative control samples were also examined. Diagnoses were further verified by clinico-pathologic correlation from patients' records or by contacting the referring clinician.	(1) **Focus floating microscopy** (FFM, a modified immunohistochemical technique); (2) Skin biopsy samples were also tested for presence of *B. burgdorferi* DNA by **PCR** (*see results below*).	*(1) Borrelia* were detected in 30 out of 32 EM **(Sn 93.8%)** and in 50 of 51 (Sn 98.0%) ACA cases by FFM. All 169 control cases, except 1 false-positive case of secondary syphilis, were negative with FFM (**Sp 99.4%).** Among all samples from patients with borreliosis and controls, focus floating microscopy (FFM) was more sensitive than PCR (96.0% vs. 45.2%) and nearly equally specific (99.4% vs. 100%). The authors concluded that FFM is an easy, quick, and inexpensive method to reliably detect *Borrelia* in cutaneous tissue sections.
B. burgdorferi **cultures**			
Skin cultures (Sn range: 25.0% - 83.3%)			
Aberer, 1996 (*see above*); Austria	(2) Skin specimens adjacent to sites biopsied for histologic procedures were taken from skin lesions of patients with ECM (n = 35) and ACA (n=20) and cultured using BSK II culture medium.	(2) *B. burgdorferi* <u>**skin** culture</u> using Barbour-**Stoenner-Kelly (BSK) II** culture medium.	2) *B. burgdorferi* was cultured from biopsies of 9 patients with ECM **(Sn 25.7%)** and from 3 patients with ACA (Sn 15%).
Berger, 1985; NY state, USA	Skin biopsy specimens of 14 patients with EM	*B. burgdorferi* <u>**skin** culture</u> using modified Kelly's **medium**	*B. burgdorferi* was cultured from biopsies of 6 out of 14 patients (**Sn 42.9%)**
Cerar, 2008 (*see below*);	Skin biopsy specimens of 150 patients with EM (periphery of the lesion).	*B. burgdorferi* <u>**skin** culture</u> using modified Kelly–**Pettenkofer medium.**	*B. burgdorferi* sensu lato was isolated from 73 (**Sn 48.7%)** of 150 skin biopsy specimens.

Slovenia		The study also tested two PCR methods on the same collection of skin biopsy samples.	Overall, **nested PCR was the most sensitive method for the demonstration of *Borrelia* spp. in EM skin lesions, followed by culture and PCR targeting the flagellin gene.**
Coulter, 2005; MD and PA, USA	This study examined and followed 86 patients who had "findings suspicious for Lyme disease" and were classified as "probable", "possible", or "unlikely" to have LD. The patients were tested with high-volume blood culture, skin biopsy culture, skin and plasma PCR, and serologic assays. The only result we were able to use was cultures from skin biopsies of those with "typical" EM (n=28).	Skin biopsy samples were **cultured using BSK II medium.**	*B. burgdorferi* was cultured from biopsies of 7 out of 28 patients with typical EM (**Sn 25.0%**) or in 15 out of 47 **(31.9%)** biopsies from those with typical and atypical lesions.
Jurka, 1998; Slovenia	Two biopsy specimens (one from the margin and the other from the center of the lesion) were taken from each of 53 adult patients with EM.	*B. burgdorferi* skin culture using modified **Kelly/Preac-Mursic (MKP) medium.**	34 (32.1%) of the 106 biopsy specimens and 23 (**Sn 43.4%**) of the 53 patients were culture-positive. Spirochetes were isolated in 19 (35.9%) of the 53 central and 15 (28.3%) of the 53 peripheral biopsies (non-significant difference).
Lebech, 2000 *(see below)*; Denmark	Skin biopsy and urine samples from 31 patients with EM. Skin biopsy specimens from 7 healthy individuals were included as controls.	Skin specimens were **cultured in BSK medium**. 16S ribosomal RNA-based PCR was also performed of skin samples. In addition, serum anti-B burgdorferi IgG and IgM were measured by ELISA assay.	*B. burgdorferi* was **cultured** in **29%** of the EM specimens; (16S rRNA PCR detected *B. burgdorferi* DNA in 71.0%, and 41% of the patients had *B. burgdorferi* -specific antibodies in serum).
Li, 2011 *(see below)*; RI and CT, USA	Skin biopsy samples from 90 patients with EM.	Skin samples were **cultured using BSK-H medium.** Standard PCR (targeting 222-bp region of the *B. burgdorferi* recA gene) and quantitative PCR (qPCR, targeting 103-bp region of *the B burgdorferi* flaB gene) techniques were also used on skin and blood samples.	75 out of 90 patients (**Sn 83.3%**) had **positive cultures** for B burgdorferi **from EM skin biopsies;** (85.6% were positive by recA PCR of the skin, 77.8% were positive by qPCR of the skin, and 37.8% were positive by recA PCR of blood samples). In EM lesions, culture and PCR results were highly concordant.
Mitchell, 1993; WI, USA	Skin biopsy specimens of 34 patients with EM (periphery of the lesion).	*B. burgdorferi* skin culture using BSK medium.	*B. burgdorferi* was isolated from 24 of 34 skin biopsy specimens (**Sn 70.6%**).

Moter, 1994 (*see below*); Germany	Skin biopsy samples from 10 untreated patients with EM and 12 patients with ACA.	**Skin** samples were **cultured in BSK medium.** Nested PCR of the skin using outer surface protein A (OspA) gene as a target was performed.	*B. burgdorferi* was **cultivated** in 3 out of 10 **(30.0%) EM biopsy** samples; (80.0% skin samples from EM patients tested positive by nested PCR).
Nadelman, 1993; NY state, USA	Skin biopsy specimens of 44 patients with EM. In culture-positive patients, subsequent biopsies adjacent to the original biopsy cite were performed upon completion of treatment with antibiotics.	*B. burgdorferi* __skin__ culture using BSK medium.	Cultures were positive in 21 of all 38 evaluable patients (**55.3%**), which amounted to **72.4%** among 29 patients who were evaluated **prior to treatment** (but in none of 9 patients evaluated during treatment). *B. burgdorferi* could not be reisolated from any of 18 evaluable subsequent biopsies of skin from 13 culture-positive patients 4 to 209 days after completion of a course of antimicrobial therapy.
O'Rourke, 2013 (*see below*); Slovenia	Skin biopsy specimens of 121 patients with solitary EM.	**Skin** samples were **cultured using modified BSK medium (BSK-B).** *Borrelia* 16S rRNA Real-Time PCR was performed (duplex quantitative real-time PCR assay targeting the *Borrelia* 16S rRNA and using human RNAseP genes as an internal positive control - to compensate for variations arising from the DNA extraction procedure and/or the size and quality of the biopsy).	65 out of 118 (**55.1%**) biopsies were **positive by culture**, and 94/121 (77.7%) were positive by PCR. PCR testing identified more positive biopsy samples than culture and correctly identified all specimens scored as culture positive. The majority of isolates were B. afzelii (96.8%).
Picken, 1997 (*see below*); Slovenia	Skin biopsy specimens from EM and ACA lesions (n=758).	Specimens were tested by PCR amplification assay and __skin__ cultures using two artificial growth media, **Barbour-Stoenner-Kelly II (BSK II) and modified Kelly-Pettenkofer (MKP).**	For classic EM lesions, **the sensitivity of MKP culture was 35.9%** (272 out of 758), and for **BSK II culture it was 23.6%** (179 out of 758). In classic EM lesions, 408 out of 758 specimens tested positive by at least one of the methods (53.8%). Among positive specimens, the highest success rate was obtained by MKP culture alone (272 of 408 positive samples (67%), followed by PCR assay alone (189 of 408 (46%), followed by BSK II culture alone (179 of 408 (44%).
Ruzic-Sabljic, 2006; Slovenia	Skin biopsy specimens of 96 patients with EM (periphery of the lesion).	*B. burgdorferi* __skin__ cultures using two media: **modified Kelly-Pettenkofer (MKP)** and **Barbour–Stoenner–Kelly II (BSK II).**	*Borrelia* strains were isolated from 48/96 skin lesions (**50%**) using **either of the mediums**. There was no difference in the isolation rate when comparing MKP and BSK-II medium (37 positive cultures in each particular medium = **38.5%**). 89% were *B. afzelii*, and 11% -*B. garinii*.

Ruzic-Sabljic, 2014; Slovenia	Skin biopsy specimens of 235 patients with EM.	*B. burgdorferi* **skin cultures using** two media: **modified Kelly-Pettenkofer (MKP)** and **Barbour–Stoenner–Kelly-H (BSK-H) .**	*Borrelia* growth was ascertained in 59/235 **(25.1%) BSK-H** and 102/235 **(43.4%) MKP** cultures (p <0.0001);
Stupica, 2015 (*see below*); Slovenia	Skin biopsy specimens of 121 patients with EM.	**Skin** biopsy specimens were **cultured using BSK-B medium** and also analyzed by quantitative PCR for the presence of *Borreliae*.	65/118 **(55.1%)** patients **had positive skin culture result** (96.8% *B. afzelii*, 3.2% *B. garinii*); (qPCR testing was positive in 77.7% patients).
Zore, 2002 (*see below*); Slovenia (?)	Skin biopsy samples from 150 patients with EM.	**Skin** biopsy specimens were **cultured using modified Kelly-Pettenkofer (MKP) medium** and also analyzed by two different PCRs using either flagellin or nested OspA primers.	**Cultures** were positive in 75 of 150 **(50%) skin samples** (*B. afzelii* 86%, *B. garinii* 14%, typed using pulsed-field gel electrophoresis (PFGE). (PCR with flagellin was positive in 28% and PCR with nested OspA primers - in 61% of skin samples).
Blood cultures (Sn range: 3.8% - 46.2%)			
Arnez, 2001; Slovenia	Blood samples from 134 consecutive patients age <15 years old with solitary EM	*B. burgdorferi* **blood culture using modified Kelly-Pettenkofer** medium. Isolated *Borreliae* were typed according to LRFP (large-restriction-fragment pattern) analysis.	*B. burgdorferi sensu lato* was isolated in 12 of 134 **(Sn 9.0%)** patients. Eleven blood isolates were typed: 10 were found to be *B. afzelii* and 1 was *B. garinii.*
Benach, 1983; NY state, USA	Blood samples from 36 patients with EM	*B. burgdorferi* **blood culture** (medium unknown).	*B. burgdorferi* was cultured from the blood of 2 out of 36 patients (**Sn 5.6%**).
Berger, 1994; NY state, USA	Blood samples from 52 patients with EM with or without extracutaneous signs and symptoms (early localized (n=18) or early disseminated (n=34) Lyme disease).	*B. burgdorferi* **blood culture using modified BSK medium.**	*B. burgdorferi* was cultured from the blood of 2 out of 52 patients with EM (**Sn 3.8%**). The authors concluded that the blood culture of *B. burgdorferi* did not appear to be an efficacious procedure to confirm the diagnosis of early Lyme disease.
Liveris, 2011; NY state, USA	Blood (plasma) samples from 65 patients with EM.	*B. burgdorferi* **blood (plasma) culture using BSK medium** with **detection amplified by testing aliquots with qPCR** (a combined culture-quantitative PCR).	When using simple **microscopic detection** of *B. burgdorferi* in BSK cultures, plasma samples of 30 patients were culture-positive (**Sn 46.2%**). The **combined culture-qPCR technique** yielded 46 positive patients, including all the (+) patients revealed with simple microscopic detection (**Sn 70.8%**).

Wormser, 1998; NY state, USA	The study determined the effect on the culture positivity rate of culturing different volumes of blood and of culturing **whole blood or serum from patients with EM.**	Three 3-ml samples of whole blood and three 3-ml samples of serum per patient were collected from a group of patients (group 1; n=31). In addition, six 3-ml samples of serum per patient were collected from another group of patients (group 2; n=26) a year later. The samples (**blood vs. serum)** were cultured for *B. burgdorferi* **using BSK medium.**	8/31 **(25.8%)** of the patients in group 1 had a positive **whole-blood or serum** culture. **Whole blood** was culture-positive for 3 (**10.0%)** of the 30 evaluable patients, whereas **serum** cultures were positive for 6/31 **(19.4%)** of patients (P=0.47). In group 2, **serum** samples of 7/26 of patients **(26.9%)** were culture positive for *B. burgdorferi.* The authors concluded that serum was preferable to whole blood as a source of culture material, and that the volume of blood cultured correlated directly with yield.
Skin or blood cultures			
Liveris, 2012 (*see below*); NY, USA	Blood (plasma) samples and skin biopsy specimens from 66 patients with untreated EM	Evaluated the sensitivity of 5 direct diagnostic methods (**culture** and nested PCR **of a 2-mm skin biopsy** specimen, nested PCR (nPCR) and quantitative PCR (qPCR) performed on the same 1-mL aliquot of plasma, and a novel **qPCR–blood culture** method)	**Standard culture of skin** samples was positive in 34 out of 55 patients **(Sn 61.8%)**; A combined **qPCR blood culture** was positive in 46 out of 65 patients **(Sn 70.8%).** Culture was more sensitive than PCR for both skin and blood, but the difference was only statistically significant for blood samples (P<0.005).
Nowakowski, 2001 (*see below*); NY state, USA	Blood (plasma) samples and skin biopsy specimens from 47 patients with EM.	**6 diagnostic modalities were compared**: ● **skin culture (BSK medium);** ● **blood culture (BSK medium);** ● quantitative PCR (qPCR) on skin biopsy–derived material; ● conventional nested PCR of skin; ● 2-stage serologic testing of convalescent-phase samples; and ● serologic testing of acute-phase samples.	Quantitative PCR on skin biopsy–derived material was the most sensitive diagnostic method (80.9%), followed by 2-stage serologic testing of convalescent-phase samples (66.0%), conventional nested PCR (63.8%), **skin culture (51.1%), blood culture (44.7%)**, and serologic testing of acute-phase samples (40.4%).
PCR			
Skin PCR (Sn range: 24.0% - 85.6%; Sp = 100%)			
Brettschneider, 1998;	Skin biopsies and urine samples of 36 patients with EM and ACA before therapy and those of 8 patients after therapy.	PCR using two different primer sets targeting **23S rRNA (PCR I)** and **66-kDa protein (PCR II)** genes.	For FFT specimens of EM, **73%** were positive by **PCR I**, **79%** were positive by **PCR II**, and **88%** were positive by combining **PCR I and II**.

Germany	Skin biopsies of 27 patients with dermatological diseases other than Lyme borreliosis and those of 10 healthy persons were examined as controls.	PCR was performed with freshly frozen tissue (FFT) and paraffin-embedded tissue (PET).	For PET specimens, PCR was less sensitive (**PCR I, 44%; PCR II, 52%; both, 56%**).
		Skin samples were also cultured using modified Barbour-Stoenner-Kelly-H (BSK-H) medium.	*B. burgdorferi* was cultured from **78.9%** (15 of 19) of the erythema migrans specimens. Cultures and PCR results of the control group were negative (**Sp 100%**)
Cerar, 2008 (*see above*); Slovenia	Skin biopsy specimens of 150 patients with EM (periphery of the lesion).	*B. burgdorferi* **skin** **samples** were analyzed using **nested PCR targeting the rrf–rrl region** and **a PCR targeting the flagellin gene**	Nested **PCR targeting the rrf–rrl region** detected 107 (**Sn 71.3%**) and a **PCR targeting the flagellin gene** - 36 (**Sn 24.0%**) positive specimens.
Eisendle, 2007 (*see above*); Austria	Archived H&E-stained sections of 309 specimens from patients with cutaneous borreliosis were re-examined and diagnoses confirmed. 109 negative control samples were also examined.	(2) **Skin biopsy** samples were tested for presence of *B. burgdorferi* DNA by **PCR** (along with floating microscopy technique).	(2) PCR detected *Borrelia* in 7 out of 15 EM (Sn **46.7%**) and 17 out of 28 ACA (Sn 60.7%) cases. PCR was negative in all 66 of negative controls (**Sp 100%).** Among all samples from patients with borreliosis and controls, focus floating microscopy (FFM) was more sensitive than PCR (96.0% vs. 45.2%) and nearly equally specific (99.4% vs. 100%).
Lebech, 2000 (*see above*); Denmark	Skin biopsy and urine samples from 31 patients with EM. Skin biopsy specimens from 7 healthy individuals were included as controls.	**16S ribosomal RNA-based PCR** of **skin** samples. Skin specimens were also cultured in BSK medium. In addition, serum anti-*B burgdorferi* IgG and IgM were measured by ELISA assay.	**16S rRNA PCR** detected *B. burgdorferi* DNA in 22/31 **(Sn 71.0%)** of the **skin** biopsy specimens from patients with EM; it was not found in any of the controls (**Sp 100%**) (*B. burgdorferi* was cultured in 29% of the EM specimens, and 41% of the patients had *B. burgdorferi* -specific antibodies in serum).
Moter, 1994 (*see above*); Germany	Skin biopsy samples from 10 untreated patients with EM and 12 patients with ACA. Negative controls included normal skin biopsy tissue culture.	**Nested PCR** of the **skin** using outer surface protein A **(OspA) gene** as a target was performed. Skin samples were also cultured in BSK medium.	8 of 10 **(Sn 80,0%) skin** samples from EM patients tested positive by **nested OspA PCR**; (*B. burgdorferi* was cultivated in 30.0% EM biopsy samples). All control specimens from individuals with normal skin yielded negative results by PCR **(Sp 100%).**
Nowakowski, 2001 (*see above*) NY state, USA	Blood (plasma) samples and **skin biopsy** specimens from 47 patients with EM.	**6 diagnostic modalities were compared**: ● **quantitative PCR (qPCR) on skin** biopsy–derived material; ● **conventional nested skin PCR**; ● skin culture (BSK medium); ● blood culture (BSK medium);	**Quantitative PCR of skin** was the most sensitive diagnostic method **(80.9%)**, followed by 2-stage serologic testing of convalescent-phase samples (66.0%), **conventional nested PCR of skin (63.8%),** skin culture (51.1%), blood culture (44.7%), and serologic testing of acute-phase samples (40.4%).

		● 2-stage serologic testing of convalescent-phase samples; and ● serologic testing of acute-phase samples.	
O'Rourke, 2013 (*see above*); Slovenia	Skin biopsy specimens of 121 patients with solitary EM.	*Borrelia* **16S rRNA Real-Time PCR** was performed on **skin** samples (duplex quantitative real-time PCR assay targeting the *Borrelia* 16S rRNA and using human RNAseP genes as an internal positive control - to compensate for variations arising from the DNA extraction procedure and/or the size and quality of the biopsy). Skin samples were also cultured using modified BSK medium (BSK-B).	94 out of 121 **skin** biopsies **(77.7%)** were **positive by PCR**, whereas only 55.1% were positive by culture. PCR testing identified more positive biopsy samples than culture and correctly identified all specimens scored as culture positive. The majority of isolates were *B. afzelii* (96.8%).
Picken, 1997 (*see above*); Slovenia	Skin biopsy specimens from EM and ACA lesions (n=758).	Specimens were tested by **PCR amplification assay (skin)** and skin culture using two artificial growth media, Barbour-Stoenner-Kelly II (BSK II) and modified Kelly-Pettenkofer (MKP).	For classic EM lesions, **the sensitivity of** PCR assay alone was **24.9%** (189 of 758). In classic EM lesions, 408 out of 758 specimens tested positive by at least one of the methods (53.8%). Among positive specimens, the highest success rate was obtained by MKP culture alone (272 of 408 positive samples (67%), followed by PCR assay alone (189 of 408 (46%), followed by BSK II culture alone (179 of 408 (44%). Differences were statistically significant.
Stupica, 2015 (*see above*); Slovenia	Skin biopsy specimens of 121 patients with EM.	**Skin** biopsy specimens were analyzed by **quantitative PCR** for the presence of *Borreliae* and also cultured in BSK-B medium.	In 94/121 **(77.7%)** patients *Borrelia* was detected in skin samples by **qPCR testing** (cultures were positive in 55.1% of patients).
von Stedingk,1995; Sweden	Skin biopsy specimens from patients with EM (n=26; 25 – solitary lesions) and ACA (n=36). 76 skin biopsies (healthy skin or from patients with non-borrelial disorders) were used as controls.	**Nested PCR** of **skin** samples	18 out of 26 (**Sn 69.2%**) EM lesions were positive by PCR. None of the controls tested positive (**Sp 100%**).
Zore, 2002 (*see above*); Slovenia (?)	Skin biopsy samples from 150 patients with EM.	**Skin** biopsy specimens were cultured using modified Kelly-Pettenkofer (MKP) medium and also **analyzed by two different PCRs using either flagellin or nested OspA primers.**	**PCR using flagellin** detected *B. burgdorferi* sensu lato DNA in **28%** of skin samples, and **PCR using nested OspA primers** - in **61%** of skin samples; (cultures were positive in 50% of skin samples)

Skin or blood PCR (blood PCR Sn range: 33.8% - 61.9%)		
Li, 2011 (*see above*); RI and CT, USA	Skin biopsy and blood samples from 90 patients with EM. For comparison, 12 normal skin biopsy samples were obtained at the edges of elliptical excision specimens of atypical melanocytic nevi, and 7 normal blood samples were obtained from healthy workers in the laboratory.	**Standard PCR** (targeting 222-bp region of the *B. burgdorferi* **recA** gene) and **quantitative PCR (qPCR**, targeting 103-bp region of *the B burgdorferi* **flaB** gene) techniques were used on **skin** and **blood** samples. Skin samples were also cultured using BSK-H medium.	77/90 (**Sn 85.6%**) were positive by **recA PCR of the skin**; 70/90 (**Sn 77.8%**) of samples from lesional **skin** were positive by **qPCR results for flaB DNA;** 34/90 (**Sn 37.8%**) **blood** samples from EM patients were positive by **recA PCR**. 75 out of 90 patients (Sn 83.3%) had positive cultures for B burgdorferi from EM skin biopsies. In EM lesions, culture and PCR results were highly concordant. Skin and blood samples from healthy donors tested negative by the PCR (**Sp 100%.**)
Liveris, 2012 (*see above*); NY, USA	Blood (plasma) samples and skin biopsy specimens from 66 patients with untreated EM	Evaluated the sensitivity of 5 direct diagnostic methods (culture and **nested PCR of a 2-mm skin biopsy** specimen, **nested PCR (nPCR)** and **quantitative PCR (qPCR)** performed on the same 1-mL aliquot of **plasma**, and a novel qPCR–blood culture method)	**nPCR of skin** samples was positive in 23 of 54 patients **(42.6%**), and **nPCR of blood** samples was positive in 26 out of 64 patients **(40.6%);** **qPCR of blood** samples was positive in 22 out of 65 cases (**33.8%**). If tested with **either nPCR or qPCR, blood** samples were positive in 29 out of 65 patients (**Sn 44.6%**). Culture was more sensitive than PCR for both skin and blood, but the difference was only statistically significant for blood samples (P<0.005).
Pauluzzi, 2004; Italy	Skin biopsies, urine and peripheral blood of 30 patients with clinically documented EM without apparent systemic involvement	**PCR using a combination of three primer sets**: the 1st set targeting a sequence of a chromosomal gene encoding for a 66 kDa protein, the 2nd set targeting flagellin gene (41 kDa protein), and the 3rd set was specific for the gene encoding the 80 kDa antigen (**B-80, 66 kDa protein, flagellin**)	*B. burgdorferi* DNA could be detected by **at least one of the three sets** in **76.7%** (23/30) of blood samples and in **100%** (30/30) of skin samples. The % of positive results for **B-80** in blood was **47%** and in skin **50%**. For **66 kDa protein** the rate of positive results was **53%** in blood and **37%** in skin. For **flagellin**, positivity was achieved in **86%** of skin samples and in **37%** in blood.
Eshoo, 2012; MD, USA (1st author is located in CA)	Blood samples collected at the initial presentation from 21 endemic area patients who had both physician-diagnosed EM and positive two-tiered serology either at the initial visit or at a follow-up visit after three weeks of antibiotic therapy.	**Multi-locus PCR** and **electrospray ionization mass spectrometry detection (PCR/ESI-MS)** assay of **blood.** This assay allowed for both detection and genotyping of B. burgdorferi. Primer sets were **targeting rpoC, rplB, leuS, flaB, ospC, hbb, and gyrB genes**. Each of the seven	Results of this **PCR/ESI-MS blood** assay showed detection of *B. burgdorferi* in 13 of 21 patients **(Sn 61.9%).** In most cases the new assay also provided the *B. burgdorferi* genotype. All control samples tested negative by the PCR/ESI-MS essay **(Sp 100%.)** In comparison, 2-tier assay detected 14/21 EM cases (66.7%), with specificity at 97.7%.

Paired whole blood and serum specimens from 44 healthy individuals residing in an endemic area served as controls.	target loci were amplified using 50 oligonucleotide primers flanking the locus.	The combined results of the direct detection assay with initial physician visit serology resulted in the detection of early LD in 19 of 21 (90%) of patients at the initial visit.

VI. What are the preferred antibiotic regimens for the treatment of erythema migrans (EM)?

DOXYCYCLINE vs. PENICILLIN V

In patients with erythema migrans, should Doxycycline be used over Penicillin V?

P: In patients with erythema migrans

I: Doxycycline

C: Penicillin V

Bibliography: 1. Eliassen, et al. Clin Microbiol Infect. 2018 Dec; 24(12):1290-1296; 2. Strle, et al. J Antimicrob Chemother. 1992 Oct; 30(4):543-50.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline	Penicillin V	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 14 days)												
1	RCT [1]	not serious	not serious	not serious	not serious [a]	none	68/68 (100.0%)	54/55 (98.2%)	RR 1.02 (0.98 to 1.06)	20 more per 1,000 (from 20 fewer to 59 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation. Mean times to resolution are shown in the order of reference.)												
2	RCT [1,2]	serious [b]	serious [c]	not serious	serious [d]	none	Mean days: 81, 8.8	Mean days: 31, 10.5	SMD 0.23 higher (0.89 lower to 1.34 higher)		⊕○○○ VERY LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
2	RCT [1,2]	serious [b]	not serious	not serious	serious [e]	none	2/91 (2.2%)	2/76 (2.6%)	RR 0.91 (0.14 to 5.92)	2 fewer per 1,000 (from 23 fewer to 129 more)	⊕⊕○○ LOW	CRITICAL
Gastrointestinal Adverse Events												
2	RCT [1,2]	serious [b, f]	not serious	not serious	serious [g]	none	25/90 (27.8%)	21/76 (27.6%)	RR 1.23 (0.36 to 4.26)	64 more per 1,000 (from 177 fewer to 901 more)	⊕⊕○○ LOW	IMPORTANT
Serious Adverse Events												

1	RCT [2]	serious [b]	not serious	not serious	serious [e]	none	1/23 (4.3%)	0/21 (0.0%)	NA [h]	43 more per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Allergic reaction												
2	RCT [1,2]	serious [b, f]	not serious	not serious	serious [e]	none	1/90 (1.1%)	0/76 (0.0%)	NA [h]	1 more per 1,000	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **SMD:** Standardized mean difference

Explanations

a. Based on an inferiority margin of 15%, not rated down for imprecision.

b. Strle 1992 study received high risk of bias ratings due to inadequate randomization technique (sequence generation and allocation sequence concealment) and potentially inadequate blinding (outcomes were self-reported).

c. I^2= 90%

d. 95% CI is wide and crossing the null value. Eliassen 2018 study reported duration of EM as median and range- Median for Doxy group was 14 days (range 3-293 days) and for PenV group was 14 days (range 5-91 days). These were converted to mean and SD using method by Hozo, et al. However, due to a wide range of EM duration in this study, the SDs are very large.

e. Due to low event rate.

f. Eliassen 2018 study received high risk of bias ratings due to single-blind design (outcomes were self-reported).

g. 95% CI crossing the null value.

h. Due to zero events in one study arm, unable to estimate relative risk.

DOXYCYCLINE vs. AMOXICILLIN

In patients with erythema migrans, should Doxycycline be used over Amoxicillin?

P: In patients with erythema migrans

I: Doxycycline

C: Amoxicillin

Bibliography: 1. Eliassen, et al. Clin Microbiol Infect. 2018 Dec; 24(12):1290-1296; 2. Dattwyler, et al. Lancet. 1990 Dec 8;336(8728):1404-6; 3. Massarotti, et al. Am J Med. 1992 Apr;92(4):396-403.

Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline	Amoxicillin	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**Nº of Events/Nº of patients**		**Effect**			
Resolution of Erythema Migrans (at 10 days (Massarotti, 1992) to 14 days (Eliassen, 2018))												
2	RCT [1,3]	serious [a]	serious [b]	not serious	not serious [c]	none	83/90 (92.2%)	78/82 (95.1%)	0.97 (0.90 to 1.05)	29 fewer per 1,000 (101 fewer to 43 more)	⊕⊕◯◯ LOW	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation: Higher values indicate longer resolution time.)												
1	RCT [1]	not serious	not serious	not serious	serious [d]	none	Mean days: 81	Mean days: 52	**MD 28.75 higher (5.32 higher to 52.18 higher)**		⊕⊕⊕◯ MODERATE	IMPORTANT
Patients Experiencing Objective Findings of Lyme (during treatment and up to 1 month)												
1	RCT [3]	serious [a]	not serious	not serious	serious [e]	none	1/22 (4.5%)	0/19 (0.0%)	NA [f]	45 more per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [e]	none	0/103 (0.0%)	0/100 (0.0%)	NA [f]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Patients Withdrawing due to Adverse Events												
2	RCT [2,3]	serious [a, g]	serious [h]	not serious	serious [e]	none	2/59 (3.4%)	5/57 (8.8%)	RR 0.50 (0.02 to 10.56)	44 fewer per 1,000 (from 86 fewer to 839 more)	⊕◯◯◯ VERY LOW	CRITICAL

Gastrointestinal Adverse Events

2	RCT [1,3]	serious [a, g]	not serious	not serious	serious [i]	none	22/89 (24.7%)	21/83 (25.3%)	RR 1.01 (0.61 to 1.68)	3 more per 1,000 (from 99 fewer to 172 more)	⊕⊕◯◯ LOW	IMPORTANT

Allergic Reaction

2	RCT [1,3]	serious [a, g]	not serious	not serious	serious [j]	none	1/89 (1.1%)	8/83 (9.6%)	**RR 0.16 (0.03 to 0.85)**	**81 fewer per 1,000 (from 14 fewer to 93 fewer)**	⊕⊕◯◯ LOW	IMPORTANT

Jarisch-Herxheimer Reaction

1	RCT [2]	serious [g]	not serious	not serious	serious [e, i]	none	3/35 (8.6%)	7/37 (18.9%)	RR 0.45 (0.13 to 1.62)	104 fewer per 1,000 (from 117 more to 165 fewer)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Massarotti 1992 received a high risk of bias due to the high number of participants excluded from final analysis due to negative serology (14 out of 55 randomized patients).

b. I^2= 80%

c. Based on an inferiority margin of 15%, not rated down for imprecision.

d. Eliassen 2018 reported duration of EM as median and range- Median for Doxy group was 14 days (range 3-293 days) and for Amoxicillin group was 13 days (range 4-179 days). These were converted to mean and SD using method by Hozo, et al. However, due to a wide range of EM duration in this study, the SDs are very large.

e. Due to low event rate.

f. Due to zero event in both arms, unable to estimate the relative risk.

g. All studies received high risk of bias ratings due to potentially inadequate blinding (for self-reported outcomes).

h. I^2= 63%

i. 95% CI crossing the null value.

j. Fragility due to low event rate.

CEFUROXIME vs. PENICILLIN V

In patients with erythema migrans, should Cefuroxime be used over Penicillins V?

P: In patients with erythema migrans

I: Cefuroxime

C: Penicillin V

Bibliography: 1. Arnez, et al. (PEDIATRIC STUDY) Wien Klin Wochenschr. 1999 Dec 10;111(22-23):916-22.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Cefuroxime	Penicillin V	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (after 1 month)												
1	RCT [1]	serious [a]	not serious	not serious	not serious [b]	none	44/46 (95.7%)	40/44 (90.9%)	1.05 (0.94 to 1.18)	47 more per 1,000 (60 fewer to 151 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation)												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	Mean days: 7.1	Mean days: 10.6	MD 3.5 lower (9.6 lower to 2.6 higher)		⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	1/45 (2.2%)	1/44 (2.3%)	RR 0.98 (0.06 to 15.15)	0 fewer per 1,000 (from 21 fewer to 322 more)	⊕⊕◯◯ LOW	CRITICAL
Patients Withdrawing due to Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	0/46 (0.0%)	0/44 (0.0%)	NA [c]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Treatment-related Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	12/46 (26.1%)	3/44 (6.8%)	RR 3.83 (1.16 to 12.65)	193 more per 1,000 (from 11 more to 794 more)	⊕⊕◯◯ LOW	IMPORTANT
Gastrointestinal Adverse Events												

1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	5/46 (10.9%)	1/44 (2.3%)	RR 4.78 (0.58 to 39.33)	86 more per 1,000 (from 10 fewer to 871 more)	⊕⊕◯◯ LOW	IMPORTANT
Jarisch-Herxheimer Reaction												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	11/46 (23.9%)	5/44 (11.4%)	RR 2.10 (0.80 to 5.57)	125 more per 1,000 (from 23 fewer to 519 more)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **MD:** Mean difference; **RR:** Risk ratio

Explanations

a. Study received high risk of bias ratings due to inadequate randomization technique (sequence generation and allocation sequence concealment) and potentially inadequate blinding (for self-reported outcomes).
b. Based on an inferiority margin of 15%, not rated down for imprecision.
c. Low event rate. 95% CI are wide and crossing the null value.
c. Due to zero events in one study arm, unable to estimate relative risk

CEFUROXIME vs. AMOXICILLIN

In patients with erythema migrans, should Cefuroxime be used over Amoxicillin?

P: In patients with erythema migrans

I: Cefuroxime

C: Amoxicillin

Bibliography: 1. Eppes, et al. (PEDIATRIC STUDY) Pediatrics. 2002 Jun;109(6):1173-7.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Cefuroxime	Amoxicillin	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 21 days)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	13/15 (86.7%)	8/12 (66.7%)	RR 1.30 (0.83 to 2.03)	200 more per 1,000 (from 117 fewer to 517 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	0/15 (0.0%)	0/12 (0.0%)	NA [c]	0 per 1,000	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Withdrawing due to Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/15 (0.0%)	0/12 (0.0%)	NA [c]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Serious Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/15 (0.0%)	0/12 (0.0%)	NA [c]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	3/15 (20.0%)	2/12 (16.7%)	RR 1.20 (0.24 to 6.06)	33 more per 1,000 (from 127 fewer to 843 more)	⊕⊕◯◯ LOW	IMPORTANT

Allergic Reaction

1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/15 (0.0%)	0/12 (0.0%)	NA [c]	0 per 1,000	⊕⊕◯◯ LOW	IMPORTANT

Jarisch-Herxheimer Reaction

1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/15 (0.0%)	1/12 (8.3%)	NA [c]	83 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT

Diarrhea

1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	3/15 (20.0%)	2/12 (16.7%)	RR 1.20 (0.24 to 6.06)	33 more per 1,000 (from 127 fewer to 843 more)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received high risk of bias ratings due to potentially inadequate blinding (for self-reported outcomes).
b. Low number of events and 95% CI is wide and crossing the null value.
c. Due to zero events in one study arm, unable to estimate relative risk

CEFUROXIME vs. DOXYCYCLINE

In patients with erythema migrans, should Cefuroxime be used over Doxycycline?

P: In patients with erythema migrans

I: Cefuroxime

C: Doxycycline

Bibliography: 1. Luger, et al. Antimicrob Agents Chemother. 1995 Mar;39(3):661-7; 2. Nadelman, et al. Ann Intern Med. 1992 Aug 15;117(4):273-80.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Cefuroxime	Doxycycline	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 30 days)												
2	RCT [1,2]	serious [a]	serious [b]	not serious	not serious [c]	none	145/155 (93.5%)	139/145 (95.9%)	RR 0.98 (0.93 to 1.03)	23 fewer per 1,000 (from 74 fewer to 27 more)	⊕⊕◯◯ LOW	CRITICAL
Patients Experiencing Objective Findings of Lyme (during treatment and up to 1 month)												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [d]	none	2/155 (1.3%)	5/145 (3.4%)	RR 0.48 (0.07 to 3.11)	18 fewer per 1,000 (from 32 fewer to 73 more)	⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [d]	none	2/113 (1.8%)	0/91 (0.0%)	NA [e]	18 more per 1,000	⊕⊕◯◯ LOW	CRITICAL
Patients Withdrawing due to Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [d]	none	8/119 (6.7%)	5/113 (4.4%)	RR 1.52 (0.51 to 4.51)	23 more per 1,000 (from 22 fewer to 155 more)	⊕⊕◯◯ LOW	CRITICAL
Treatment-related Adverse Events												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [f]	none	39/182 (21.4%)	51/173 (29.5%)	RR 0.74 (0.47 to 1.19)	77 fewer per 1,000 (from 156 fewer to 56 more)	⊕⊕◯◯ LOW	IMPORTANT

Gastrointestinal Adverse Events											
2	RCT [1,2]	serious [a]	serious [g]	not serious	serious [f]	none	23/182 (12.6%)	16/173 (9.2%)	RR 1.37 (0.54 to 3.49)	34 more per 1,000 (from 43 fewer to 230 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Allergic Reaction											
2	RCT [1,2]	serious [a]	not serious	not serious	serious [h]	none	4/182 (2.2%)	20/173 (11.6%)	**RR 0.19 (0.07 to 0.55)**	**94 fewer per 1,000 (from 146 fewer to 42 fewer)**	⊕⊕◯◯ LOW	IMPORTANT
Jarisch-Herxheimer Reaction											
2	RCT [1,2]	serious [a]	serious [i]	not serious	serious [f]	none	32/182 (17.6%)	18/173 (10.4%)	RR 1.80 (0.55 to 5.92)	83 more per 1,000 (from 47 fewer to 512 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Diarrhea											
2	RCT [1,2]	serious [a]	not serious	not serious	serious [h]	none	18/163 (11.0%)	4/154 (2.6%)	**RR 3.58 (1.32 to 9.70)**	**67 more per 1,000 (from 8 more to 226 more)**	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Both studies received high risk of bias ratings due inadequate reporting of withdrawals and to the high number of participants excluded from final analysis due to enrollment violation, deviation from protocol (adherence pro), adverse events or lost-to-follow-up (Luger 1995: early endpoint : 38 of 232 randomized patients and late endpoint: 62 of 180 remaining patients; Nadelman 1992: 1-month endpoint : 17 of 123 randomized patients and 12-month endpoint: 10 of 96 remaining patients) and due to potentially inadequate blinding (for self-reported outcomes).
b. I^2= 68%
c. Based on an inferiority margin of 15%, not rated down for imprecision.
d. Due to low event rate.
e. Due to zero event in one arm, unable to estimate relative risk.
f. 95% CI is wide and/or crossing the null value.
g. I^2= 56%
h. Fragility due to low event rate.
i. I^2= 76%

AZITHROMYCIN vs. PENICILLIN V

In patients with erythema migrans, should Azithromycin be used over Penicillin V?

P: In patients with erythema migrans

I: Azithromycin

C: Penicillin V

Bibliography: 1. Arnez, et al. (PEDIATRIC STUDY) Wien Klin Wochenschr. 2002 Jul 31;114(13-14):498-504; 2. Weber et al. Infection. 1993 Nov-Dec;21(6):367-72; 3. Strle, et al. J Antimicrob Chemother. 1992 Oct;30(4):543-50.

Certainty assessment							№ of Events/№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Azithromycin	Penicillin V	Relative (95% CI)	Absolute (95% CI)		
Resolution of Erythema Migrans (at 10 days)												
1	RCT [2]	not serious	not serious	not serious	serious [a]	none	14/32 (43.8%)	4/33 (12.1%)	**RR 3.61 (1.33 to 9.80)**	**316 more per 1,000 (from 112 more to 521 more)**	⊕⊕⊕◯ MODERATE	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation: Mean times to resolution are shown in the order of reference)												
2	RCT [1,3]	serious [b]	not serious	not serious	serious [c]	none	5.6, 8.6	6.3, 10.5	MD 1.59 lower (4.16 lower to 0.98 higher)		⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
2	RCT [1,3]	serious [b]	not serious	not serious	serious [c]	none	1/60 (1.7%)	3/62 (4.8%)	RR 0.50 (0.07 to 3.71)	24 fewer per 1,000 (from 45 fewer to 131 more)	⊕⊕◯◯ LOW	CRITICAL
Patients Withdrawing due to Adverse Events												
2	RCT [1,2]	serious [b, d]	not serious	not serious	serious [c]	none	1/74 (1.4%)	0/75 (0.0%)	NA [e]	14 more per 1,000	⊕⊕◯◯ LOW	CRITICAL
Total Adverse Events												

1	RCT [2]	serious [d]	not serious	not serious	serious [c]	none	12/32 (37.5%)	5/33 (15.2%)	RR 2.47 (0.98 to 6.23)	223 more per 1,000 (from 3 fewer to 792 more)	⊕⊕◯◯ LOW	IMPORTANT
Serious Adverse Events												
2	RCT [1,2]	serious [b, d]	not serious	not serious	serious [c]	none	0/72 (0.0%)	0/74 (0.0%)	NA [e]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Gastrointestinal Adverse Events												
3	RCT [1-3]	serious [b, d]	not serious	not serious	serious [a]	none	17/92 (18.5%)	8/95 (8.4%)	**RR 2.19 (1.00 to 4.83)**	**100 more per 1,000 (from 4 more to 198 more)**	⊕⊕◯◯ LOW	IMPORTANT
Allergic Reaction												
2	RCT [2,3]	serious [b, d]	not serious	not serious	serious [c]	none	2/52 (3.8%)	0/54 (0.0%)	NA [e]	38 more per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Diarrhea												
1	RCT [2]	serious [d]	not serious	not serious	serious [c]	none	4/32 (12.5%)	3/33 (9.1%)	RR 1.38 (0.33 to 5.66)	35 more per 1,000 (from 61 fewer to 424 more)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Fragility due to small number of events.
b. Anez 2002 and Strle 1992 studies both received high risk of bias ratings due to inadequate randomization technique (sequence generation and allocation sequence concealment).
c. Low event rate, 95% CI is wide and crossing the null value.
d. All 3 studies received high risk of bias ratings due to potentially inadequate blinding (for self-reported outcomes).
e. Due to zero events in one study arm, unable to estimate relative risk.

AZITHROMYCIN vs. AMOXICILLIN

In patients with erythema migrans, should Azithromycin be used over Amoxicillin?

P: In patients with erythema migrans

I: Azithromycin or clarithromycin or erythromycin)

C: Amoxicillin

Bibliography: 1. Arnez, et al. Pediatr Infect Dis J. 2015 Oct;34(10):1045-8; 2. Luft, et al. Ann Intern Med. 1996 May 1;124(9):785-91; 3. Massarotti, et al. Am J Med. 1992 Apr;92(4):396-403.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	№ of Events/№ of patients		Effect		Certainty	Importance
							Azithromycin	Amoxicillin	Relative (95% CI)	Absolute (95% CI)		
Resolution of Erythema Migrans (at 20 days)												
2	RCT [2,3]	not serious	not serious	not serious	serious [a]	none	97/127 (76.4%)	109/125 (87.2%)	**RR 0.88 (0.78 to 0.99)**	**108 fewer per 1,000 (from 203 fewer to 14 fewer)**	⊕⊕⊕◯ MODERATE	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation)												
1	RCT [1]	serious [b]	not serious	not serious	serious [c]	none	4.7	5.9	MD 1.2 lower (3.35 lower to 0.95 higher)		⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (during treatment and up to 1 month)												
3	RCT [1-3]	serious [b]	not serious	not serious	serious [d]	none	5/211 (2.4%)	2/209 (1.0%)	RR 1.87 (0.44 to 7.95)	8 more per 1,000 (from 5 fewer to 67 more)	⊕⊕◯◯ LOW	IMPORTANT
Patients Experiencing Objective Findings of Lyme (6 months and beyond)												
1	RCT [2]	not serious	not serious	not serious	serious [a]	none	17/106 (16.0%)	4/103 (3.9%)	**RR 4.13 (1.44 to 11.86)**	**122 more per 1,000 (from 17 more to 422 more)**	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Withdrawing due to Adverse Events												

2	RCT [2,3]	serious [e]	not serious	not serious	serious [d]	none	2/140 (1.4%)	10/141 (7.1%)	RR 0.26 (0.07 to 1.05)	52 fewer per 1,000 (from 4 more to 66 fewer)	⊕⊕◯◯ LOW	CRITICAL
Total Adverse Events												
2	RCT [1,2]	serious [b, e]	not serious	not serious	serious [a]	none	61/195 (31.3%)	42/190 (22.1%)	**RR 1.41 (1.01 to 1.96)**	**91 more per 1,000 (from 2 more to 212 more)**	⊕⊕◯◯ LOW	IMPORTANT
Gastrointestinal Adverse Events												
2	RCT [1,2]	serious [b, e]	not serious	not serious	serious [d]	none	6/195 (3.1%)	7/190 (3.7%)	RR 1.04 (0.16 to 6.97)	1 more per 1,000 (from 31 fewer to 220 more)	⊕⊕◯◯ LOW	IMPORTANT
Jarisch-Herxheimer Reaction												
1	RCT [1]	serious [b, e]	not serious	not serious	serious [d]	none	6/84 (7.1%)	13/84 (15.5%)	RR 0.46 (0.18 to 1.16)	84 fewer per 1,000 (from 25 more to 127 fewer)	⊕⊕◯◯ LOW	IMPORTANT
Allergic Reaction												
3	RCT [1-3]	serious [b, e]	not serious	not serious	serious [d]	none	0/211 (0.0%)	14/209 (6.7%)	NA [f]	67 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT
Diarrhea												
2	RCT [2,3]	serious [e]	not serious	not serious	serious [d]	none	5/127 (3.9%)	2/125 (1.6%)	RR 2.24 (0.52 to 9.60)	20 more per 1,000 (from 8 fewer to 138 more)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Fragility due to small number of events and IOS criteria not met.
b. Arnez 2015 study received a high risk of bias ratings due to inadequate randomization technique (sequence generation and allocation sequence concealment).
c. 95% CI is wide and crossing the null value.
d. Low event rate, and 95% CI is wide and crossing the null value.
e. The majority of studies received at least one high risk of bias rating due to potentially inadequate blinding (for self-reported outcomes).
f. Due to zero events in one study arm, unable to estimate relative risk.

Supplement Materials for the IDSA/AAN/ACR 2020 Draft Lyme Disease Guidelines

AZITHROMYCIN vs. DOXYCYCLINE

In patients with erythema migrans, should Azithromycin be used over Doxycycline?

P: In patients with erythema migrans

I: Azithromycin

C: Doxycycline

Bibliography: 1. Barsic, et al. Infection. 2000 May-Jun;28(3):153-6; 2. Massarotti, et al. Am J Med. 1992 Apr;92(4):396-403; 3. Strle, et al. J Antimicrob Chemother. 1992 Oct;30(4):543-50; 4. Strle, et al. Infection. 1993 Mar-Apr;21(2):83-8; 5. Strle, et al. Infection. 1996 Jan-Feb;24(1):64-8.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Azithromycin	Doxycycline	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (10 to 15 days)												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [b]	none	55/64 (85.9%)	44/62 (71.0%)	**RR 1.21 (1.00 to 1.46)**	**150 more per 1,000 (from 8 more to 291 more)**	⊕⊕○○ LOW	CRITICAL
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
4	RCT [1,3,4,5]	serious [a]	not serious	not serious	serious [c]	none	1/180 (0.6%)	7/152 (4.6%)	RR 0.28 (0.06 to 1.23)	33 fewer per 1,000 (from 11 more to 43 fewer)	⊕⊕○○ LOW	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation: Mean times to resolution are shown in the order of reference.)												
4	RCT [1,3,4,5]	serious [a]	not serious	not serious	serious [b]	none	5.5, 9.2, 7.5, 7.8	7.9, 5.7,11.4, 10.7	**MD 2.39 lower (1.22 lower to 3.56 lower)**		⊕⊕○○ LOW	IMPORTANT
Patients Withdrawing due to Adverse Events												
4	RCT [1,2,4,5]	serious [a]	not serious	not serious	serious [c]	none	0/177 (0.0%)	1/156 (0.6%)	NA [d]	6 fewer per 1,000	⊕⊕○○ LOW	CRITICAL
Treatment-related Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	3/47 (6.4%)	5/35 (14.3%)	RR 0.45 (0.11 to 1.75)	79 fewer per 1,000 (from 107 more to 127 fewer)	⊕⊕○○ LOW	IMPORTANT

Gastrointestinal Adverse Events											
4	RCT [1,3,4,5]	serious [a]	not serious	not serious	not serious	none	17/180 (9.4%)	32/152 (21.1%)	**RR 0.46 (0.27 to 0.80)**	**114 fewer per 1,000 (from 42 fewer to 154 fewer)**	⊕⊕⊕◯ MODERATE	IMPORTANT
Allergic reaction											
5	RCT [1,2,3,4,5]	serious [a]	not serious	not serious	serious [c]	none	0/196 (0.0%)	14/174 (8.0%)	NA [d]	80 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. All trials received at least one high risk of bias rating due to potentially inadequate blinding (self-reported outcomes).
b. Fragility due to small number of events and IOS criteria not met.
c. 95% CI is wide and crossing the null value.
d. Due to zero events in one study arm, unable to estimate relative risk.

VII. How long should a patient with EM be treated?

10 DAYS DOXYCYCLINE vs. 20 DAYS DOXYCYCLINE
In patients with erythema migrans, should doxycycline be used for 10 days over 20 days?
P: In patients with erythema migrans
I: 10-day course of Doxycycline
C: 20-day course of Doxycycline

Bibliography: 1. Wormser, et al. Ann Intern Med. 2003 May 6; 138(9): 697-704.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline 10 days	Doxycycline 20 days	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
			Certainty assessment				№ of Events/№ of patients		Effect			
Resolution of Erythema Migrans (at 20 days)												
1	RCT [1]	not serious	not serious	not serious	not serious [a]	none	47/48 (97.9%)	45/45 (100.0%)	RR 0.98 (0.92 to 1.04)	20 fewer per 1,000 (from 80 fewer to 40 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Patients Withdrawing due to Adverse Events												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	1/61 (1.6%)	0/59 (0.0%)	NA [c]	16 more per 1,000	⊕⊕⊕◯ MODERATE	CRITICAL
Total Adverse Events												
1	RCT [1]	not serious	not serious	not serious	serious [d]	none	27/61 (44.3%)	25/59 (42.4%)	RR 1.04 (0.69 to 1.57)	17 more per 1,000 (from 131 fewer to 242 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Gastrointestinal Adverse Events												
1	RCT [1]	not serious	not serious	not serious	serious [d]	none	21/61 (34.4%)	22/59 (37.3%)	RR 0.92 (0.57 to 1.49)	30 fewer per 1,000 (from 160 fewer to 183 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Photosensitivity Reaction												

| 1 | RCT [1] | not serious | not serious | not serious | serious [d] | none | 5/61 (8.2%) | 2/59 (3.4%) | RR 2.42 (0.49 to 11.98) | 48 more per 1,000 (from 17 fewer to 372 more) | ⊕⊕⊕◯ MODERATE | IMPORTANT |

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Based on an inferiority margin of 15%, not rated down for imprecision.
b. Due to low event rate.
c. Due to zero events in one study arm, unable to estimate relative risk.
d. 95% CI wide and crossing the null value.

10 DAYS DOXYCYCLINE vs. 15 DAYS DOXYCYCLINE

In patients with erythema migrans, should doxycycline be used for 10 days over 15 days?

P: In patients with erythema migrans

I: 10-day course of Doxycycline

C: 15-day course of Doxycycline

Bibliography: 1. Stupica, et al. Clin Infect Dis. 2012 Aug; 55(3): 343-50.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline 10 days	Doxycycline 15 days	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 14 days)												
1	RCT [1]	serious [a]	not serious	not serious	not serious [b]	none	82/108 (75.9%)	84/117 (71.8%)	RR 1.06 (0.91 to 1.24)	43 more per 1,000 (from 65 fewer to 172 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	0/96 (0.0%)	0/101 (0.0%)	NA [d]	0 per 1,000	⊕⊕◯◯ LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [e]	none	14/108 (13.0%)	25/117 (21.4%)	RR 0.61 (0.33 to 1.11)	83 fewer per 1,000 (from 24 more to 143 fewer)	⊕⊕◯◯ LOW	IMPORTANT
Allergic reaction												
1	RCT [1]	serious [a]	not serious	not serious	serious [e]	none	0/108 (0.0%)	7/117 (6.0%)	NA [d]	60 fewer per 1,000	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received multiple high risk of bias ratings due to open label allocation and unblinded trial design.

b. Based on an inferiority margin of 15%, no rated down for imprecision.
c. Due to low event rate.
d. Due to zero events in one arm, unable to estimate relative risk.
e. 95% CI wide and crossing the null value.

14-DAY DOXYCYCLINE vs. 14-DAY PENICILLIN V

In patients with erythema migrans, should 14 days of Doxycycline be used over 14 days of Penicillin V?

P: In patients with erythema migrans

I: 14-day course of Doxycycline

C: 14-day course of Penicillin V

Bibliography: 1. Eliassen, et al. Clin Microbiol Infect. 2018 Dec; 24(12):1290-1296.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline 14 days	Penicillin V 14 days	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
			Certainty assessment				**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 14 days)												
1	RCT [1]	not serious	not serious	not serious	not serious [a]	none	68/68 (100.0%)	54/55 (98.2%)	RR 1.02 (0.97 to 1.07)	20 more per 1,000 (from 29 fewer to 69 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation: Higher values indicate longer resolution time.)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	Mean days: 81	Mean days: 31	**MD 50 higher (29.05 higher to 70.95 higher)**		⊕⊕⊕◯ MODERATE	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	not serious	not serious	not serious	serious [c]	none	0/68 (0.0%)	0/55 (0.0%)	NA [d]	0 per 1,000	⊕⊕⊕◯ MODERATE	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [e]	not serious	not serious	serious [c]	none	21/67 (31.3%)	20/55 (36.4%)	RR 0.86 (0.52 to 1.42)	51 fewer per 1,000 (from 153 more to 175 fewer)	⊕⊕◯◯ LOW	IMPORTANT
Allergic Reaction												
1	RCT [1]	serious [e]	not serious	not serious	serious [c]	none	0/67 (0.0%)	0/55 (0.0%)	NA [d]	0 per 1,000	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Based on an inferiority margin of 15%, not rated down for imprecision.

b. Eliassen 2018 reported duration of EM as median and range- Median for Doxy group was 14 days (range 3-293 days) and for PenV group was 14 days (range 5-91 days). These were converted to mean and SD using method by Hozo, et al. However, due to a wide range of EM duration in this study, the SDs are very large**.**

c. Due to low event rate and/or 95% CI crossing the null value.

d. Due to zero events in one study arm, unable to estimate relative risk.

e. Due to single-blind design (outcomes were self-reported).

14-DAY DOXYCYCLINE vs. 14-DAY AMOXICILLIN

In patients with erythema migrans, should 14 days of Doxycycline be used over 14 days of Amoxicillin?

P: In patients with erythema migrans

I: 14-day course of Doxycycline

C: 14-day course of Amoxicillin

Bibliography: 1. Eliassen, et al. Clinical Microbiology and Infection. 2018 Dec;24(12):1290-1296.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline 14 days	Amoxicillin 14 days	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 14 days)												
1	RCT [1]	not serious	not serious	not serious	not serious [a]	none	68/68 (100.0%)	62/63 (98.4%)	RR 1.02 (0.97 to 1.06)	20 more per 1,000 (from 30 fewer to 59 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Time to Resolution of Erythema Migrans (Days since treatment initiation: Higher values indicate longer resolution time.)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	Mean days: 81	Mean days: 52		**MD 28.75 higher (5.32 higher to 52.18 higher)**	⊕⊕⊕○ MODERATE	IMPORTANT
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	not serious	not serious	not serious	serious [c]	none	0/68 (0.0%)	0/63 (0.0%)	NA [d]	0 per 1,000	⊕⊕⊕○ MODERATE	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [e]	not serious	not serious	serious [c]	none	21/67 (31.3%)	19/64 (29.7%)	RR 1.06 (0.63 to 1.77)	18 more per 1,000 (from 110 fewer to 229 more)	⊕⊕○○ LOW	IMPORTANT
Allergic Reaction												
1	RCT [1]	serious [e]	not serious	not serious	serious [c]	none	0/67 (0.0%)	2/64 (3.1%)	NA [d]	31 fewer per 1,000	⊕⊕○○ LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio; **MD:** Mean difference

Explanations

a. Based on an inferiority margin of 15%, not rated down for imprecision.

b. Eliassen 2018 reported duration of EM as median and range- Median for Doxy group was 14 days (range 3-293 days) and for Amoxicillin group was 13 days (range 4-179 days). These were converted to mean and SD using method by Hozo, et al. However, due to a wide range of EM duration in this study, the SDs are very large.

c. Due to low event rate and/or 95% CI crossing the null value.

d. Due to zero events in one study arm, unable to estimate relative risk.

e. Due to single-blind design (outcomes were self-reported).

14-DAYS CEFTRIAXONE vs. 21-DAYS DOXYCYCLINE

In patients with erythema migrans, should 14-day course of Ceftriaxone be used over 21-day course of Doxycycline?

P: In patients with erythema migrans

I: 14-day course of Ceftriaxone

C: 21-day course of Doxycycline

Bibliography: 1. Dattwyler, et al. N Engl J Med. 1997 Jul 31;337(5):289-94.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	14 Days Ceftriaxone	21 Days Doxycycline	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of Events/№ of patients**		**Effect**			
Resolution of Erythema Migrans (at 90 days)												
1	RCT [1]	not serious	not serious	serious [b]	not serious [c]	none	55/60 (91.7%)	63/67 (94.0%)	RR 0.97 (0.88 to 1.07)	28 fewer per 1,000 (from 66 more to 113 fewer)	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Experiencing Objective Findings of Lyme (at 6 months and beyond)												
1	RCT [1]	not serious	not serious	serious [b]	serious [d]	none	1/68 (1.5%)	1/72 (1.4%)	RR 1.06 (0.07 to 16.59)	1 more per 1,000 (from 40 fewer to 39 more)	⊕⊕◯◯ LOW	CRITICAL
Patients Withdrawing due to Adverse Events												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [d]	none	4/68 (5.9%)	0/72 (0.0%)	NA [g]	59 more per 1,000	⊕◯◯◯ VERY LOW	CRITICAL
Treatment-related Adverse Events												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [e]	none	39/68 (57.4%)	31/72 (43.1%)	RR 1.33 (0.95 to 1.86)	142 more per 1,000 (from 22 fewer to 370 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [f]	none	28/68 (41.2%)	18/72 (25.0%)	**RR 1.65 (1.01 to 2.69)**	**162 more per 1,000 (from 3 more to 423 more)**	⊕◯◯◯ VERY LOW	IMPORTANT

Allergic reaction											
1	RCT [1]	serious [a]	not serious	serious [b]	serious [e]	none	4/68 (5.9%)	9/72 (12.5%)	RR 0.47 (0.15 to 1.46)	66 fewer per 1,000 (from 57 more to 106 fewer)	⊕◯◯◯ VERY LOW	IMPORTANT
Diarrhea											
1	RCT [1]	serious [a]	not serious	serious [b]	serious [f]	none	25/68 (36.8%)	4/72 (5.6%)	**RR 6.62 (2.43 to 18.03)**	**312 more per 1,000 (from 79 more to 946 more)**	⊕◯◯◯ VERY LOW	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Trial received a high risk of bias rating due to unblinded design (self-reported outcomes).

b. Patients in this trial have evidence of disseminated Lyme disease at baseline rather than localized Lyme disease.

c. Based on an inferiority margin of 15%, not rated down for imprecision.

d. Due to low event rate.

e. 95% CI crossing the null value.

f. Fragility due not small sample size (OIS criteria not met).

g. Due to zero events in one study arm, an absolute risk reduction was not estimable.

VIII. Should patients with Southern Tick-Associated Rash Illness (STARI) be treated with antibiotics?

Bibliography: 1. Kirkland, et al.Arch Intern Med. 1997 Dec 8-22; 157(22): 2635-41; 2. Felz, et al. ; 3. Wormser, et al. Clin Infect Dis. 2005 Feb 1;40(3):423-8; 4. Wormser, et al. Clin Infect Dis. 2005 Oct 1; 41(7): 958-65; 5. Philipp, et al. Clin Vaccine Immunol. 2006 Oct; 13(10): 1170-1; 6. Clark, et al. Int J Med Sci. 2013 May 23; 10(7): 915-31; 7. Lantos, et al. Vector Borne Zoonotic Dis. 2013 Dec; 13(12): 877-83.

Study and Location	Study Design	Risk of bias*	Lyme Disease/STARI Diagnosis method, Patient characteristics	Lyme vs. STARI characteristics	Study conclusions
Kirkland, et al., 1997 North Carolina	Case series (14 cases)	NA	8 cases of suspected erythema migrans (EM) were observed in a camp in the central Piedmont region of North Carolina in 1994, all with onset between May 14 and July 5. 6 additional cases were observed in the same area in 1995, with onset between May 1 and August 12. Of the 14 cases, 10 people were residents of the area and 4 were staff members of the camp. Thirteen case-patients were female, with a median age of 15 years (range, 13-49 years). 13 patients were evaluated for presence of antibodies against *B. burgdorferi* by ELISA, confirmed by Western Blot. Skin biopsies were collected from five patients (4 of whom had not yet received antibiotics).	The EM-like lesions were erythematous, had irregular borders, and had central clearing. Several patients first noted a small papule around which a red patch developed and then expanded, leaving an area of central clearing. Other case-patients first noticed the rash when it was larger and annular in appearance. Several case-patients reported that the lesions were tender or pruritic. The rash lesions were on the ankles or feet in 8 case-patients, other parts of the leg in 2 case-patients, on the trunk in 3 case-patients, and on the wrist in 1 case-patient. The lesions ranged in size from 3 x 5 cm to 6 x 7 cm. Most of the case-patients had mild systemic illnesses at the time of their rash. Ten case-patients reported headache, 8 reported musculoskeletal pain, 7 reported fatigue, and 6 reported nausea. One case-patient had a temperature of 38.7°C; the rest were afebrile. Four of the 14 case-patients had no manifestations of illness other than the skin lesion.	None of the biopsy specimens from 5 patients were positive by culture. 4 of 14 case-patients had positive or equivocal results by ELISA, but these results were not confirmed by Western Blot in any case. Of 9 patients with negative ELISA, one patient had positive IgM results by Western Blot both in the acute and convalescent phase. None of the patients tested (N=13) had positive IgG Western Blot. All 14 case-patients were treated with a 10-day course of doxycycline with resolution of symptoms within the 10-day period. Based on negative serologic and culture findings, the authors conclude that there is evidence of a tick-associated EM-like rash illness in Southern states which is not caused by *B. burgdorferi.*

| Felz, et al., 1999

Georgia and South Carolina | Case series (23 cases) | NA | 23 patients presenting with an expanding erythematous rash ≥5 cm in diameter, physician diagnosis of erythema migrans (EM), and tick bite or exposure within the preceding month.

Patients were excluded if the rash had resolved prior to enrollment, if they had received antibiotics within the prior two weeks, or if they were ≤2 years old, pregnant, or nursing.

Skin biopsy specimens were collected and histologically examined and cultured using BSK II medium. Biopsy specimens were also subject to PCR analysis. Serologic analysis was performed with a *fla*-EIA, confirmed by Western Blot, and results were available for 22 patients.

30 healthy blood donors served as positive controls. | Clinical manifestations were limited to solitary, annular, erythematous skin lesions at the site of a tick bite and mild systemic complaints. 78% of patients had rash with central clearing. 91% of patients recalled a tick bite, and verbal reports of two patients suggested that the tick may have been *A. americanum*. 26% of patients reported malaise, and 26% of patients reported headache. 22% of patients were febrile (>38°C). 17% of patients reported arthralgia or myalgia, and 17% of patients reported sore neck.

Secondary or multiple erythematous lesions, neuropathy, arthritis, and bradycardia were not observed.

Lesions ranged in size from 5 x 4 cm to 20 x 17 cm, with a mean of 9.6 cm. Lesions had been observed by patients for a mean of 9.3 days (range 3-21 days). Lesions occurred on the leg (N=8), back (N=6), shoulder (N=4), abdomen (N=4), and chest (N=1). | Spirochetes suggestive of *Borrelia* were demonstrated in replicate kin sections stained with the Steiner silver procedure in 3 (13%) of 23 patients.

Biopsy specimens were positive by PCR in 5 (22%) patients. Only 1 of 23 (4%) patients was culture-positive for *B. burgdorferi*.

Only 2 of 23 (9%) patients was serologically confirmed to have *B. burgdorferi* infection. 8 patients had equivocal results by EIA. Across all methods, only 7 patients had confirmed *B. burgdorferi* infection.

The authors concluded that EM in this region may represent Lyme infection in some cases, but is of undetermined origin for most patients. |
| Wormser 2005 and Wormser 2005*

Valhalla, NY and Cape Girardeau, MO

*Same cohort, | Prospective cohort study | 6 | Microbiologic Diagnosis:
21 patients from Missouri and 143 New York patients presented with erythema migrans (EM)-like skin lesions. Skin biopsy specimens were cultured and evaluated by PCR, and serum tested by ELISA.

Clinical Diagnosis:
At study onset and up to three months, 21 Missouri and 97 (of 143, with similar lesions to those of MO patients) New York patients were physically examined and photographs were taken of the presenting lesion(s). | Microbiologic Diagnosis:
Of 19 cultures, none of the Missouri skin samples were positive by PCR for *B. burgdorferi*. 63% of skin samples from New York patients were positive.

None of the Missouri patients were seropositive for antibodies against *B. burgdorferi* by ELISA, whereas 75% of New York patients were (P< 0.001).

Clinical Diagnosis:
Statistically significant differences were found between the clinical presentations of the two | Microbiologic Diagnosis:
B. lonestari and *B. burgdorferi* are not likely cases of EM-like skin lesions in patients from Missouri. The etiology of the condition is unknown.

No evidence of *B. lonestari* was found in any of 312 field-collected *A. americanum* ticks or in 2 *A. americanum* ticks removed from Missouri patients who later developed EM-like lesions.

Clinical Diagnosis:
Missouri patients recovered more rapidly than NY patients after antibiotic therapy (p=0.037). |

					groups. 85.7% of Missouri and 19.8% of New York case patients recalled a tick bite. The authors found that the time period from tick bite to onset of the skin lesion was shorter among Missouri patients (mean 6.1 days vs. mean 10.4 days in NY patients; p=0.011). Missouri patients presented with milder symptoms than those from New York (P= 0.005). Missouri patients were less likely to be symptomatic than NY patients (p<0.001), and were less likely to have multiple skin lesions (p=0.042). The skin lesions of Missouri patients were more likely to show central clearing (p< 0.001) and were typically more circular in shape (p=0.004).	The authors noted several statistically significant differences in clinical presentation of EM-like lesions between patients in Missouri and patients in New York.
reports different results						
Philipp, et al., 2006 Maryland, Missouri, New York, and North Carolina	Prospective cohort study	4	Serum specimens were evaluated for presence of antibodies against *B. burgdorferi* by the C6 Lyme ELISA. Focus Diagnostics, Inc. (FDI): Specimens from 9 STARI patients from Missouri and from one patient who had a proven *B. lonestari* infection acquired in Maryland or North Carolina. 9 acute-phase and 9 convalescent-phase specimens from patients with erythema migrans and culture-confirmed Lyme disease patients from New York. Tulane Nat'l. Primate Research Center (TNPRC): 70 acute- or convalescent-phase samples from 63 STARI patients from Missouri were tested.	ND	FDI: All 10 serum samples from STARI patients were negative by C6 Lyme ELISA. In contrast, 88.9% (8 of 9) of serum samples from New York patients with Lyme disease were positive by C6 ELISA. TNPRC: 98.4% (62 of 63) serum samples from STARI patients were negative by C6 Lyme ELISA. The authors concluded that patients who presented with an "erythema-migrans-like" rash, or "Lyme-like" illness in Southern states such as Missouri or North Carolina do not have Lyme disease.

Clark, 2013 Florida and Georgia, USA	Case series (10 cases)	NA	Clinical presentation, PCR, and DNA sequence used to identify *B. burgdorferi* in samples of blood and skin in 10 patients (6 of 10 female) from Florida or Georgia. In 4 patients with Lone Star tick bites, *B. burgdorferi* sensu lato DNA was found and confirmed by PCR.	ND	Several *B. burgdorferi* sensu lato species may be associated with Lyme disease-like signs and symptoms in southern states. Lyme borreliosis occurs in Florida and Georgia. Some cases of Lyme-like illness referred to as STARI in southern United States may actually be prior, undetected infections with Lyme *Borrelia* strains. Study found no evidence to support *B. lonestari* as cause of STARI.
Study and Location	**Study Design**	**Risk of bias***	**Population Characteristics and Treatment Decision Options**	**Study Results**	**Study Conclusions**
Lantos, et al., 2013 15 USA states	Decision Analysis Study	NA	Patients presenting with an EM-like skin lesion in endemic and non-endemic areas were studied. Three possible decisions were assessed: 1. **Treat all:** all patients are given standard course of antibiotics intended to treat EM due to early Lyme disease 2. **Observe All:** patients are observed, and treated only if disseminated Lyme disease develops 3. **Serology and treat seropositive patients:** patients are tested using two-tier serology (ELISA and Western blot). Seropositive patients are treated with antibiotics, and seronegative patients are observed.	1. **Treat all:** If the probability that EM is Lyme= 1, the model predicts 78,000 cases of disseminated Lyme disease would be averted for every 100,000 treated patients. If the probability that EM is Lyme= 0.0001, only 8 cases of disseminated Lyme disease would be prevented for every 100,000 patients treated. Additionally, one patient would have a major adverse medication event for every averted case of disseminated Lyme disease, making such an event 64 more times likely than an averted case of disseminated Lyme disease. 2. **Observe all:** Observing 100,000 patients where the probability that EM is Lyme= 1 would result in 83,000 cases of disseminated	"Treat All" was the most affordable strategy whenever the probability of EM being Lyme disease exceeded 0.0061. "Observe All" was the least costly strategy below this value. Regardless of the probability of EM being Lyme, "Serology" was never the most cost-effective strategy..

				Lyme disease, as compared with 5,000 under a Treat All strategy . **3. Serology and treat seropositive patients**: Serology would substantially reduce the number of adverse treatment events per averted case of disseminated Lyme disease. **4. Cost Effectiveness:** In cases when EM cases are definitely due to Lyme disease, the cost for Treat All strategy would be $219 per patient. Serology-guided care would cost $2,315 per patient. The Observe strategy would cost $3,320 per patient. In areas where EM-like lesions are never due to Lyme disease (the probability that EM is Lyme=0), "Treat All" would cost $19 per patient, Serology would cost $80, and Observe would cost $0 per patient. As the probability that EM is Lyme disease increases, all strategies become costlier. Treat all is the least expensive strategy for all probability values of EM being Lyme greater than 0.0061.	

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Neurologic Lyme disease

IX. What is the preferred diagnostic testing strategy for Lyme neuroborreliosis?

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Study and Location	Study Design	Risk of bias*	Population Characteristics	Diagnosis Method, % Positive	Study Conclusions
			Culture		
Karlsson, et al., 1990 Stockholm, Sweden	Prospective cohort study		Cerebrospinal fluid (CSF) samples were obtained from 105 patients with a preliminary clinical diagnosis of Lyme borreliosis with neurological complications. Final diagnoses were established retrospectively by reviewing the records of each patient. The diagnosis of neuroborreliosis required at least two of the following criteria: (i) pleocytosis in CSF together with neurological signs and symptoms and/or general symptoms compatible with neuroborreliosis, (ii) neurological signs and symptoms or pleocytosis within 3 months after	Samples were cultivated on Kelly-modified medium, with and without rabbit serum. All cultures were incubated at 35°C and examined by dark-field microscopy every 2 weeks for 2 months. After three to four passages, the isolates were stored at -70°C. Paired serum and CSF samples of the patients from whom spirochetes were cultivated were analyzed by Western Blot. Paired serum and CSF samples from the 105 patients included in the study were screened in a routine ELISA.	The 4 culture-positive patients were among the 38 patients that retrospectively fulfilled criteria for neuroborreliosis. Two of the thirty-eight patients were receiving oral treatment with antibiotics at the time of lumbar puncture, and as a result, their cultures were negative. Positive IgM or IgG titers were found in both serum and CSF from 17 of 38 patients with retrospectively confirmed neuroborreliosis. Positive antibody titers

			the onset of erythema migrans, (iii) positive antibody titers against *B. burgdorferi* in serum and/or CSF.	Spirochetes were cultured from CSF samples from 4 of the 36 patients (11%) with untreated neuroborreliosis.	were found by routine ELISA in serum from one of the four patients from whom spirochetes were cultured. Positive antibody titers in CSF were found in another two of these four patients. The authors concluded that isolation of spirochetes from CSF is not suitable as a routine diagnostic test. They noted that isolation of spirochetes from CSF might prove successful in clinically selected cases.
Cerar, et al., 2008 Ljubljana, Slovenia	Case-control study		48 adults with a working clinical diagnosis of Lyme neuroborreliosis (median duration 10 days, range 2-90), defined by erythema migrans ≤4 months before the appearance of neurological symptoms and signs, including radiculoneuritis and/or peripheral facial palsy, and pleocytosis, were assessed. 45 adults with a working clinical diagnosis of suspected Lyme neuroborreliosis (median duration 14 days, range 1-120), defined by erythema migrans ≤4 months before the appearance of neurological symptoms, but without pleocytosis. Erythema migrans was still present in 13 of 48 patients with a working clinical diagnosis of Lyme neuroborreliosis and in 40 of 45 patients with suspected Lyme neuroborreliosis. 42 patients with tick-borne encephalitis (TBE) and 21 neurosurgical patients served as controls.	Specimens were cultivated in modified Kelly-Pettenkofer (MKP) medium and were analyzed by nested PCR which amplified two DNA targets—the intergenic *rrf-rrl* region and the gene *OspA* with restriction fragment length polymorphism (RFLP) analysis. *Borreliae* were only isolated from 1 out of 135 blood samples by culture. PCR targeting OspA (Blood): ● Working diagnosis LNB: 14.6% positive ● Suspected LNB: 11.1% positive ● TBE: 4.8% positive PCR targeting OspA (CSF): ● Working diagnosis LNB: 20.8% positive ● Suspected LNB: 15.6% positive ● TBE: 4.8% positive PCR targeting *rrf-rrl* (Blood): ● Working diagnosis LNB: 10.4% positive ● Suspected LNB: 15.6% positive ● TBE: 4.8% positive PCR targeting *rrf-rrl* (CSF): ● Working diagnosis LNB: 20.8% positive	Results of the two PCR methods were concordant in 131 of 135 (97%) blood samples and in 146 of 156 (93.6%) CSF samples. PCR targeting OspA detected the presence of borrelial DNA in 14 of 135 (10.4%) blood samples and in 19 of 156 (12.2%) CSF specimens. Frequency of positive results did not significantly differ between CSF and blood. PCR targeting OspA found no borrelial DNA in the CSF samples of the 21 patients in the neurosurgical control group. Only half of the culture-positive CSF and blood specimens were positive with at least one of the two PCR approaches.

				● Suspected LNB: 17.8% positive ● TBE: 2.4% positive	
Nowakowski, et al., 2009 Westchester County, NY	Prospective cohort study		26 untreated adult patients (57.7% male; mean age 49 years) with objective neurologic, cardiac, or musculoskeletal manifestations consistent with Lyme disease were enrolled. 13 patients (50%) had ≥1 early neurologic manifestations of Lyme disease, including cranial nerve palsy, meningitis, or radiculopathy.	Plasma samples were cultured in Barbour Stoenner Kelly (BSK) media. Cultures were examined microscopically at 2, 4, and 8 weeks for the presence of spirochetes, which were confirmed as *B. burgdorferi* by PCR evaluation. Of 13 patients with neuroborreliosis, 3 (23.1%) had a blood culture positive for *B. burgdorferi*; all of these patients had multiple erythema migrans skin lesions.	Patients with positive blood culture results were symptomatic for a significantly shorter duration of time than were those with negative blood culture results (p=0.04). Positive blood culture results were not associated with age (p=0.87), sex (p>0.99), or number of viral-like symptoms (p=0.53). The association of positive culture results with shorter symptom duration and presence of erythema migrans led the author to believe that blood culture is more likely to be positive during the early stages of infection with Lyme disease.
Maraspin, et al., 2011 Ljubljana, Slovenia	Retrospective study		Documentation of all patients ≥15 years of age who were diagnosed with borrelial lymphocytoma, Lyme neuroborreliosis, Lyme arthritis, or acrodermatitis chronica atrophicans (ACA) was reviewed. 176 patients were documented as having neuroborreliosis. In order to be diagnosed with neuroborreliosis, patients were required to have CSF pleocytosis and the presence of erythema migrans or borrelial IgG antibodies in serum.	All serum samples were cultured for presence of spirochetes using Kelly Pettenkofer medium. Cultures were examined microscopically every week for 12 weeks for the presence of spirochetes, which were confirmed as *B. burgdorferi* by PCR evaluation. *B. burgdorferi* was isolated from the blood in 6 of 176 (3.4%) patients with neuroborreliosis. Two of these six patients had erythema migrans at the time of blood culture.	Overall, the authors found that in European patients with manifestations of Lyme borreliosis other than erythema migrans, the isolation rate of *B. burgdorferi* by blood culture is low (11 of 442, or 2.5% overall). Successful culture was associated with presence of erythema migrans (36.4% vs. 13.2%, p=0.0513) and a shorter duration of symptoms (median 3.5 weeks).
Polymerase Chain Reaction (PCR)					
Dumler, 2001	Systematic Review and Meta-analysis		Search dates unclear (Latest included article was published in 2000; published in 2001). Included any studies evaluating published PCR assays of skin (N=8 studies), plasma or serum (N=3 studies), synovial fluid (N=7 studies), cerebrospinal fluid (CSF) (N=10 studies), or urine	Published PCR assays for plasma or serum overall (113 tested): ● Weighted mean sensitivity: 26% (median sensitivity 29%, range: 0-52%). ● Weighted mean specificity: 100%.	The authors found that there is a significant lack of sensitivity for PCR detection of *B. burgdorferi* in the CSF of patients with Lyme disease, and concluded that a negative PCR result does not exclude the diagnosis of neuroborreliosis. They state that since most patients with neurologic involvement are

		(N=) as a diagnostic tool for various stages of Lyme disease. Cerebrospinal fluid analysis was specifically described in relation to disseminated infection manifesting as meningitis, radiculoneuritis, and cranial nerve palsies, as well as rarer manifestations, such as central nervous system involvement or chronic forms of the disease. Details of the meta-analysis were not described. Patients in the cerebrospinal fluid analysis were divided by disease stage ("Overall"= all stages, and "Non-chronic" referring to patients experiencing more acute forms of disseminated Lyme infection related to the nervous system).	Published PCR assays for urine overall (405 tested): ● Weighted mean sensitivity: 68% (median sensitivity 74%, range: 13%-100%). ● Weighted mean specificity: 99%. Published PCR assays for CSF overall (705 tested): ● Weighted mean sensitivity: 19% (median sensitivity 24%; range: 6%-91%) ● Weighted mean specificity: 100% Published PCR assays for CSF for "non-chronic" patients (650 tested): ● Weighted mean sensitivity: 17% (median sensitivity 21%, range: 6%-76%).	serologically reactive and also have specific intrathecal *B. burgdorferi* antibody production, CSF PCR for Lyme disease should be reserved for patients with a high clinical index of suspicion for neurologic involvement early on in the disease process.	
Avery, et al., 2005 Wilmington, DE	Retrospective study	108 pediatric patients (69.4% male; mean age 9.5 years, range 2.7 years-17.8 years) with meningitis qualified for the study. 20 patients met the criteria for Lyme meningitis (positive serology confirmed by Western Blot), and 88 were classified as having aseptic meningitis. Of 20 Lyme meningitis patients, 12 had erythema migrans and 6 had both ELISA and Western blot reactivity. *Exclusion criteria included a past history of Lyme meningitis; evaluation for an ongoing chronic neurologic condition, traumatic lumbar puncture, or a positive CSF Gram stain for bacteria.*	CSF samples were assessed by a PCR (Lyme PCR) assay which amplified a *B. burgdorferi* DNA flagellin gene sequence. Lyme PCR: ● Sensitivity: 5% ● Specificity: 99%	The Lyme meningitis patient with positive Lyme CSF-PCR had the highest CSF white blood cell count and CSF protein values compared with the other 19 Lyme meningitis patients. The authors concluded that PCR of CSF is not a helpful laboratory test in identifying Lyme meningitis or differentiating Lyme meningitis from aseptic meningitis because of its low sensitivity. Of 20 patients diagnosed with Lyme meningitis, the Lyme CSF-PCR result was a false negative in 95%, leading the authors to conclude that a negative Lyme CSF-PCR result does not exclude the diagnosis of Lyme meningitis. The authors observed a rate of positive Lyme CSF-PCR which was lower than those reported in European children with Lyme meningitis.	

Pícha, et al., 2005 Nymburk, Czech Republic	Prospective cohort study		57 hospitalized patients (52.6% male; mean age 42 years; range 12–71 years) with active neuroborreliosis, defined as actual symptoms of nervous system disturbances <u>with</u> positivity of specific antibody index in cerebrospinal fluid (CSF) or serum (N=51) <u>or</u> single isolated proof of specific antibodies in CSF (N=6). Symptom presentation included Bannwarth's syndrome (N=29), acute meningoencephalitis (N=5), subacute encephalitis (N=3), meningitis (N=6), multiplex neuritis (N=9), and facial palsy (N=5). Symptoms were assessed as mild in 25 patients (43.8%), and moderate in 17 patients (29.8%). Eleven patients (19.2%) were classified as more severe and five patients had extensive neurological findings.	Nested PCR targeting *B. burgdorferi* flagellin, 16SrDNA, and OspC genes was used for the detection of specific DNA in plasma, CSF and urine. Before treatment: ● All specimens: Sensitivity= 63.1% ● Urine: Sensitivity= 49.1% ● CSF: Sensitivity= 35% ● Plasma: Sensitivity= 28% After treatment: ● Urine (immediately post-treatment): 30% positive ● Urine (3 months post-treatment): 14% positive ● Urine (6 months post-treatment): 1.8% positive	The authors concluded that the highest sensitivity of PCR was achieved in the acute period of neuroborreliosis in three body fluids comparing with CSF antibody synthesis.
Babady, et al., 2008 Rochester, MN	Retrospective study		23,777 cerebrospinal fluid (N=15,939), blood (N=5,703), synovial fluid (N=1,976), tissue (N=92), and other sample sources (N=67) were tested by a reference laboratory. For assay validation, results of the real-time PCR assay were compared with those obtained by conventional PCR using blood, cerebrospinal fluid, synovial fluid, and tissue.	Samples were tested by a real-time PCR encoding for the borrelial plasminogen binding protein. Synovial fluid: 6.4% positive (127 of 1,976) Tissue: 6.5% positive (6 of 92) Blood: 0.1% positive (6 of 5,703) Cerebrospinal fluid: 0.09% (14 of 15,939) Among patients with a positive PCR in synovial fluid or tissue, 8 also had blood or CSF submitted for testing by PCR with negative results.	Though cerebrospinal fluid and blood are most frequently submitted for Lyme PCR, they demonstrate the lowest positivity rates. Detection of *B. burgdorferi* was highest in synovial fluid (6.4%) and tissue (6.5%), even though these specimen types making up a small percentage (8.3% and 0.4%, respectively) of the total number of specimens submitted for testing by PCR. The authors note that their data are in agreement with results reported in previous studies using either real-time or conventional PCR, which have shown a low positivity rate for blood and cerebrospinal fluid, even in patients with known Lyme disease. The authors state that blood submitted for Lyme PCR is rarely useful and recommend synovial fluid PCR in the context of a positive serologic test.

Maes, et al., 2017 Diepenbeck, Belgium	Retrospective study		103 cerebrospinal fluid samples of patients with clinical suspicion of neuroborreliosis (classic triad of peripheral paresis, lymphocytic CSF pleocytosis, and radiculitis) were analyzed retrospectively. "The age range of the patients was 5–91 years (average 47). Intrathecal antibody production was found for 16 % of the patients, meaning that the patients in this study have a possible Lyme neuroborreliosis."	The O-DiaBorburg kit (DIA) targeting ospA, *Borrelia burgdorferi* PCR kit–ISEX (GENE) targeting the 16S rDNA gene, and the *Borrelia burgdorferi* sensu lato Real-TM PCR (SAC) also targeting the 16S rDNA gene were selected for validation. The analytical sensitivity was evaluated for *B. afzelii*, *B. garinii*, and *B. burgdorferi sensu stricto*. The LOD_{95} is defined as the lowest detectable DNA concentration with a 95 % chance of a positive result. The study aimed at defining an LOD95 at least as sensitive as described in the kit insert of the assay which was 532 copies/ml, 500 copies/ml, and 20,000 copies/ml for the GENE, SAC, and DIA kit respectively. The specificity was evaluated by analyzing organisms that are genetically related to *Borrelia* spp. (*B. duttonii*, *B.miyamotoi*, *B. japonica*, *B. hermsii*, *Treponema phagedenis*, *T. pallidum*, *Leptospira spp.*) and other pathogens present in CSF that cause similar symptoms.	"LOD95 experiments showed that the GENE kit was the most sensitive real-time PCR when it was performed on RGQ. Although the exact LOD95 was not determined for the SAC and DIA kits for some *Borrelia* strains, it was seen that the obtained LOD95 results of the SAC and DIA kits on LC480 were already higher than the obtained LOD95 values for these kits on RGQ." No cross-reactivity was found for genetically related organisms or other pathogens. Both the GENE and SAC kit were able to detect *B. valaisiana*, *B. bavariensis*, *B. spielmanii*, and *B. lusitaniae*. The DIA kit failed to detect *B. lusitaniae*
Barstad, et al., 2018 Southwest Norway	Prospective cohort study		Children aged 3 months to 18 years with symptoms suggestive of LNB who were admitted to the pediatric departments of five hospitals from autumn 2011 to spring 2014 were eligible. Children who had been given antibiotics prior to admission were excluded. Children with confirmed and probable LNB were included as LNB patients, and children with non-Lyme aseptic meningitis (NLAM) and negative controls were included as non-LNB controls. CSF samples of 117 children were analyzed by PCR. 76 were diagnosed as LNB patients (58 confirmed and 18 probable), 13 were diagnosed with possible LNB conditions, and 28 as non-LNB	DNA samples were tested using two independent real-time assays targeting the OspA and 16S rRNA genes to detect *B. burgdorferi*. Samples were considered positive for *B. burgdorferi* DNA if both assays gave positive results after one or two runs, or if more than one replicate in one assay was positive after two runs. ● Sensitivity: 45% ● Specificity: 100%	In this study, *B. burgdorferi* DNA was detected in the CSF of 46% of children diagnosed with LNB, which is a higher proportion than previously reported in most studies of children and adults. *B. garinii* was the predominant genotype associated with LNB in children in southwest Norway. This study supports the use of PCR as a supplemental diagnostic tool for children with suspected LNB, particularly in the early phase, when the antibody index is negative and the diagnosis is inconclusive.

		controls (12 with NLAM and 16 negative controls).		
		Antibody Index and CSF Analysis		
Halperin, et al., 1989 Stony Brook, NY	Prospective cohort study	85 patients with serological evidence of *B. burgdorferi* infection presenting with central nervous system manifestations. Diagnosis was based on 1) history of well-documented erythema migrans or evidence of immunoreactivity against *B. burgdorferi*, using either (a) a specific ELISA or (b) a specific lymphocyte proliferation assay; and (2) prominent nervous system abnormalities. **Group I**: Patients who described subjective difficulties with memory and cognition, and who had demonstrable cognitive or memory deficits; **Group II**: Patients with typical relapsing-remitting MS; **Group III**: Patients with an acute, monophasic illness affecting the CNS, apparently caused by *B burgdorferi* infection (Lyme meningitis- N=2, optic neuropathy- N=2, focal brainstem lesion- N=1); **Group IV**: Patients with peripheral nervous system dysfunction; **Group V**: These patients did not appear clinically to have CNS disease, but had had lumbar punctures because of (a) peripheral immunoreactivity against *B burgdorferi*, and (b) a concern that they might have *B burgdorferi* infection involving the CNS. Three had psychiatric disorders (2 with recurrent depression, 1 with a diagnosis of schizoaffective disorder), 1 had severe, persistent headaches, 1 had vitritis, and 1 had an acute, transient confusional state; **Group VI**: Patients who met the same criteria as those in group I, but did not undergo lumbar puncture or brain MRI.	**Group I:** "In 10 of the 13 patients in whom CSF was studied, the CSF Lyme antibody index was greater than 1.0, indicating intrathecal synthesis of anti-*B burgdorferi* antibody" **Group II:** "The CSF Lyme antibody index was consistently less than 1.0 (range, 0.19 to 0.73; mean, 0.39) in all 5 patients in whom this was measured. The 6th patient declined a lumbar puncture." **Group III:** "Anti-*B burgdorferi* antibody was elevated in the serum of 2 of the 5... One of this lacked evidence of antibody synthesis in the CSF, but this may have been artifactual." **Group IV:** "Only 2 patients had elevated CSF Lyme antibody indices. Both had moderately severe chronic polyneuropathy." **Group V:** Only one patient presented with a Lyme antibody index greater than 1. **Group VI:** These patients did not undergo lumbar puncture	"Several previous studies suggested that active CNS Lyme borreliosis is frequently associated with evidence of intrathecal synthesis of anti-*B burgdorferi* antibody. In our study, 10 of the 13 patients with encephalopathy and one-half the patients with focal CNS disease had evidence of intrathecal synthesis of specific antibody. In contrast, this was observed in only 2 of 24 with peripheral or cranial neuropathies. (Interestingly, 3 patients with radicular pain did not have evidence of intrathecal synthesis of specific antibody. This observation, in conjunction with published pathologic studies demonstrating striking abnormalities in biopsied sural nerves in patients with Lyme radiculitis, suggests that this "radiculitis" may actually be a manifestation of a more widespread neuropathy, and may not necessarily involve nerve root inflammation.) High CSF concentrations of specific antibody in patients with encephalopathy suggest that CSF anti-*B burgdorferi* antibody concentration is a useful indicator of CNS involvement. Moreover, it provides considerable support to the hypothesis that the cognitive difficulties described by our patients reflect active CNS disease. This type of CSF abnormality could be due either to a chronic, active CNS infection or to an abnormality of the immune response."

Steere, et al., 1990 Boston, MA and Cologne, Germany	Prospective cohort study	37 U.S. patients were evaluated for early or late neurologic abnormalities. 13 patients had early Lyme neuroborreliosis, defined as neurologic symptoms occurring within weeks or months of illness accompanied by a positive antibody response to *B. burgdorferi* in serum or cerebrospinal fluid (CSF). These patients had meningitis, sometimes accompanied by cranial or peripheral neuropathy. 18 U.S. patients had symptoms suggestive of late neuroborreliosis, defined as neurologic symptoms occurring ≥1 year after disease onset accompanied by a positive antibody response to *B. burgdorferi* in serum or CSF. Of these, 12 had central nervous system (CNS) syndromes with subtle cognitive impairment, headache, seizures, unilateral hearing loss, or somnolence; 6 of them also had peripheral nervous system abnormalities. Serum and CSF samples were also collected from 30 West German patients with early (N=20) or late (N=10) neuroborreliosis.	Routine laboratory determinations done on blood and CSF included white blood cell count, total protein, glucose, albumin, and total IgG. From these data, the CSF IgG index was calculated according to the following formula: (CSF IgG x serum albumin)/(CSF albumin x serum IgG). ● 92% of patients from the U.S. with Lyme meningitis were found to have intrathecal antibody production by Antibody capture EIA ● 42% of patients with late central nervous system manifestations of Lyme disease had local production of IgG or IgA spirochetal antibody ● CSF abnormalities could not be demonstrated in 6 patients with late peripheral nervous system manifestations.	30 European patients with neuroborreliosis had significantly higher CSF serum antibody ratios in both early and late disease stages. The authors concluded that intrathecal antibody determinations are the most specific diagnostic test currently available for Lyme neuroborreliosis, but local antibody production in CSF is an inconsistent finding in American patients with late neurologic manifestations.
Halperin, et al., 1991 Stony Brook, NY	Prospective cohort study	103 Lyme patients from hyper-endemic areas in the northeast United States with clinical evidence of central nervous system (CNS) dysfunction and definite immunologic evidence of exposure to *B. burgdorferi* (based on presence of antibody in serum or CSF, or peripheral blood T-cell responsiveness to *B. burgdorferi*). Patients presented with the following CNS abnormalities: Meningitis (N= 15, mean age: 37.2, 93% seropositive); Encephalitis (N= 35, mean age: 42.3, 69% seropositive); Multiple Sclerosis (MS) (N= 12, mean age: 41, 50% seropositive);	Out of 94 paired serum-CSF specimens, "Lyme antibody index was less than 1.0 in 63, between 1.0 and 6.0 (the usual diagnostic range) in 26, and above 6.0 in five." "…using a cutoff value of 1.3, the sensitivity of the method is 53%, the specificity 100%, and the overall accuracy 87%." "If, instead, a negative cutoff of 0.9 is used, the sensitivity improves to 87%, the specificity decreases only to 93%, and the overall accuracy improves slightly to 91%."	"Application of this method permits the identification of a rare *B burgdorferi*-associated multifocal encephalitis (brain infection) and its differentiation from a milder encephalopathy, or confusional state; the latter may not require CNS bacterial invasion. The encephalitis involves white matter more often than gray; severity varies widely. Of six patients with this antibiotic-responsive encephalitis, five were positive for HLA DQw3(DQw7)." The authors "conclude that (1) measurement of intrathecal antibody production is a reliable

		Encephalopathy (N= 41, mean age: 43.7, 80% seropositive). Samples were also taken from 73 non-Lyme controls, including 21 patients with relapsing-remitting MS and 52 with other disorders, including intracranial tumors, CNS lupus, cerebral sarcoidosis, spinal stenosis, inflammatory demyelinating polyneuropathies, and headaches.	"Since this lower cutoff improves overall accuracy and sensitivity, with a minimal loss of specificity, we have adopted this value as indicative of CNS infection, recognizing that occasionally CNS infection may be over-diagnosed in patients with indices in the range 0.9 to 1.3."	indicator of CNS infection, (2) North American neuroborreliosis includes the same spectrum of neurologic dysfunction as described in Europe, and (3) HLA typing may be useful in furthering our understanding of severe CNS involvement."
Tumani, et al., 1995 Göttingen, Germany	Prospective cohort study	24 patients with acute neuroborreliosis were included. Presentations included peripheral facial nerve paresis, myalgia, radiculopathy, headache, back pain, fatigue, abducens nerve paresis, and polyneuroradiculitis. 25% of patients with neuroborreliosis recalled a tick bite. 45 "disease controls" (relevant differential diagnosis of neuroborreliosis), consisting of patients with bacterial (N=4) and viral (N=4) infections of the central nervous system, multiple sclerosis (N=71), idiopathic facial nerve paresis (N=81), nucleus pulposus prolapse (N=4), neurosyphilis, diabetic or virus-induced radiculopathy, tuberculous meningitis, and polyneuropathy of various etiologies were included. 28 "normal controls" (patients without inflammatory signs in the cerebrospinal fluid [CSF] and with intact blood-CSF barrier function) were also analyzed.	CSF and serum samples were analyzed for presence of antibodies against *B. burgdorferi* by a modified ELISA. The antibody concentration ratio, or antibody index (AI) was calculated as (Concentration of antibody CSF/Concentration of antibody serum). IgM antibody index (pathologic value > 1.4): • Sensitivity: 79% • Specificity: 96% IgG antibody index (pathologic value > 1.4): • Sensitivity: 63% • Specificity: 89% Both antibody Index and CSF diagnostic markers: • Sensitivity: 80% • Specificity: 98%	"Combined evidence of an elevated CSF cell count, IgM-class dominance in both the cellular and intrathecal humoral immune response, and blood-CSF barrier dysfunction yielded 70% diagnostic sensitivity and 98% diagnostic specificity for detection of neuroborreliosis." The authors concluded, after analysis of CSF variables over a disease course, that acute versus past disease could be discriminated by a combination of basic CSF variables and *B. burgdorferi* antibody index.
Maraspin, et al., 2002 Ljubljana, Slovenia	Prospective cohort study	200 adult patients with multiple erythema migrans (median number of lesions: 3, range: 2-60) who received lumbar puncture were assessed. Sixty-three (31.5%) patients had no associated symptoms. 137 (68.5%) patients (including two with arthritis, six with radicular pain, a patient	Patients had routine blood and cerebrospinal fluid (CSF) tests performed, and borrelial antibody titers in CSF and blood were determined In the majority of these patients. Skin, blood, and CSF specimens were cultured in MKP medium for the presence of *Borrelia*.	CSF examination revealed abnormal results in 62 of 200 (31%) patients: lymphocytic pleocytosis was found in 15 (7.5%) patients (six were clinically without systemic symptoms, six had mild systemic symptoms, and three reported radicular pains), and elevated CSF protein concentration was present in 52 (26%)

		with facial palsy, another patient with foot palsy and a patient with transitory diplopia) reported local and/or constitutional symptoms.	Intrathecal borrelial antibody production was demonstrated in 8 (4%) patients (three of whom had elevated CSF cell counts) and *B. burgdorferi* was isolated from skin lesions, blood, and CSF in 77 of 191 (40.3%), 3 of 154 (1.95%), and 2 of 200 (1%) patients, respectively.	patients (nine of whom also had elevated CSF cell counts). The authors found that abnormal CSF findings were not rare in patients with multiple erythema migrans and may be present without clinical signs of central nervous system involvement.
Pícha , et al., 2000 Prague, Czech Republic	Prospective cohort study	88 patients with symptoms of neurological involvement and the presence of anti-borrelial IgM or IgG antibodies in the cerebrospinal fluid (CSF), as detected by enzyme immunoassay (EIA) were included. Diagnoses included acute meningoencephalitis, meningoradiculitis, encephaloradiculitis, radiculomyelitis, meningitis, and polyneuritis. Thirty-four patients had erythema migrans lesions, 21 were positive for neuroborreliosis by PCR, and 9 were positive by immune-electron microscopy using monoclonal antibodies. In patients whose CSF and serum samples were IgG positive (N=74), the antibody index (AI) was calculated but the IgM AI was not.	Antigen-nonspecific oligoclonal IgG (OIgG) was examined in all patients. The functional status of the blood-brain barrier was examined in all patients by establishing an albumin-globulin quotient. The antibody index was calculated according to the Reiber and Lange method: AI= (Concentration of IgG antibody CSF/IgG fraction in CSF originating only from blood); normal reference range= 0.7-1.3; antibody synthesis indicated by values ≥1.5. <u>Antibody index (N=72):</u> 54.1% positive Antigen nonspecific OIgG was detected in 33 (44.6%) patients and *B. burgdorferi*-specific OIgG was found in 31 (41.9%).	The frequency of specific OIgG positivity increased from 32.1% in patients with meningoencephalitis to 46.6% and 58.3% in patients with encephaloradiculitis and meningoradiculitis, respectively. By using both the calculation of AI and the detection of specific OIgG, verification of the diagnosis of neuroborreliosis was possible in this study for more than 50% of patients with CSF positivity for anti-borrelial antibodies. The authors concluded that calculation of antibody index is helpful in distinguishing non-spirochetal central nervous system inflammation from neuroborreliosis, particularly in regions with a high prevalence of seropositivity for anti-borrelial antibodies.
Tuerlinckx, et al., 2003 Yvoir, Belgium	Retrospective study	Patients with Lyme meningitis (N=6; 100% female; median age 9.3 years, range 3-13.3 years), both isolated and associated with peripheral facial palsy, and patients with aseptic meningitis (N=7; 100% female; median age 5.9, range 1.7-13 years) were enrolled. Diagnosis with Lyme meningitis required recent onset of compatible neurological symptoms and	The laboratory workup included white blood cell (WBC) count and differentiation, C-reactive protein (CRP) determination, cerebrospinal fluid (CSF) findings (WBC count, differentiation, protein dosage), and serological tests for antibodies to *B. burgdorferi* in simultaneous serum and CSF samples. Normal CSF cell count was <10*10^6 cells/l and protein level below 0.4	*B. burgdorferi* DNA was only detected by PCR in one CSF sample. The authors suggest that some clinical data and laboratory findings may help the physician to diagnose aseptic or Lyme meningitis prior to completion of serologic testing. They note that Lyme meningitis should be suspected in cases of meningitis with very low CSF neutrophilic

			cerebrospinal fluid (CSF) pleocytosis with positive Lyme serology (serum and CSF). Patients without intrathecal antibodies were included if they had specific IgM antibodies against *B. burgdoferi* by immunoblot. Diagnosis with aseptic meningitis required pleocytosis in the CSF without any bacterial growth on culture of CSF and with negative serologic tests for *B. burgdorferi*. *Exclusion criteria included antibiotic treatment prior to lumbar puncture.*	g/l. IgG and IgM antibodies to *B. burgdorferi* were detected by ELISA. All patients had CSF pleocytosis. The mean cell count was not statistically different between patients with Lyme meningitis and aseptic meningitis, but the neutrophilic component was significantly lower in patients with Lyme disease (mean 3.86%) compared with the aseptic meningitis group (mean 56%). Patients with isolated Lyme meningitis had higher protein levels (mean 1.12 g/l) compared with aseptic meningitis patients (mean 0.39 g/l) (p=0.003).	counts and high protein levels associated with prolonged duration of symptoms, low grade fever, and absence of pronounced signs of meningitis.
Blanc, et al., 2007 Strasbourg, France	Retrospective study		123 patients with clinical signs of neurologic involvement, with *B. burgdorferi* antibodies in cerebrospinal fluid (CSF) who were tested for anti-*Borrelia* antibody index (AI) were included. The patients were divided into three groups: Group 1 consisted of 40 patients who definitely had neuroborreliosis, defined as presentation with typical meningoradiculitis (spinal and/or cranial meningoradiculitis) <u>and/or</u> the disappearance of neurologic signs after ceftriaxone treatment (2 g/day for ~2 weeks), <u>with</u> the presence of *B burgdorferi* antibodies in the CSF. Group 2 consisted of 9 people with possible neuroborreliosis (*B. burgdorferi* antibodies present in the CSF <u>and</u> no other neurologic disease, but no response to ceftriaxone.) Group 3 comprised 74 patients with *B. burgdorferi* antibodies in the CSF who were classified as negative controls due to a proven etiologic diagnosis.	The antibody index (AI) was calculated as the ratio of (ELISA titer CSF/ELISA titer serum) to (total IgG titer CSF/total IgG titer serum). <u>"Definite neuroborreliosis":</u> ● Sensitivity Antibody Index: 75% <u>"Possible neuroborreliosis":</u> ● 5 of 9 patients had positive antibody index. ● 1 patient was intermediate, 1 was negative. <u>"Other neurologic diagnosis":</u> ● Specificity Antibody Index: 97%	The authors concluded that though the antibody index had an excellent specificity, it was too insensitive to diagnose all patients with neuroborreliosis. The authors suggest that it could be a useful adjunct to a pragmatic diagnostic plan.

Schwenkenbecher, et al., 2017 Hannover, Germany	Retrospective study	68 patients who were hospitalized with neuroborreliosis were retrospectively analyzed. Patients were categorized into five groups depending on the dominant neurological deficit leading to hospital admission: cranial nerve palsy (50%), symptoms/signs of radiculitis (25%), symptoms/signs of meningitis (29%- sum of all "meningitis" from Table 2), symptoms/signs of encephalitis (12%), and symptoms/signs of myelitis (7%). "Twenty-three patients (34%) reported previous tick bite and/or erythema chronicum migrans. Nine of them remembered only tick bite, seven remembered the occurrence of erythema chronicum migrans with previous tick bite, while seven remembered erythema chronicum migrans without previous tick bite." ELISA testing for *Borrelia burgdorferi sensu lato* specific antibodies: ● IgG or IgM: 94% ● IgG and IgM: 50% ● IgG+, IgM-: 34% ● IgM+, IgG-: 10%	Intrathecal synthesis of IgG, IgA, and IgM was calculated based on the method of Reiber-Felgenhauer referring the IgG, IgA, and IgM quotients to the albumin quotient. Elevated CSF cell count: 98.5% Blood-CSF barrier dysfunction: 87% CSF oligoclonal bands: 90% Quantitative intrathecal synthesis of immunoglobulins: 81% *Quantitative intrathecal synthesis of specific immunoglobulins:* IgM in 74%, IgG in 47%, and IgA in 32%	The authors concluded that neuroborreliosis is typically accompanied by profound immunological changes in the cerebrospinal fluid. The authors specifically addressed the issue of pleocytosis in active neuroborreliosis: "Repeated CSF analyses after 6 or 14 days indeed discovered an increase in cell counts and an elevation of the *Borrelia*-specific antibody index confirming active neuroborreliosis. Our cohort included only one patient without pleocytosis whose symptoms resolved completely after treatment with ceftriaxone." Conclusion: "...most, but not all patients with neuroborreliosis presented with typical neurological findings and consistent CSF changes. Uncommon presentations such as acute delirium and stroke-like symptoms underline the variety of symptoms. A thorough CSF analysis is considered essential for a reliable diagnosis of neuroborreliosis."
French, et al., 2018 New York, USA	Retrospective study	CSF samples from 27 patients with different neurological diseases were assessed: CNS Lyme disease (n = 5), West Nile Virus (WNV) meningoencephalitis (n = 5), Clinically Isolated Syndrome (CIS) of multiple sclerosis (MS, n = 4), rabies (n = 10), or Histoplasma meningitis (n = 3). Healthy controls were not available.	Samples were analyzed by 1H-NMR spectroscopy. A total of 57 compounds were identified and quantified in CSF samples. Quantification for 13 metabolites was considered not to be exact but still useful in detecting differences between groups. Lyme disease and WNV patients shared higher levels of formate and glycine compared to controls. High pyroglutamate discriminated WNV, Lyme, and histoplasmosis from controls.	NMR metabolomics of CSF is a potentially important tool for emergent diseases and distinguishing between autoimmune and infectious EM. Decision analytical approaches such as CART offer diagnostic flow charts that are easily implemented once validated, with quantifiable diagnostic probabilities.

				Classification and regression trees (CART) analysis differentiated infection status with 100% sensitivity and 93% specificity.	
				CXCL13	
Ljøstad and Mygland, 2008 Kritstiansand, Norway	Case-control study		Paired serum and cerebrospinal fluid (CSF) samples were collected from adults with acute neuroborreliosis (LNB) who had participated in a clinical trial comparing ceftriaxone and doxycycline. Patients were categorized as having "definite LNB" (N=37), "probable LNB" (N=7), or "possible LNB" (N=7); for this study, patients with "definite LNB" were used as the reference standard. The average symptom duration at baseline was 4.3 weeks (range 1-16). Eight patients who did not fulfill case definitions were used as controls. Serum and CSF samples were tested for presence of antibodies against *B. burgdorferi* by two different ELISA tests, and CSF was assessed for intrathecal antibody production. For this study, 31 patients with multiple sclerosis, 11 with non-inflammatory neurological disorders, and 10 with verified viral (enterovirus, N=3; Herpes Simplex virus (HSV) 1, N=1; HSV 2, N=2; Varicella Zoster virus, N=2) or bacterial meningoencephalitis (N=2) and high CSF cell count also served as controls.	Pre-treatment sensitivity of elevated CXCL13 in the CSF: 100% (95% CI: 91%–100%) Pre-treatment specificity of elevated CXCL13 in the CSF: 63% (95% CI: 31%–86%) Pre-treatment sensitivity of positive CSF *B. burgdorferi* antibody index: 78% (95% CI: 75%-96%) Pre-treatment specificity of positive CSF *B. burgdorferi* antibody index: 63% (95% CI: 31%-86%)	Pre-treatment sensitivity of elevated CXCL13 in the CSF was significantly higher than a positive antibody index for *B. burgdorferi* (p = 0.053). Specificity was not different between the two tests. At presentation, CSF CXCL13 was elevated in all patients with "definite LNB", as compared to a positive CSF *B. burgdorferi* antibody index in only 33 of 37 "definite LNB" patients. Four months post-treatment CSF CXCL13 was normalized in 82% of patients with "definite LNB", as compared to a negative antibody index in only 10% of patients (p<0.001) and a normal CSF cell count in only 60% of patients (p=0.092) at four months. CSF CXCL13 was very slightly elevated in 15 of 31 multiple sclerosis patients and in 9 of 10 patients with non-Lyme meningitis, and was normal in all patients with non-inflammatory neurological disorders. Mean CSF CXCL13 was significantly higher in patients with "definite LNB" than in patients with multiple sclerosis or non-Lyme meningitis (p<0.001). The authors concluded that CSF levels of CXCL13 are an accurate diagnostic marker for the early diagnosis of neuroborreliosis, and that measuring CXCL13 may also be a useful tool for measuring treatment success, since it reverts to normal more quickly than the intrathecal antibody index.

Tjernberg, et al., 2011 Kalmar and Jönköping, Sweden	Case-control study		124 patients with a positive *B. burgdorferi* antibody index were identified and matched to 124 patients with negative antibody index. An additional 90 patients with intrathecal antibody production from another county were also identified and were included together with 25 matching negative patients. In total, 261 of 363 patients had sufficient cerebrospinal fluid (CSF) and serum material and were included. Patients were divided into three main diagnostic groups based on original results of CSF pleocytosis and intrathecal anti-B*orrelia* antibodies: Definite neuroborreliosis (LNB) (N=124), Possible LNB with anti-B*orrelia* antibodies (N=29), Possible LNB without anti-*Borrelia* antibodies (N=16), and non-LNB (N=92). Median symptom duration before lumbar puncture ranged from 1 week to 4 weeks, with maximum symptom duration of 730 weeks reported. Median age ranged from 8 years to 55 years, with a minimum age of 3 and a maximum age of 87 years reported.	CSF-Serum CXCL13 ratio: ● Sensitivity: 99% ● Specificity: 96% Serum CXCL13 alone: ● Sensitivity: 47% ● Specificity: 80% CSF-C6 antibodies: ● Sensitivity: 99% ● Specificity: 88% CSF-Serum CXCL13 ratio and CSF-C6 antibodies in parallel: ● Sensitivity: 99% ● Specificity: 98%	In addition to high sensitivity and specificity noted among "definite LNB" and "non-LNB" patients, the CSF-Serum CXCL13 ratio was able to detect highly probable cases of LNB among children with short symptom duration in whom CSF anti-*Borrelia* antibodies were still negative. The authors note that levels of CXCL13 in LNB had previously primarily been reported as a ratio between CSF levels of CXCL13 and total protein count in the CSF to compensate for damage to the blood-brain barrier, but that the CSF-Serum CXCL13 ratio has been reported to differentiate patients with neurosyphilis better from LNB patients compared with the CSF-CXCL13/total protein ratio. Although CXCL13 in CSF is a more specific marker for LNB compared to pleocytosis, the suggest that increased CXCL13 levels found in multiple sclerosis and neurosyphilis point to the benefit of combining results of CXCL13 with intrathecal antibody production in order to gain diagnostic specificity. The authors concluded that the CSF-Serum CXCL13 ratio is a reliable diagnostic tool in patients with suspected neuroborreliosis.
Schmidt, et al., 2011 Dachau, Germany	Case-control study		192 patients with suspected neuroborreliosis (LNB) for whom the *B. burgdorferi*-specific antibody index had been requested who presented with cerebrospinal fluid (CSF) pleocytosis were analyzed. In this cohort, 19 patients presented with an antibody index >1.5. Of these, 12 (63.2%) fulfilled the criteria for definite LNB, defined by new neurologic symptoms suggestive of LNB, lymphocytic	CXCL13 CSF in patients with acute and untreated cases of LNB (N=17): ● Sensitivity: 94.1% ● Specificity: 96.1% ● CSF CXCL13 cutoff:1,229 pg/mL CSF *B. burgdorferi* antibody index in patients with acute and untreated cases of LNB (N=17): ● Sensitivity: 85.7%	There were 27 patients with clinically suspected LNB in the overall sample, 17 of whom had not received treatment. 4 of the 14 patients with definite LNB had not received antibiotic treatment prior to study entry. The pretreatment CSF CXCL13 levels were markedly elevated in all 4 untreated patients. The 13 patients with definite and untreated LNB who were retrospectively

| | | | pleocytosis, and antibody index >1.5. In addition,CSF/serum samples of 13 patients with definite and untreated LNB, which had been previously collected, were also retrospectively analyzed.

178 patients with diagnoses other than LNB were included as controls (encephalomyelitis disseminata, N=63; viral meningitis or encephalitis, N=32; bacterial meningitis, N=6; Candida meningitis, N=1; other chronic autoimmune inflammatory diseases, N=11; malignancies of the white blood cell line, N=6; CSF pleocytosis of unclear etiology, N=4; other diagnosis, e.g. Guillain-Barré syndrome, N=55). | ● Specificity: 96.1%

CXCL13 CSF in all patients with LNB, untreated and treated (N=27):
● Sensitivity: 92.6%
● Specificity: 83.7%
● CSF CXCL13 cutoff: 155 pg/mL

Serum concentrations of CXCL13 were not significantly different between the patient groups. | analyzed also showed significantly elevated CSF CXCL13 levels.

Five patients had already taken antibiotics for at least 14 days and did not show a substantial elevation of CSF CXCL13 levels. Three patients had received antibiotic treatment (ceftriaxone or doxycycline) for less than 14 days, as had 2 patients with clinical symptoms of acute LNB without an antibody index >1.5; the CSF CXCL13 levels in this group were found to be slightly increased.

Depending on the duration of antibiotic treatment, there was no elevation or only a borderline elevation of CSF CXCL13 in the pretreated patients with LNB, which is in line with the findings of other studies which show that CXCL13 levels decline quickly with treatment.

The authors conclude that CSF CXCL13 has a high sensitivity and specificity for untreated acute European LNB and can be a useful tool to monitor treatment progress. The authors recommend its use in patients with typical clinical symptoms and CSF pleocytosis but with a negative antibody index, or in patients with atypical clinical symptoms and CSF pleocytosis. |
| Sillanpää, et al., 2013

Helsinki, Finland | Case-control study | | 57 cerebrospinal fluid (CSF) samples from pediatric patients (ages 2-17) with clinically suspected neuroborreliosis (LNB) living in a highly endemic area were tested.

Based on the presence or absence of anti-flagella antibodies and lymphocytic pleocytosis in CSF, | Based on the cut-off value of 103 pg/m, all 24 "confirmed LNB" samples and 11 of 16 "possible LNB" were positive.

"Confirmed LNB":
● Sensitivity CXCL13: 100%
● Specificity CXCL13: 98.5% | The differences in CXCL13 levels between "confirmed LNB" patients and controls or between "possible LNB" patients and controls were significant (p<0.0001). During the course of LNB, CXCL13 seemed to be detectable earlier than specific intrathecal antibodies. |

		patients were divided into 3 different groups: "confirmed LNB" (N=24), "possible LNB" (N=16), and "non-LNB" (N=17). None of the patients had reported erythema migrans. Disease control CSF samples were obtained from children with other neurological diseases such as viral meningitis or convulsions/epilepsy (N=13), and from adults with no proven infection (N=16), adults with viral central nervous system infections (N=20), and from one patient with syphilis. None of the disease control samples had anti-*Borrelia* antibodies in CSF or serum. Of the 50 controls, 24 had CSF pleocytosis.	"Confirmed" and "Possible" LNB: ● Sensitivity CXCL13: 88% ● Specificity CXCL13: 98.5%	The authors concluded that in children with typical symptoms of LNB, elevated CXCL13 levels seem to be compatible with lymphocytic pleocytosis and elevated anti-*Borrelia* antibodies in CSF. They recommend this test as an additional marker to improve diagnostic accuracy in children with clinically suspected LNB.
Bremell, et al., 2013 Gothenburg, Sweden	Mixed; Longitudinal and Cross-sectional elements	**Longitudinal:** 25 neuroborreliosis (LNB) patients (68% male; mean age 50 years, range 12-74) who had undergone CSF sampling before and after treatment with 200–400 mg of oral doxycycline daily for 10–14 days were observed. The mean duration of LNB symptoms at study onset was 28 days (5-360 days). 60% (15 of 25) of patients were experiencing radiculitis at baseline; 3 patients were experiencing facial palsy. 23 patients (92%) had a positive antibody index. **Cross-sectional:** Patients with untreated LNB (N=16; CSF sampling prior to treatment initiation required), untreated HIV-1 infection (N= 27; asymptomatic infection, no antiretroviral treatment, no clinical signs of neurologic disease, and no syphilis required), and controls with no infectious or inflammatory disease (two subgroups: patients with neurological symptoms such as headache, vertigo and radiculitic pain, where an underlying organic neurological disease had been ruled out and where CSF sampling had been undertaken, N=18; no neurological disease or symptoms who had undergone lumbar	CXCL13 was measured by ELISA. For the assessment of the diagnostic performance of CSF CXCL13, data on LNB patients from the cross-sectional study and the pre-treatment part of the longitudinal study were combined and analyzed against the combined group of HIV patients and controls. CSF CXCL13 (cut-off: 61 pg/mL): ● Sensitivity: 90% ● Specificity: 88%	In the longitudinal LNB study, initially high CSF CXCL13 levels declined significantly (p<0.001) after doxycycline treatment. The quotients before and after treatment of CSF CXCL13 and CSF mononuclear cells were calculated as (CSF CXCL13 before treatment)/(CSF CXCL13 after treatment) and (CSF mononuclear cell before treatment)/(CSF mononuclear cells after treatment). The quotients correlated significantly (p= 0.036) In the cross-sectional study, all the LNB patients had CSF CXCL13 levels elevated above the lowest standard point of the assay (7.8 pg/mL); of HIV patients, 52% had elevated CSF CXCL13 levels. There was a clear overlap in CSF CXCL13 concentrations between LNB patients and asymptomatic HIV patients. 38 of the 39 controls had CSF CXCL13 levels below 7.8 pg/mL. For LNB patients, the correlation between CSF mononuclear cells and CXCL13 was significant

		puncture for CSF sampling as part of a research project unrelated to this study, N=21).			both in the cross-sectional study and in the pre-treatment part of the longitudinal study. The combined analysis of the two groups of untreated LNB patients also produced a significant correlation (Spearman r= 0.55, p<0.001). For LNB patients, there was no significant correlation between the duration of neurological symptoms and CSF CXCL13 levels, either in the longitudinal study or the cross-sectional study. The authors note that when prior proposed cut-offs for CXCL13 levels (such as 142 pg/mL or 1,229 pg/mL) were used in their study, they resulted in sensitivity below 83%, making them less useful. A cut-off value at a low level of 61 pg/mL was needed to obtain acceptable sensitivity in their study, when LNB was compared with a mixed population of non-infectious controls and asymptomatic HIV patients.
Hytönen, et al., 2014 Turku, Finland	Retrospective study	390 cerebrospinal fluid (CSF) samples from 366 individuals were retrospectively analyzed for neuroborreliosis (LNB), tick-borne encephalitis, central nervous system (CNS) varicella zoster, CNS Herpes simplex virus 1 and 2, CNS human Herpesvirus 6, CNS enterovirus, neurosyphillis, multiple sclerosis, all of which were untreated. 31 samples were *B. burgdorferi* antibody positive and were confirmed to have come from LNB patients; of these 31, 24 post-treatment CSF samples were available for CXCL13. LNB patients were diagnosed using the following criteria: 1) The patients had symptoms consistent with LNB, 2) other relevant diagnoses were excluded, 3) there was mononuclear pleocytosis in CSF, 4) CSF samples were at a positive level for *Borrelia*		A CXCL13 cut-off 415 pg/ml resulted in nearly perfect discrimination between the LNB patients and the other groups: ● Sensitivity: 100% ● Specificity: 99.7%	LNB patients were treated with either intravenous ceftriaxone, oral doxycycline, or with a combination of both of these drugs. The decline in CSF CXCL13 levels during and after the treatment was statistically significant (p<0.001). The difference in CSF CXCL13 concentrations between the untreated LNB patients and the non-LNB group was statistically significant (p<0.001). The statistical difference between the viral CNS infection samples and the LNB samples was highly significant, with p values of 0.0013 or less in all cases. CSF CXCL13 concentrations in the 34 samples from patients with non-infectious neuroinflammatory conditions was also

| | | specific antibodies of IgM and/or IgG by whole *Borrelia* antigen (I B31 sonicate) ELISA and/or with the commercial C6-peptide based assay, and 5) intrathecal antibody production as indicated by a positive antibody index (AI).

The presenting symptom in 30 of the LNB patients was radiculitis or pain radiating to the upper or lower limbs or to the trunk. Nineteen patients had facial nerve paralysis. Thirteen patients had both symptoms. The median duration of symptoms before the first CSF sample was 30 days (range 0-120 days). | | significantly different from those from LNB patients (p<0.001).

Neurosyphilis was the only other disease among the conditions investigated in this study that led to markedly increased CSF CXCL13 concentration.

The level of CSF CXCL13 was highly elevated in patients with untreated LNB and decreased rapidly after treatment, which is consistent with other studies which measure treatment progress using CXCL13. The authors concluded that CSF CXCL13 may be an excellent biomarker in differentiating LNB from viral CNS infections and from other neuroinflammatory conditions when locally determined cut-offs are used, and that it may also be a useful tool for follow-up of LNB patients after antibiotic treatment. The authors recommend that CSF CXCL13 results be interpreted in conjunction with intrathecal antibody production, CSF pleocytosis, and ideally also borrelia nucleic acid amplification result. |
| Rupprecht, et al., 2014

Dachau, Germany | Case-control study | CSF samples from all patients (N=204) with suspected acute neuroborreliosis (LNB) were analyzed.

This study included 179 patients who were not pretreated with antibiotics. Of these patients, 15 were defined as having "definite LNB", 3 had "probable LNB", and all had a CXCL13 value above the cut-off level. Only 2 of the 161 patients with a non-LNB diagnosis (both with a lymphoma) had a CXCL13 value in the CSF higher than 250 pg/ml. | The CXCL13 cut-off level for acute LNB was set at 250 pg/m.

The biomarker CXCL13 has a higher sensitivity (100% vs. 87%) with a specificity (99%) comparable with the established diagnostic markers for LNB, e.g. CSF pleocytosis and *Borrelia*-AI in the investigated patient population.

The negative predictive value of CXCL13 is 100%. | The authors conclude that a normal CXCL13 level virtually excludes LNB. They consider CXCL13 to be a valuable tool in clinical practice and a practical diagnostic marker for LNB which can even detect an acute LNB in patients without CSF pleocytosis. |

Wutte, et al., 2014 Graz, Austria	Cross-sectional study		50 patients (56% male; 54% adult; median age 11 years, range 6-69) clinically classified as having neuroborreliosis (LNB) (N=22) or as neurologic control patients with suspected viral meningitis, or facial palsy (N=31) were assessed. "Definite LNB" was defined by both CSF pleocytosis and intrathecal antibody production as determined by ELISA. "Possible LNB" was defined in one of two ways: 1) one CSF test was positive without pleocytosis and 2) pleocytosis was present and CSF tests were negative. 80% of patients showed CSF pleocytosis. The median disease duration for adults was 4 days, and 77% recalled a tick bite 2 weeks to 4 months before onset of neurologic symptoms.37% of adult patients had evidence of erythema migrans. The median disease duration for children was 3 days, and 52% recalled a tick bite 2 weeks to 4 months before onset of neurologic symptoms. 34.7% of children had evidence of erythema migrans.	Patients were tested for *Borrelia*-specific intrathecal antibodies by flagellum ELISA antibody index (flELISA-AI), a recombinant ELISA antibody index (rELISA-AI), immunoblot, and CXCL13 levels were tested by ELISA. Antibody indices for both ELISAs were considered positive ≥1.5. rELISA-AI: • Sensitivity: 58% • Specificity: 82% fELISA-AI: • Sensitivity: 34% • Specificity: 80% Immunoblot: • Sensitivity: 40% • Specificity: 82% CSF CXCL13: • Sensitivity: 44% • Specificity: 80%	CSF CXCL13 was elevated above the cut-off in 22 patients with definite LNB (44%). No control patient or possible LNB patient was positive by CXCL13 testing. Antibody index results were more often positive with rELISA than with immunoblot (p=0.039) or compared to CXCL13 levels (p=0.022). There were no significant differences when agreement between flELISA, immunoblot, and CXCL13 was calculated. The authors found that the rELISA-AI test appeared to be the most sensitive and that the flELISA-AI was the least sensitive. When the ELISA-AIs were confirmed by immunoblot, different patients were identified as LNB, while only 26% were identified by all test methods. All patients classified as "definite LNB", and only those patients, had elevated CXCL13 levels, but classification of the patients was not influenced by CXCL13 levels. The authors concluded that immunoblot could be an important supplement to recombinant ELISA antibody index to rule out the diagnosis of LNB in children and adults suggestive symptoms or in unclear clinical cases. The authors also noted that CXCL13 levels in CSF may be useful as an additional non-*Borrelia* specific determinant in early NB.
Lindström, et al., 2016 Gothenburg, Sweden	Retrospective study		28 patients with varicella zoster facial palsy, which was diagnosed by detection of varicella zoster DNA in cerebrospinal fluid (CSF) by PCR, and 21 patients with facial palsy caused by neuroborreliosis (LNB), who were included from two patient cohorts previously included in unrelated prospective studies on LNB, were	Median CSF concentrations of CXCL13 for facial palsy caused by LNB were 1808 pg/mL, and for VZV facial palsy were 9 pg/mL. All control samples except one were below the detection limit.	This was the first comparative study on CSF levels of CXCL13 in patients with facial palsy caused by LNB versus varicella zoster. The authors found significantly higher concentrations of CXCL13 in CSF of patients with LNB compared to patients with varicella zoster. Previously proposed cut-off levels for

		retrospectively assessed. A control group with 52 patients without central nervous system infection was included. The median number of days between onset of facial palsy and CSF sampling was 2 days for varicella zoster patients and 4 days for LNB patients.	CSF CXCL13 (cut-off: 34.5 pg/mL): ● Sensitivity: 82.6% ● Specificity: 82.1% Patients whose CSF was sampled within one week of facial palsy onset CXCL13 (cut-off: 61 pg/mL): ● Sensitivity: 92.9% Patients whose CSF was sampled within one week of facial palsy onset CXCL13 (cut-off: 1,224 pg/mL): ● Sensitivity: 64.3%	CXCL13 have ranged from 61 pg/mL to 1,224 pg/mL, but since cutoffs this high would have led to unacceptably low sensitivity in this population, the authors derived a new cutoff of 34.5 pg/mL using receiver operating characteristic (ROC) analysis. The authors suggest that further studies on CXCL13 concentrations in central nervous system infections need to be conducted in order to inform its use in a clinical setting, and that such studies should focus on central nervous symptom infections with similar clinical presentations.
Barstad, et al., 2017 Southwest Norway	Prospective cohort study	217 children who were hospitalized with symptoms suggestive of LNB were included, and 210 children were eligible for laboratory analysis and categorized into the following diagnostic groups: Confirmed LNB (n=59), "probable" LNB (n=18), "possible" LNB (n=7), Non-Lyme aseptic meningitis (NLAM) (n=12), "possible" peripheral LNB (n=7), non-meningitis (n=91), and negative controls (n=16).	Serum and CSF analyses of *B. burgdorferi* antibody levels and antibody index were conducted using the Liaison Borrelia immunoglobulin (Ig)M and Borrelia IgG, Enzygnost Lymelink VlsE/IgG and Borreliose IgM tests. In addition, IDEIA Lyme Neuroborreliosis and Reibergram were used. CXCL13 analyses were performed by ELISA. For this analysis, the highest combined sensitivity and specificity of 91% and 100%, respectively, were found with a CXCL13 cutoff level of 213 pg/mL.	CSF CXCL13 levels were substantially higher in children with LNB compared with children with other diagnoses. If a low CSF CXCL13 cutoff level is chosen to obtain a high sensitivity, the specificity to discriminate LNB from NLAM may be more moderate. This suggests that CSF CXCL13 should be used as a supplementary tool in the diagnosis of LNB in children. With a low cutoff level, CSF CXCL13 had a high sensitivity to diagnose LNB but a more moderate specificity to discriminate LNB from NLAM.
Gyllemark, et al., 2017 Jönköping County, Sweden	Retrospective study	165 patients who had been investigated by lumbar puncture (LP) and blood sampling during 2007–2009 for suspected LNB were assessed. Patients in group 1 ("definite" LNB, n= 49) had CSF pleocytosis and *Borrelia*-specific antibodies. Group 2 ("possible" LNB pleocytosis, n= 14) had short duration of symptoms and CSF pleocytosis but no *Borrelia*-specific antibodies. Group 3 ("possible" LNB Ab+, n= 14) had Borrelia specific	CSF levels of CXCL13 were significantly elevated in all LNB groups compared to the non-LNB group, while there were no differences in serum. Children <15 years of age in groups 1, 2 and 3 (n= 34) had significantly higher levels of CXCL13 in serum and CSF (920 pg/mL, 398–1706, p= 0.03) compared to adults. CXCL13 in CSF was positively correlated with pleocytosis (rho= 0.55, p<0.001).	Levels of CXCL13 are raised in CSF from patients with LNB, strengthening the involvement of B cell immunity in LNB. The lack of correlations between cytokine/chemokine levels in serum versus CSF indicates an intrathecal source of the cytokines and chemokines present in CSF, thus reflecting the pathological process in the CNS.

		antibodies in CSF, but no pleocytosis and less suggestive symptoms. 88 gender- and age-matched patients from the same cohort were selected as a reference group: neurological diagnosis unverified (n= 56), Bell's palsy (n= 18), Alzheimer's disease/Parkinson's disease/stroke (n= 14).		
Remy, et al., 2017 Bern, Switzerland	Retrospective case-control	CSF of 185 children with suspected LNB was analyzed for CXCL13. 53 patients (29%) were diagnosed with "definite LNB," while 91 patients (49%) were classified as controls in a "non-LNB" group (negative AI and no pleocytosis or presence of a differential diagnosis). Some control patients had viral meningitis or encephalitis due to varicella-zoster virus (VZV, n= 4), enterovirus (n= 20), or tick-borne encephalitis virus (TBEV, n= 5), while others had inflammatory diseases (n= 8), idiopathic facial palsy (n= 19), primary headache (n= 11), or other diseases (n= 29). Forty-one additional patients (22%) were classified as "possible LNB" as they fulfilled only two out of three criteria for LNB.	ELISA was used to measure CXCL13 concentrations in the CSF. The median CXCL13 concentration in the CSF of patients with definite LNB was 774.7 pg/ml and values ranged from 58.9 to 13487.0 pg/ml. In comparison, non-LNB patients including patients with other confirmed CNS diseases had a median CSF CXCL13 concentration of 4.5 pg/ml (range 4.5–816 pg/ml). Means were significantly different (p<0.001)	CSF CXCL13 is highly elevated in children during early LNB, as previously shown in adults. CXCL13 is a highly sensitive and specific marker that helps to differentiate LNB from other CNS affections in children.
Skogman, et la., 2017 Central and Southeast Sweden	Prospective laboratory study	146 children who were evaluated for LNB at seven pediatric departments in a Lyme endemic area in Central and Southeast Sweden during the years 2010–2013 were included in the study. 37 children were diagnosed with "definite" LNB, and 21 children were diagnosed with "possible" LNB (neurological symptoms and pleocytosis). 88 children without LNB were included as controls, and an additional 15 children with other confirmed diagnoses were included as a reference.	Definite LNB (n=37): ● Sensitivity: 89% Possible LNB (n-21): ● Sensitivity: 48% Non-LNB (n=88): ● Specificity: 97% Other diagnoses (n=15):	The recomBead Borrelia antibody index (AI) assay performs with moderate sensitivity in pediatric LNB patient, with the major advantage being increased sensitivity in the "possible" LNB group compared to the IDEIA assay. The recomBead Borrelia AI assay is more expensive and requires measurements of albumin and total immunoglobulin levels in serum and cerebrospinal fluid (CSF). The authors suggest that the recomBead Borrelia antibody index (AI) assay may be recommended in IDEIA-negative pediatric patients with CSF pleocytosis. The diagnostic

126

				● Specificity: 100% Overall Performance: ● Sensitivity: 74% ● Specificity: 97%	sensitivity may be further increased by using a combination of early markers, such as CXCL13 in CSF and total IgM index.
Rupprecht, et al., 2018	Meta-analysis		The literature research identified 200 potential articles on CXCL13. Ultimately, 18 studies involving a total of 2944 individuals (618 with LNB and 2326 non-LNB patients) were included.	In all included studies, CXCL13 was determined by commercially available ELISA kits. An optimal cut-off of 162 pg/mL was identified. Its corresponding sensitivities and specificities were 89% and 96%, respectively. With respect to the optimal CSF CXCL13 cut-off of 162 pg/mL, the overall specificity was low when considering individuals suffering from neurosyphilis (16%) or CNS lymphoma (18%).	The overall sensitivity and specificity of CSF CXCL13 were 89% and 96%, respectively, indicating a sufficient level for overall diagnostic accuracy.
Henningsson, et al., 2018 Southeast Sweden	Prospective observational study		Children with suspected LNB were recruited from seven pediatric clinics in a Lyme endemic area in southeast Sweden during the period 2010 to 2014. Patients were clinically evaluated on admission and underwent a lumbar puncture as part of the routine investigation. Additional blood and CSF samples were taken at the same occasion for the purposes of this study. All children in the study were followed for 2 months. 44 patients had "definite" LNB, 22 had "possible" LNB, 102 children did not have LNB, and 23 had other neurological diagnoses.	The difference in CXCL13 concentrations between LNB patients ("definite" and "possible" LNB) and controls (non-LNB and other specific diagnoses) was statistically significant (p<0.001). Overall performance of the CXCL13 recomBead test in CSF: ● Sensitivity of 88% ● Specificity of 89%.	Concentrations of CXCL13 in CSF were significantly higher in LNB patients compared to controls. The overall sensitivity (88%) and overall specificity (89%) observed in this study were moderate, compared to previous studies on CXCL13 in CSF which reported sensitivities ranging between 88 and 100% and specificities between 89 and 100%. In assessing the "definite" LNB group exclusively, the sensitivity of CXCL13 in CSF was very high (95%). The sensitivity in the "possible" LNB group was as high as 73%.

Maric, et al., 2018 Zagreb, Croatia	Prospective observational study		84 children were included: 23 with acute disseminated encephalomyelitis (ADEM), 20 with non-polio enterovirus aseptic meningitis (NPEVAM), 21 with LNB, and 20 controls (acute infectious disease not affecting the CNS). Only patients who fulfilled criteria for definite or possible LNB were included in the study. In all LNB patients, enterovirus infection was excluded by negative CSF NPEV PCR.	Serum and CSF concentrations of CXCL13 ranged widely in all four groups. CSF concentrations of CXCL13 distinguished between children with LNB and all other children. At the optimal cut-off concentration, the sensitivity was low, but specificity was 96.8%, resulting in reasonably high positive and negative predictive values.	The potential use of chemokines may aid in the differential diagnosis of conditions that largely overlap in (early) clinical and laboratory presentation. CSF levels of CXCL13 may help to discriminate between LNB and other conditions with high specificity and sensitivity
Markowicz, et al., 2018	Retrospective study		100 CSF specimens were obtained from patients with "proven LNB" (n=25), "suspected LNB" (n=25), tick-borne encephalitis (TBE) (n= 25), and aseptic meningitis/meningoencephalitis other than TBE (n= 25). CSF specimens from 45 women and 55 men were included in the study. The median age of the patients was 45 years. The median duration of neurological symptoms prior to CSF examination was 7 days. The longest duration of neurological symptoms prior to LNB was observed in the LNB group (median 30 days). All 25 patients in the "suspected" LNB group and 13 patients in the "proven" LNB group reported EM. The median time that elapsed from the onset of EM to lumbar puncture was 17 days. Eleven patients received antibiotics prior to lumbar puncture.	RecomBead CXCL13 assay and CXCL13-ELISA were used to determine the concentration of CXCL13 in CSF. Overall performance of the CXCL13 recomBead assay in CSF: ● Sensitivity: 88% (68.8–97.5%) ● Specificity: 94% (83.5–98.7%) *After the inclusion of the CSF lymphocyte/monocyte cell count, the sensitivity of recomBead CXCL13 increased to 92% and specificity increased to 98%.* Overall performance of the CXCL13 ELISA: ● Sensitivity: 98% (86–100%) ● Specificity: 100% (93–100%)	Overall, both assays performed similarly in terms of time consumption, robustness and requirements on technical experience of the laboratory staff. CXCL13 may be useful for the diagnosis of LNB in particular in patients exhibiting pleocytosis. Both assays performed very well in excluding TBE. Aseptic meningitis other than TBE can be associated with mildly elevated CXCL13 CSF concentration, which suggests that the chemokine is not specific for LNB. The analysis demonstrated the combined dynamics of the concentrations of leukocytes/monocytes and CXCL13 in the CSF. Using linearized CXCL13 cut-off values depending on the CSF cell count may further improve the laboratory diagnosis of LNB.

Wagner, et al., 2018	Retrospective study		459 patients in whom CSF analysis for CXCL13 was performed between May 2015 and November 2016 and for whom documentation on clinical symptoms and diagnosis was available were included. 255 (55.6%) patients were male, and the median age was 57 years. 20 patients were diagnosed with LNB.	The median CSF CXCL13 concentration in all patients was 10 pg/ml (range 0–6548 pg/ml), and 900 pg/ml (range 10–6500 pg/ml) in NB patients. Duration of symptoms in LNB and CSF CXCL13 concentration did not show a significant correlation. There was not a significant difference between patients with the diagnoses of "likely" and "confirmed" LNB. Performance of the CXCL13 ELISA (optimal cut-off point of 93.83 pg/ml): ● Sensitivity: 95% ● Specificity: 97%	The results of the present study confirm the high diagnostic value of CSF CXCL13 in LNB, reproducing its high sensitivity and specificity in an unselected group of patients, with a similar specificity but higher sensitivity than Borrelia IgG/IgM-antibody index (97% vs. 89% and 95% vs. 80%, respectively) and a higher sensitivity than Borrelia burgdorferi PCR (95% vs. 15%).
				Serologic Testing Data	
Study and Location	**Study Design**	**Risk of bias***	**Population Characteristics**	**Meta-analysis details**	**Results and Study Conclusions**
Waddell, et al., 2016	Systematic Review and Meta-analysis	*see footnote	Searched from 1995 – Sep. 2013 Included 48 North American diagnostic test studies that compared results of one test using a validated test panel, results of clinical diagnosis, or a gold standard test result or investigated inter-test agreement. No studies were excluded based on their quality assessment. Studies evaluating in-house tests were included; however, heterogeneity analyses on the impact of the non-commercial tests were performed, where applicable. The following disease stages were addressed: Early/acute (*Stage 1*; <30 days; includes EM); Early disseminated (*Stage 2*) (neurologic/cardiac/multiple EM); Late (*Stage 3*) (late neuroborreliosis/arthritis).	The included tests were evaluated in the context of clinical diagnosis or compared with one another. No studies addressed serologic testing of cerebrospinal fluid (CSF). Meta-analysis was conducted using hierarchical logistic regression and bivariate models that account for the correlation between sensitivity and specificity. Due to broad inclusion criteria, many studies received downgraded risk of bias ratings in the selection, performance (inadequate blinding), reporting, and/or funding domains.	1. **Two-tier test vs. clinical diagnosis** *Stage 2 (Early neurologic/cardiac)(N=8 studies):* ● Sensitivity 89.7% (95% CI: 78.3%-95.4%) ● Specificity 99.7% (95% CI: 98.4%-99.9%) 2. **EIA (1st tier tests, including ELISA) vs. clinical diagnosis** *Stage 2 (Early neurologic or cardiac)(N=5 studies):* ● Sensitivity 79.1% (95%CI: 66.1%-88.0%) ● Specificity 99.7% (95% CI: 96.8%-98.4%) 3. **Nested PCR primer sets targeting OspA vs. Clinical diagnosis (N=1 study)**

					● *Acute Neurologic Lyme*: Sensitivity of 37.5% – 50% Across all studies, the sensitivity for C6 ELISA was highest, with the lowest variability over other tests and test protocols.

Cook and Puri, 2016	Meta-analysis	High risk of bias for all studies	Search dates unclear: 1995 - unknown (Latest included article was published in 2015; Epub in Jul 2014).
			Included any studies (N=18 studies, 12 from US) evaluating commercially available serologic tests.
			The included studies did not evaluate the tests in clinical settings, where the use of antibiotics or other factors may influence the antibody response. The review did not evaluate microscopy, culture, PCR, or novel technologies (LTT etc.).
				Samples were proved positive based on records of erythema migrans, positive serology and/or culture, or CDC-certified panels.
				Only studies in which test specificity was reported to be at least 85% were included, to avoid overinflated sensitivity at the cost of lowered thresholds and too many false-positives. Sensitivities of each test were not evaluated within every stage of borreliosis due to the lack of standard definitions of disease stages and the possibility of retrospective selection bias.
					1. **Weighted mean (All studies, all disease stages)**
					  ● Sensitivity 59.5% (95% CI: 55.6%-63.5%)
					  ● Specificity 96.1%
					2. **Weighted Mean Sensitivities by test (all disease stages)**
					  ● Western Blot: 62.4% (95% CI: 54.2%-70.7%)
					  ● ELISA (any variety): 62.3% (56.6-68.1)
					  ● C6 ELISA: 53.9% (48.3-61.1)
					  ● Two-tier: 53.7% (49.9-57.4)
					3. **Weighed Sensitivities for Neurologic Lyme (all test types)**
					  ● Neurologic Lyme (stage unknown): 87.3% (95%CI: 71.4%-97.5%)
					  ● Neurologic/arthritis/carditis: 92.2% (78.4-100.0)

Leeflang, et al., 2016	Systematic Review and Meta-analysis	*see footnote	Last search date: Feb. 2014. The oldest included study was published in 1987. Only European studies evaluating the diagnostic accuracy of serologic assays for Lyme borreliosis against a reference standard for clinical criteria (sometimes combined with positive serology) in	Meta-analysis was performed using Hierarchical Summary ROC (HSROC) model, a hierarchical meta-regression method incorporating both sensitivity and specificity while taking into account the correlation between the two.	20 case-control studies addressing diagnosis of neuroborreliosis included healthy controls. The overall sensitivity for serologic tests in neuroborreliosis patients was 77% (95%CI: 67%-85%), and overall specificity was 93% (95% CI: 88%-96%).

			"possible" or "suspected" Lyme patients were included (N=75 studies); these patients counted as "cases". Indirect fluorescent antibody assays were not evaluated because of the rare use in practice.	The authors noted that the included studies had high levels of heterogeneity and bias and did not represent the tests in true clinical settings.	The summary sensitivity for any serologic test done in serum in neuroborreliosis patients (N=6 studies) was 78% (53-92), and specificity was 78% (40-95). Sensitivity was similar for IgG and IgM, with higher specificity for IgG. Commercial ELISA tests (N=11 studies) had sensitivity of 81% (70-89) and specificity of 94% (91-96). Sensitivity and specificity of two-tier testing (N=1 study) ranged from 41%-87% and 88%-94%, respectively. 6 studies addressed any ELISA done on CSF, and the sensitivity of ELISA of CSF was 74% (38-93), with specificity of 96% (85-99). Sensitivity and specificity of specific antibody index (AI) tests (serum/CSF) were analyzed by study type: for case-control (N=7 studies), Sensitivity was 86% (63-95), and Specificity was 94% (85-97); for cross-sectional (N=4 studies), Sensitivity was 79% (34-97) and Specificity was 96% (64-100). Specific AI test did not outperform single tests.
Serologic Testing in Neuroborreliosis					
Study and Location	**Study Design**	**Risk of bias***	**Population Characteristics**	**Diagnosis Method, % Positive**	**Study Conclusions**
Adoracion Pegalajar-Jurado, et al., 2017 CDC Lyme Serum Repository	Retrospective study		10 serum samples of patients with Lyme neuroborreliosis were taken from the CDC Lyme Serum Repository. Only two Lyme neuroborreliosis patients had duration of illness of ≥30 days when the serum samples were collected. The LD samples were not tested for co-infections. Serum samples for control (n=347) were collected from patients with fibromyalgia (n=31), infectious mononucleosis (n=30),	Samples were subject to serologic testing using either the LIAISON *Borrelia burgdorferi* CLIA (Stillwater, MN) that detects IgM and IgG antibodies against VlsE, and the Captia *Borrelia burgdorferi* IgG/IgM EIA (Trinity Biotech, Jamestown, NY) that detects IgM and IgG antibodies against whole cell lysate. <u>First-Tier Tests:</u> ● VlsE: Sensitivity- 100%; Specificity- 98%	"When MTTT algorithms were compared to STTT algorithms, the percent difference in proportion of samples correctly classified was always in favor of the former when all samples from patients with LD were considered. This was not the case when the results of MTTT and STTT algorithms were compared for other diseases and healthy controls. For these two groups, the use of an MTTT algorithm did not always result in

131

			multiple sclerosis (n=22), rheumatoid arthritis (n=21), severe periodontitis (n=20) or syphilis (n=20) and from healthy donors from regions endemic (n=101) or non-endemic (n=102) for LD.	• C6: Sensitivity- 100%; Specificity- 95% • WCS (Whole Cell Sonicate): Sensitivity- 100%; Specificity- 56% Modified Two-Tiered Testing Algorithms (MTTT): • VlsE/C6: Sensitivity- 100%; Specificity- 100% • WCS/C6: Sensitivity- 100%; Specificity- 97% • WCS/VlsE: Sensitivity- 100%; Specificity- 98% Standardized Two-Tiered Testing Algorithms (STTT): • VlsE/ViraStripe: Sensitivity- 90%; Specificity- 100% • C6/ViraStripe: Sensitivity- 90%; Specificity- 99% • WCS/ViraStripe: Sensitivity- 90%; Specificity- 92%	significantly higher proportions of correctly classified samples when compared to the use of an STTT algorithm. Although the MTTT algorithms did not have an inferior performance as compared to the STTT algorithms, there was an overall trend for better specificity when the all MTTTs were compared to all STTTs. In particular, the STTT that incorporated WSC as the first tier test did not perform as well as the two MTTTs that used WSC."
Molins, et al., 2017 CDC Lyme Serum Repository	Retrospective study		Serum samples from 124 well-characterized patients with Lyme disease (LD) were analyzed: acute LD with erythema migrans (EM)- N=40, Convalescent LD with EM- N= 38, Lyme neuroborreliosis- N= 10, Lyme carditis- N= 7, Late Lyme disease, Lyme arthritis- N= 29. 347 negative controls were also analyzed. 144 control samples originated from patients with other diseases, and the rest of the samples came from healthy controls from endemic and non-endemic regions. The IgM/IgG Vidas test (LYT) is the most commonly used first-tier EIA in the US. Recently launched dissociated first-tier tests, the Vidas Lyme IgM II (LYM) and IgG II (LYG) EIAs (use purified recombinant test antigens and a different algorithm than STTT) were evaluated	***Sensitivity results are shown for neuroborreliosis patients ONLY*** First-Tier Tests: • Vidas LYT: Sensitivity- 90% • Vidas LYM: Sensitivity- 100% • Vidas LYG: Sensitivity- 90% Standardized Two-Tiered Testing Algorithms (STTT): • LYT-ViraStripe: Sensitivity- 80% • LYM/LYG-ViraStripe: Sensitivity- 90% • C6-ViraStripe: Sensitivity- 90% Modified Two-Tiered Testing Algorithms (MTTT): • LYT-C6: Sensitivity- 90% • LYM/LYG-C6: Sensitivity- 100%	"The difference between the proportion of samples called positive by the combined LYM/LYG EIAs and that by the LYT EIA was calculated to be 3.4% (95% Confidence interval [CI] of -7.6% to 0.8%) with a P value of 0.12, indicating that the numbers of samples called positive are similar between the two EIA approaches regardless of whether the samples were true positives or controls. The percent agreement between LYM/LYG and LYT for all samples was 80% (95% CI of 77% to 83%), and the percentages of disagreement for each possible result were similar at 8% and 10%, respectively. The percent agreement for Lyme disease patient samples ranged from 70% to 100%, with the lowest percentage resulting when samples

		against the combined LYT EIA. Standardized Two-Tiered Testing (STTT) was undertaken with Western Blotting as the second-tier test, and Modified Two-Tier Testing (MTTT) was undertaken, using the C6 EIA as the second-tier test. **Results are shown for neuroborreliosis patients ONLY**	Specificity: ● First-Tier Tests: "When all negative controls were tested, the specificities of the two EIA strategies were the same (85% specificity) when duration of illness was not considered for LYM testing in patients with other diseases." ● STTT: "The overall specificity was slightly lower when using the combined LYT EIA (97% specificity) or the dissociated LYM/LYG assays (97% specificity) than with the C6 EIA (99% specificity)." ● MTTT: "The overall specificity for both algorithms was 98 to 99%, and this was similar to the specificities obtained when immunoblot assays were used as the second-tier test (specificities of 97 to 99%)."	from early Lyme disease patients with EM were tested." "Overall, the dissociated LYM/LYG EIAs performed, with minor exceptions, equivalently to the LYT in test-to-test comparisons or as first-tier assays in STTT or MTTT. An advantage to users who already have the Vidas instrument in their laboratories is that these first-tier EIA approaches use the same automated platform. To the extent that they may reduce the number of Western immunoblots required, this approach will remove technical time and complexity associated with immunoblotting."	
van Gorkom, et al., 2017 The Netherlands	Retrospective study	Patients were classified into four groups: Active LNB patients (n= 27; median age 57.8 years; 51.9% male), Treated LNB patients (n= 36; median age 59.1 years; 52.8% male), Treated healthy individuals (n= 27; median age 53.1 years; 44.4% male), and Healthy individuals (n= 147; median age 40.9 years; 38.8% male). 4/27 (25%) active LNB patients reported having had an EM and 9/27 (56.3%) recalled a tick bite. 9/36 (25%) treated LNB patients reported having had an EM and 27/36 (75%) recalled a tick bite. 22/27 (81.5%) treated healthy individuals reported having had an EM, 4/27 (14.8%) reported a diffuse redness after a tick bite, and 1/27 (3.7%) had flu-like symptoms after a tick bite. 4/147 (2.7%) healthy individuals reported	Serum samples of all study subjects were tested in two ELISAs (C6 ELISA and SERION ELISA) and one immunoblot (RecomLine IgM/IgG Immunoblot). Three different strategies were used: STTT (C6 ELISA ▢ Immunoblot), STTT (SERION ELISA ▢ Immunoblot), and MTTT (C6 ELISA+SERION ELISA ▢ Immunoblot). High concordances between the results of the test strategies were found for healthy individuals and active Lyme neuroborreliosis patients groups (range 98.6–100%); however, low concordances were observed for Lyme neuroborreliosis patients and healthy individuals	Discordant test results represent variability in the amount and type of antibodies, which may be influenced by antibiotic treatment and/or the natural course of clearance of infection. Of the investigated factors affecting the natural clearance of the infection, only age contributed to discordant ELISA or test strategy results within both treated groups. Older age was associated with an increase of discordant ELISA results among treated LNB patients and with a decrease of discordant test strategy results among treated healthy individuals	

		having had an EM and 86/147 (58.5%) recalled a tick bite. The 147 healthy individuals were significantly younger than the other three groups (p ≤ 0.001).	who had been treated for Lyme borreliosis in the past (range 77.8–88.9%).	
van Gorkom, et al., 2018 The Netherlands	Retrospective study	Whole-blood and serum samples were obtained from hospital patients diagnosed with active LNB, hospital patients previously treated for LNB, and healthy individuals (all 18 years old). 33 active Lyme neuroborreliosis patients were included; their median age was 56.7 years. They were included before, during, or shortly after antibiotic treatment started (median, 7.0 days after the start of antibiotic therapy). Antibiotic therapy consisted of intravenous ceftriaxone for 14 or 30 days. Two patients switched to doxycycline because of an adverse reaction to ceftriaxone. One patient was given doxycycline from the start (21 days). The clinical symptoms among active Lyme neuroborreliosis patients mostly consisted of radicular disease (15/33 [45.5%]) and/or cranial nerve paresis (15/33 [45.5%]).	Borrelia-specific serum antibodies were detected using a two-tier serology protocol. The first test used was the C6 enzyme-linked immunosorbent assay (ELISA), which was confirmed by using the recomLine IgM/IgG immunoblot tests. Detection of intrathecally produced Borrelia-specific antibodies was done using the IDEIA test. Antibody index (AI) scores of 0.3 were considered positive. The Borrelia ELISpot assay was performed on peripheral blood isolated from all study participants. Most of the active LNB patients were seropositive (30/33 [90.9%]). 6/36 (16.7%) assessable treated LNB patients had a positive result. Of 173 healthy individuals, 23 (13.3%) had *Borrelia*-specific antibodies.	Positive Borrelia ELISpot assay results are, in general, associated with exposure and/or (past) infection with B. burgdorferi sensu lato. The diagnostic performance of the Borrelia ELISpot assay for the detection of active disease was determined by calculation of the ROC curve, which resulted in an AUC of 0.591, suggesting that this assay is unsuitable for the diagnosis of active Lyme neuroborreliosis.
Dersch, et al., 2019 Freiburg, Germany	Retrospective study	A total of 43 patients with definite LNB who were treated with antibiotics and 40 healthy controls were included. For definite LNB, patients were required to have compatible neurological symptoms, CSF pleocytosis, antibodies against *Borrelia burgdorferi* in serum and CSF, and evidence of a *Borrelia*-specific intrathecal synthesis of immunoglobulins (defined as an antibody index ≥2). Healthy controls came from the same region as the LNB patients. LNB patients were statistically significantly older than the healthy controls. The mean follow-up period for the LNB patients was 4.9 years after the initial diagnosis.	Ten LNB patients (22.7%) had persisting antibodies in serum at follow-up (5 IgM, 3 IgG, and 2 IgM & IgG). Serum samples from LNB patients at follow-up were positive for IgM antibodies in seven patients (16.3%) and positive for IgG antibodies in five patients (11.6%). Serum samples from healthy controls were positive for IgM antibodies in three cases (7.5%) and positive for IgG antibodies in seven (17.5%) cases. Overall, six healthy controls (15%) showed positive anti-borrelial antibodies in immunoblot (1 IgM, 3 IgG, and 2 IgM&IgG).	Seroprevalence of anti-borrelial antibodies in serum of treated LNB patients at five-year follow-up did not have a statistically significant difference from those of healthy controls from the same area with endemic Lyme borreliosis. At a mean follow-up period of 4.9 years (SD: 3.3), 10 LNB patients (22.7%) had persisting antibodies in serum (5 IgM, 3 IgG, and 2 IgM&IgG). The prevalence of IgM or IgG antibodies at follow-up showed no statistically significant difference between LNB patients and healthy controls (IgM p=0.32, IgG p=0.54).

Phospholipidomic Analysis of Plasma				
Łuczaj, et al., 2017 Bialystok, Poland	Retrospective study	Samples were collected from eight patients with neuroborreliosis (three female and five male); mean age of 48 years (range 21–83). Neuroborreliosis diagnosis was confirmed by epidemiological anamnesis. 50% of patients reported "previous tick bites, clinical manifestations of Bannwarth's syndrome, lymphocytic meningitis with or without nerves paresis", and positive detection of anti-*B. burgdorferi* IgM and IgG antibodies by ELISA confirmed by Western Blot. The control group consisted of eight healthy subjects (three female and five male); mean age 47 years (range 22–72).	"Total lipids from all plasma samples were extracted using a modified Folch method." "Silica gel TLC plates were used to separate the PL classes… Identification of the different PL classes was performed by comparison with PL standards applied to the same plate. Estimation of the total amount of PL in total lipid extracts and in the spots after TLC separation was performed according to Bartlett and Lewis [8]. The relative abundance (%) of each PL class was calculated by relating the amount of phosphorous in each spot to the amount of total phosphorous in each plasma lipid extract." "PL classes were separated by hydrophilic interaction liquid chromatography (HILIC), performed on an Ultra high-performance liquid chromatography (UPLC) system."	"Significant increases in the lysophosphatidylcholines LysoPtdCho 16:0 and LysoPtdCho 18:2 were observed. The plasma of neuroborreliosis patients appeared to have an increased relative abundance of sphingomyelin CerPCho d18:1/24:1 and a decrease in CerPCho d18:0/18:0. Principal components analysis of the relative abundances of all PL class species distinguished between neuroborreliosis patients and healthy subjects."

*Risk of bias of studies included in Waddell, 2016 systematic review and meta-analysis were evaluated with QUADAS-2 tool; 8 were deemed to have low risk of bias and 40 were assessed as unclear risk of bias; Quality of studies included in Leeflang, 2016 systematic review and meta-analysis were evaluated with QUADAS-2 tool, and none of the studies had low bias risk in all four QUADAS-2 domains.

XI. For which neurological presentations should patients be tested for Lyme disease?

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Demyelinating Disease (First episode vs. Relapsing-Remitting Multiple Sclerosis [RRMS] vs. Acute Disseminated Encephalomyelitis [ADEM])						
Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Population characteristics and Observed Neurologic Presentation	Prevalence of Lyme in Patients with Disorder *or Prevalence of Disorder in Lyme patients*	Study Conclusions
Schmutzhard, 1988 Vienna, Austria	Case control study	8	Antibodies to *B. burgdorferi* were tested in serum by ELISA. Results were considered as positive when units were calculated ≥4.5 at a cut off at 0-2 nm.	106 patients from the province of Tyrol (high risk area) who had been diagnosed with probable or definite multiple sclerosis. 27.4% of patients were suffering from chronic progressive MS, and 63.2% had a relapsing-remitting form of the disease. 9.4% of patients could not be classified. 103 matching controls (typically family members or neighbors) were also included.	14.2% (15 of 106) patients with MS had IgG antibodies above threshold, whereas 25.2% (26 of 103) healthy controls had IgG antibodies above threshold.	There was no significant difference between MS patients and healthy controls with regard to antibody prevalence. The authors found no significant association between either relapsing-remitting MS or chronic progressive MS with the presence of *B. burgdorferi* antibodies.
Coyle, 1989 Stony Brook, NY	Prospective cohort study	5	Serum antibody titer.	100 patients living in a Lyme-endemic region who had been referred to a clinic for possible MS.	1.1% (1 of 89) definite MS patients were positive for antibodies to *B. burgdorferi.* 18% (2 of 11) non-MS patients were antibody positive.	The authors determined that Lyme Disease does not play a significant role in the differential diagnosis of MS due to the rarity of infection with *B. burgdorferi* in MS patients.

Halperin, et al, 1989 Stony Brook, NY	Prospective cohort study	5	Diagnosis was based on 1) history of well-documented erythema migrans or evidence of immunoreactivity against *B. burgdorferi*, using either (a) a specific ELISA or (b) a specific lymphocyte proliferation assay; and (2) prominent nervous system abnormalities.	85 patients with serological evidence of *B. burgdorferi* infection presenting with central nervous system manifestations (encephalopathy [41], neuropathy [27], meningitis [2], multiple sclerosis (MS) [6], and psychiatric disorders [3]).	7.1% (6 of 85) patients with serological evidence of neurologic Lyme disease presented with MS-like symptoms.	Patients with an MS-like illness had abnormal evoked potentials (EP)s, elevated IgG index, and oligoclonal bands in the cerebrospinal fluid. MRIs were abnormal in 5/6 patients with an MS-like illness. Authors concluded that MS patients with serum immunoreactivity against *B burgdorferi* lack evidence of CNS infection with this organism.
Chmielewska-Badora, et al., 2000 Poland	Prospective cohort study	5	Serum IgM and IgG antibodies for *B. afzelii* were measured by ELISA. Clinical presentation and tick bite history were also assessed.	769 adult neurological patients with various diagnoses living in an endemic area. 26 patients were diagnosed with multiple sclerosis based on clinical examination and MRI, and 25 patients were diagnosed with neuroborreliosis.	38.5% (10 of 26) of MS patients were seropositive. 19.4% (149 of 769) of the overall examined neurological patients were seropositive.	The relationship between diagnosis of MS and presence of anti-*Borrelia* antibodies was found to be statistically significant in this cohort of patients (p= 0.04). The authors concluded that MS may be associated with *Borrelia* infection but indicated that further studies were required to suggest any recommendation.
Mosayebi, et al., 2009 Arak, Iran	Case control study	6	IgM and IgG antibodies for *B. burgdorferi* were detected in serum by ELISA.	31 new cases of MS patients and 65 healthy controls.	ND	The authors found a significant difference between case and control groups with regard to prevalence of IgM antibody titer against *B. burgdorferi* (p=0.0001). They concluded that the probability of MS may be increased by acute Borrelia infection.
O'Mahony, et al., 2010 Canada	Prospective cohort study	4	ND	19 children (median age 11.7 years; range 1-15) initially diagnosed with acute demyelinating syndromes (ADS).	One of 19 (0.05%) children initially diagnosed with ADS was subsequently diagnosed with Lyme disease manifesting as transverse myelitis.	The authors concluded that patients presenting with symptoms of ADS should be assessed for atypical presentation and that their clinical, laboratory, and MRI features should be monitored to exclude alternate diagnoses.

137

Spirin, et al., 2010 Yaroslavl, Russia	Prospective cohort study	5	ND	10 patients with primary progressive multiple sclerosis (PPMS). 10 patients with chronic progressive borrelial encephalomyelitis (CPBEM).	ND (30% of patients with PPMS recalled a tick bite, whereas 80% of patients with CPBEM recalled a tick bite. Difference not significant)	The authors compared the clinical features of patients with PPMS to those with CPBEM. Patients with multiple sclerosis showed some significant differences from patients with borrelial encephalomyelitis, including the absence of erythema migrans (p= 0.015). These patients also were less likely to experience arthralgia (p= 0.005) or sensory dysfunction and did not have *B. burgdorferi* antibodies in their CSF.
Radolovic Prenc, et al., 2011 Croatia	Prospective cohort study	4	ND	121 patients with clinically and laboratory-supported definite MS.	A faulty MS diagnosis was found in 14 (16.9%) of 121 patients. 21.4% (3 of 14) faulty diagnoses were in patients diagnosed with Lyme disease.	The authors concluded that the most commonly indicated examinations and diagnostic tests are not sufficient, and that serologic tests for *B. burgdorferi* were recommended in differential diagnosis of relapsing-remitting MS.

Parkinsonism						
Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Population characteristics and Observed Neurologic Presentation	Prevalence of Lyme in Patients with Disorder *or Prevalence of Disorder in Lyme patients*	Study Conclusions
Baranova and Bykanova, 2012 Russia	Prospective cohort study	3	Chronic neuroborreliosis was defined by presence of neurologic symptoms ≤ 6 mo from appearance of EM and/or a tick bite or symptoms persisting for ≥6 mo; presence of antibodies to *B. burgdorferi* in serum or CSF; clinical improvement from specific antibiotic therapy; the exclusion of other causes, which could explain	164 patients diagnosed with chronic neuroborreliosis participated in the study (70% female, 30% male, mean age 49.6 years old).	Only 1 patient of 82 (1.2%) chronic neuroborreliosis cases reported Parkinson's as a manifestation of Lyme disease.	Parkinson's as a manifestation of neuroborreliosis is relatively rare. Treatment with antibiotic therapy significantly improved the symptoms in this patient.

			development of existing symptoms			
				Dementia		
Study name, Location	**Study Design**	**Risk of bias***	**Lyme Disease Diagnosis method**	**Population characteristics and Observed Neurologic Presentation**	**Prevalence of Lyme in Patients with Disorder _or Prevalence of Disorder in Lyme patients_**	**Study Conclusions**
Blanc, 2014 Strasbourg, France	Prospective cohort study	7	_B. burgdorferi_ ELISA, Antibody index, and Western Blot were performed on serum and CSF for IgG	1,594 patients presenting with a dementia consistent with the DSM-IV definition. 65% of patients had Alzheimer's disease dementia.	1.25% (20 out of 1,594) of dementia patients also had neuroborreliosis. 0.44% (7 out of 1,594) patients reported a "pure Lyme dementia."	Patients with "pure Lyme dementia" had a stable outcome or mild improvement after treatment with ceftriaxone. The authors indicated that in endemic areas, serological testing for antibodies to _B. burgdorferi_ should be done on dementia patients and should be confirmed by CSF analysis. The presence of "pure Lyme dementia" which was manageable with antibiotics in this cohort was the primary reason for this recommendation.
				Amyotrophic Lateral Sclerosis (ALS)		
Study name, Location	**Study Design**	**Risk of bias***	**Lyme Disease Diagnosis method**	**Population characteristics and Observed Neurologic Presentation**	**Prevalence of Lyme in Patients with Disorder _or Prevalence of Disorder in Lyme patients_**	**Study Conclusions**
Halperin, et al., 1990 Suffolk County and Nassau County, NY and Boston, MA	Case control study	8	Patients were serologically tested by ELISA. Cerebrospinal fluid samples were taken from 24 patients and examined for cell count, Lyme titer, and other indicators of Lyme disease. Medical histories were reviewed for history of tick bite, arthritis, meningitis, radicular	52 patients (19 from Suffolk County, 14 from Nassau County, and 19 from Boston, MA) who had been diagnosed with ALS (had signs and symptoms consistent with ALS). 38 age-, sex-, and residency-matched controls were matched to the 19 patients from Suffolk County.	9 of 19 (47%) ALS patients from Suffolk County were seropositive. 4 of 38 (10.5%) matched controls were seropositive. 3 of 14 (21.4%) Nassau County residents with ALS were seropositive.	Patients with ALS living in Suffolk County (a highly endemic area) were significantly more likely to be seropositive for Lyme disease than healthy controls (p=0.0053; OR 7.65 [95%CI 1.65, 38.46]). ALS patients from Suffolk County were significantly more likely to be seropositive than patients from Boston

| | | | pain, or erythema migrans. None of the patients across all groups reported typical symptoms of Lyme disease. | 37 patients from Suffolk County with other neuromuscular diseases were selected as a separate control group. | One of 19 (5.3%) Boston residents with ALS was seropositive. | (p=0.0078, OR 16.2 [95%CI 1.68, 747.7]). There was no significant difference in the rates of seropositivity between the Suffolk County and Nassau County residents (p=0.12).

14 of 15 seropositive ALS patients received ceftriaxone (2g/day for 14 days); 1 other patient received other antibiotics non-specific to Lyme due to another medical condition. 3 of 14 (21.4%) seropositive ALS patients improved with ceftriaxone treatment. 5 of 14 (35.7%) patients treated with ceftriaxone died.

The authors found that in hyperendemic regions, there is a statistically significant association between ALS and seropositivity for *B. burgdorferi*. The authors did not conclude that such infection is a frequent cause of ALS and noted that the association may be coincidental. |
| ALS Untangled Group, 2009

United States and Ireland | Retrospective cohort study | 2 | Patients were tested for Lyme by ELISA, confirmed by Western Blot.

Per the authors: "(Of ten clinician-scientists within this group) Only 3 clinician-scientists have routinely tested most or all newly diagnosed patients with ALS for Lyme, the other 7 test it only when there are other symptoms or signs of possible Lyme, or when a patient asks for it." | 4,000 patients with confirmed ALS across multiple clinics. | 30 of 4,000 (0.075%) of patients with confirmed ALS were positive for Lyme disease by ELISA and Western Blot. | Of 30 ALS with confirmed Lyme disease, most were treated with intravenous antibiotics, but none of the patients' neurologic symptoms improved with treatment.

The authors concluded that there is no convincing evidence that ALS can be caused by Lyme disease, and warned clinicians against suggesting that antibiotic treatment may reverse or improve the symptoms of ALS. |

Qureshi, et al., 2009 Boston, MA	Retrospective cohort study	3	Serum IgG antibody titers against *B. burgdorferi* were measured by ELISA, and positive or borderline results were confirmed by Western Blot.	The charts of 1,760 patients with confirmed or suspected ALS were reviewed. 414 patients (23.5%) with confirmed or suspected ALS who underwent at least one laboratory test for Lyme disease.	24 of 414 (5.8%) of patients with ALS were positive by ELISA. 4 of 414 (0.97%) had positive ELISA which was confirmed by Western Blot. Of these 4 patients, only 3 reported clinical symptoms and/or exposure to ticks in the recent past. None of these 4 patients had a family history of ALS.	Two of 4 ALS patients with exposure to *B. burgdorferi*, confirmed by ELISA and Western Blot, received treatment with ceftriaxone for 4-5 weeks. Antibiotic treatment did not lead to improvement of neurologic symptoms. All 4 ALS patients positive for antibodies against *B. burgdorferi* died of ALS. The authors found that the prevalence rate of confirmed previous Lyme disease in ALS patients was similar to the occurrence of Lyme disease in endemic regions. The authors conclude that testing for Lyme disease should not be performed in ALS patients unless clear symptoms and history are suggestive of Lyme infection.
Visser, et al., 2017 Utrecht, The Netherlands	Case control study	8	Serum IgG antibody titers against *B. burgdorferi* were measured by ELISA, and positive or borderline results were confirmed by Western Blot.	491 patients (median age 64.9 years; range 19.5-90.5) were diagnosed with definite (22.6%, 111 of 491), probable (34%, 167 of 491), laboratory-supported probable (29.1%, 143 of 491), or possible (14.3%, 70 of 491) ALS were observed. 982 age-, sex-, and residency-matched controls were selected.	58 of 491 (11.8%) of ALS patients had a positive or borderline ELISA. 20 of 491 (4.1%) ALS patients were confirmed IgG positive by Western Blot. 107 of 982 (10.9%) of controls had a positive or borderline ELISA. 58 of 982 (5.9%) controls were confirmed IgG positive by Western Blot.	The authors found no significant difference in seroprevalence of *B. burgdorferi* between ALS patients and healthy controls (p=0.60, ELISA; p=0.17, Western Blot). The seroprevalence of 4.1% in ALS patients was similar to that of the normal population in The Netherlands. The authors conclude that there is no association between *B. burgdorferi* antibodies and ALS, and do not recommend routine serologic testing for patients with classical ALS.
Sensorineural Hearing Loss						

Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Population characteristics and Observed Neurologic Presentation	Prevalence of Lyme in Patients with Disorder _or Prevalence of Disorder in Lyme patients_	Study Conclusions
Hanner, et al.,1989 Sweden	Prospective cohort study	4	Serum antibody titer against _B. burgdorferi_ ≥320 was the criteria for positive infection.	98 patients who had a history of sudden hearing loss, disorders similar to Meniere's disease, or hearing loss with acute facial palsy or vertigo.	17% (17 of 98) of patients showed serological evidence of borreliosis. 82% (14 of 17) of patients with borreliosis also experienced vertigo.	All 17 patients received treatment with high dose IV benzylpenicillin. In 5 patients, treatment led to improved hearing. The authors concluded that serological testing for _B. burgdorferi_ is worthwhile in patients with unexplained hearing disorders.
Richardson, et al., 1994	Prospective cohort study	4	ND	100 patients presenting with asymmetrical sensorineural hearing loss.	One patient of 100 (1%) had positive Lyme serology.	The patient who had positive serology for Lyme disease was treated with antibiotics, but experienced no improvement in hearing after treatment. The authors concluded that routine screening for Lyme disease was not cost effective in non-endemic regions.
Hydén, 1995 Sweden	Prospective cohort study	4	Serological testing of CSF and serum	21 patients with sudden unilateral deafness and 16 patients with vestibular neuritis with "typical clinical history and findings".	None of the patients presenting with sudden deafness had positive Lyme serology.	Despite a lack of findings, the authors concluded that testing for _Borrelia_ was prudent in endemic areas.
Peltomaa, et al., 2000 Finland	Prospective cohort study	6	ND	165 patients with acute idiopathic sensorineural hearing loss.	2.4% (4 of 165) patients in this cohort had confirmed Lyme Disease.	The prevalence of positive antibody titer against _B. burgdorferi_ was four times higher in patients with sensorineural hearing loss than that of the general population of Finland. The authors concluded that Lyme Disease is a rare, but treatable cause of sudden hearing loss, and that

						serologic testing is warranted in endemic areas.
Finizia, et al., 2001 Sweden	Prospective cohort study	6	Serological testing of CSF and serum, and/or elevated CSF proteins and/or pathological CSF cell counts	19 patients with sudden sensorineural hearing loss, both seropositive and seronegative.	There was a high prevalence (68%) of pathology in serum and CSF. In 54% of the patients, elevated levels of CSF proteins and/or pathological CSF cell counts were present without positive antibodies to Bb.	"Positive levels of antibodies against Bb or pathological proteins in CSF were associated with better hearing recovery (means of 47.2% and 51.7%, respectively) ... Patients with positive serology to *B. burgdorferi* who received antibiotic treatment (oral tetracycline), with or without steroids, had the best hearing recovery (61.7 and 48.4%, respectively)" The authors concluded that more liberal testing guidelines would be helpful in encouraging earlier antibiotic treatment for patients with Lyme Disease.
Lorenzi, et al., 2003	Prospective cohort study	6	Serological testing for *B. burgdorferi*	47 patients with sudden deafness, both seropositive and seronegative.	21.3% of cases had positive antibody titers to *B. burgdorferi*	The authors reported no distinct differences in clinical presentation between the seropositive and seronegative groups. Both groups experienced a similar clinical outcome.
Walther, et al., 2003	Retrospective study	5	IgM and IgG antibodies for *B. burgdorferi* were detected in serum by ELISA.	344 patients with acute sensorineural hearing loss and 66 patients with vestibular neuronitis. Seropositivity frequencies were compared against those of healthy individuals in the literature.	IgG antibodies were elevated in 15.7% of patients with sensorineural hearing loss. IgM antibodies were elevated in 4.7% of patients.	Patients with sensorineural hearing loss experienced a higher prevalence of IgG seropositivity than the healthy population but showed a comparable prevalence of IgM seropositivity. The authors concluded that low frequency hearing loss may be a sign for a *Borrelia* infection and suggest treatment with oral antibiotics in the presence of IgM antibodies.

| Wentland, et al., 2018

Boston, MA | Retrospective study | 7 | Serological testing for *B. burgdorferi* | 746 patients (newborn to age 18) with a diagnosis of sensorineural hearing loss or hearing loss.

Lyme titers were performed in 54 patients. | 3 of 54 patients (6%) had positive antibody titers to *B. burgdorferi*, and 2 (4%) patients had equivocal results.

One patient with positive antibody titers also had an ipsilateral enlarged vestibular aqueduct (passed newborn hearing screen). It was unknown if the other two positive cases had passed the newborn hearing screen. | The authors stress that cytomegalovirus is the most common infectious cause of sensorineural hearing loss.

In the context of the current study, testing for Lyme titers was only conducted in patients at high risk of Lyme disease (children who acquired hearing loss with residence in or travel to Lyme-endemic areas).

Children with positive results were referred to pediatricians or infectious disease specialists for management, and clinical outcomes are not reported. |

| Radiographic White Matter Disease ||||||

Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Population characteristics and Observed Neurologic Presentation	Prevalence of Lyme in Patients with Disorder _or Prevalence of Disorder in Lyme patients_	Study Conclusions
Fernandez, et al., 1990 Toms River, NJ	Retrospective study	4	Patients had positive serology and history of Lyme disease or suspected Lyme disease	14 patients with Lyme disease who had been referred for an MRI examination due to complaints of headache, blurred vision, hearing loss, radiculopathy, or transverse myelitis.	42.9% (6 of 14) of patients with neurologic Lyme disease had MRI abnormalities.	In the six patients who demonstrated an abnormal MRI, lesions in the brain were widely distributed. The authors noted that most of these patients had lesions which involved the subcortical white matter of the frontal and parietal lobes. The authors concluded that Lyme Disease of the central nervous system should be considered as a differential diagnosis of white matter lesions, and that MRI may be useful in differentiating Lyme

						Disease from other white matter diseases.
Agosta, et al., 2006	Case control study	7	No information given. 11/20 had confirmation of neuroborreliosis by IgG Western Blot. 2/11 patients had presented with CSF pleocytosis and increased CSF protein count.	20 patients with neuroborreliosis.15/20 patients presented with focal neurologic syndromes (13 had sensorimotor deficits, one had optic neuritis, and one had a brain stem syndrome), whereas 5 patients presented with non-focal symptoms (3 had fatigue and sleep disorders, and 2 had meningoencephalitis). 11 healthy controls matched for age and sex were selected.	80% (12 of 15) patients with neuroborreliosis had white matter abnormalities.	The authors focused on white matter differences between patients with neuroborreliosis and patients with MS. They found that patients with neuroborreliosis did not report cervical cord pathology or occult brain tissue damage, which are commonly associated with MS. The authors concluded that MRI evaluation could be a valuable diagnostic tool in patients with focal neurologic syndromes, absence of systemic manifestations, and multiple white matter lesions
Aalto, et al., 2007 Linköping, Sweden	Case control study	7	Patients had a combination of CSF IgM or IgG antibody production for *Borrelia*, CSF lymphocytic pleocytosis, and relevant longstanding symptoms (persisting for 3-6 months or longer).	16 patients with chronic neuroborreliosis and 16 matched healthy controls received an MRI examination focused on white matter lesions and lesions in the basal ganglia.	75% (12 of 16) of patients with chronic neuroborreliosis had white matter lesions.	Patients with chronic neuroborreliosis had slightly more frequent and more advanced subependymal white matter lesions than matched controls, but the difference was not statistically significant (p= 0.12). White matter lesions were generally detected in control subjects age 43 and older, but they were detected at younger ages in the patient group. The authors concluded that there were no significant differences in prevalence or severity of white matter lesions between patients and controls. The correlation of age with white matter lesions led the authors to suggest that MRI may not be

					useful in differentiating chronic neuroborreliosis in older patients.
Monteventi, et al., 2018 Switzerland	Retrospective case series	NA	Demonstration of *B. burgdorferi* specific antibodies in both the serum and in the CSF	The clinical records of 4 children who had serologically confirmed neuroborreliosis and had suffered arterial ischemic childhood stroke that was attributed "with confidence" to LNB were reviewed. All patients underwent MRI, and two patients had supplemental vessel imaging with MRA.	1 of 4 patients (25%) who had LNB-related stroke presented with scattered white matter lesions, in addition to multifocal vasculitis with prominent basilar artery involvement. All cases, except for the child with white matter lesions, displayed CSF pleocytosis; however, the authors attribute the absence of pleocytosis potentially to the delay in diagnosis for this case.	The child received IV ceftriaxone (2g QD for 2 weeks) with 100 mg aspirin QD. The child experienced mild sequelae after discharge, despite treatment. Imaging at 10 months revealed stable vascular lesions and no new parenchymal lesions. Though LNB is a rare cause of stroke (and white matter lesions), the authors recommend serological testing of serum and CSF in patients with unexplained cerebral vasculitis, particularly involving posterior circulation.

Seizures					
Study name, Location	**Study Design**	**Risk of bias***	**Lyme Disease Diagnosis method**	**Patient characteristics and Observed Neurologic Presentation**	**Clinical course and author statements**
Baumann, 2010 Austria	Case report	NA	The patient was tested for IgM and IgG antibodies for *B. burgdorferi* by ELISA of serum and CSF, and positive results were confirmed by Western blot. CSF was analyzed for pleocytosis. The patient's status was monitored by MRI. IgM and IgG were positive in serum, Western Blot was borderline for IgG alone. IgM and IgG were positive in CSF, but only positive 3 weeks after the initial test.	6-year-old boy had experienced two epileptic seizures. He did not have a fever, meningitis, or neurological abnormalities upon physical examination. A cranial MRI revealed an increased signal in the sulci of the right parietal region.	The patient received IV ceftriaxone for 14 days. After treatment, the patient did not experience further seizures. Cranial MRI was conducted at a 4-month follow-up and was normal.
Markeljević, 2011 	Case report	NA	The patient was tested for IgM and IgG antibodies for *B. burgdorferi* by ELISA of serum. CSF was analyzed for pleocytosis.	45-year-old man presented with a severe tremor, myoclonic jerks, and psychosis. His symptoms had begun to develop ten months earlier. He was first tested for	The patient was treated with IV ceftriaxone (2g/day), followed by doxycycline (100 mg BID) over a 35-day period. He showed rapid functional and clinical improvement but required further psychiatric

146

Zagreb, Croatia			The patient did not recall a tick bite or skin lesion. IgG was positive in serum, IgM was negative. The patient presented with high pleocytosis.	brucellosis and tularemia, but these tests were negative.	treatment. Combination of psychiatric treatment with antibiotics led to further improvement. The patient was readmitted and treated again in the same manner, but he continued to experience cognitive symptoms for over a year.
Schober, 2012 Feldkirch, Austria	Case report	NA	The patient had positive serology in CSF and elevated CSF protein count.	13-year-old boy showed a generalized seizure and was treated with anti-epileptic therapy. One month later, he had weakness of his left arm, which prompted an MRI examination. MRI examination revealed a subcortical white matter lesion.	The patient was treated with IV ceftriaxone for 3 weeks, which led to resolution of neurological symptoms. MRI examinations in follow-up were normal and no further seizures or neurological abnormalities occurred.
Juric, 2014 Zagreb, Croatia	Case report	NA	The patient was tested for IgM and IgG antibodies for *B. burgdorferi* by ELISA of serum, and positive results were confirmed by Western blot. CSF was analyzed for protein count. IgM and IgG were positive by ELISA and Western Blot. The patient showed elevated CSF protein	46-year-old man experienced a partial motor epileptic seizure with secondary generalization. He had experienced general weakness for several days prior to this. He recalled several tick bites in the past and had had a rash on his abdomen and chest one year prior to the seizure. Upon admission, he had a reddish-purple rash on his abdomen.	The patient received anti-epileptic treatment and IV ceftriaxone (2 g/day for 17 days). Over the course of a four-year follow-up, he experienced no further seizures and eventually discontinued anti-epileptic therapy. The authors noted that cerebral vasculitis, which was suggested by MRI changes in this patient, may have been a cause of the seizure.
Matera, 2014 Catanzaro, Italy	Case report	NA	The patient was tested for serum and CSF IgM and IgG antibodies for *B. burgdorferi* and *B. garinii* by the "VIDAS Lyme Screen" enzyme-linked fluorescence assay, and positive results were confirmed by Western Blot. CSF was tested by PCR for *Borrelia* DNA. The patient was weakly positive by ELFA and Western Blot. PCR analysis of CSF revealed late infection with *B. garinii*.	26-year-old man experienced a generalized seizure before admission to the hospital and again upon admission. He had experienced a facial paralysis two years prior to admission, but it had resolved spontaneously. He recalled no tick bites, erythema migrans, prior fatigue, arthralgia, or myalgia. Multifocal white matter lesions were detected by MRI.	The patient received doxycycline (200 mg/day for 21 days). MRI was performed two months later and showed substantial improvement. The patient was followed up for 4 months and was in full clinical remission throughout.
Trigeminal Neuralgia					

Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Patient characteristics and Observed Neurologic Presentation	Clinical course and author statements
Fritz, et al., 1996 Marburg, Germany	Case report	NA	Neurological examinations were normal, including CSF protein and pleocytosis. Patient's serum and CSF were tested for *B. burgdorferi* antibody by ELISA. IgG antibodies were elevated in serum and CSF. IgM were found in serum, but not CSF.	58-year-old previously healthy man noticed a rash on his back with slight headache and fatigue, which progressed over two weeks to an intense stabbing pain in the distribution of his fifth cranial nerve. He did not recall a tick bite. 4 weeks after finding the rash, it was still there.	After diagnosis of Lyme Disease, patient was treated with IV ceftriaxone (2g/day) for 14 days. He began to experience improvement three days after treatment initiation and had completely recovered after one week of treatment. The authors suggest that *Borrelia* infection be considered as a differential diagnosis in the event of craniofacial pain.
Knudtzen, et al., 2017 Funen and Langeland, Denmark	Retrospective case series	NA	Clinical data and information about risk factors for and history of tick bites were collected. IgG and IgM antibodies were collected in serum and CSF. Leukocyte counts were also registered from CSF.	The characteristics of all participants (N= 442 with positive CSF for *Borrelia* AI) are as follows: median age was 47 years (IQR, 50 [12–62] years), 60.8% male, and 126 children <18 years. 36.9% had a history of tick bite registered, and 19.5% had a history of erythema migrans. 2 (0.05%) patients had a documented palsy of the trigeminal nerve.	The course of treatment is not documented specifically for individuals with palsy of the trigeminal nerve. The authors made note that overall, there was a significant delay in treatment for Lyme neuroborreliosis (median 24 days). Approximately one third of all observed patients experienced persistent symptoms and sequalae after treatment. The authors suggest that the treatment delay may have contributed to the occurrence of residual symptoms.

*Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XII. Should adult patients with psychiatric illnesses be tested for Lyme disease?

Bibliography: 1. Halperin, et al. Neurology. 1989 Jun; 39(6): 753-9; 2. Nadelman, et al. Mt Sinai J Med. 1997 Nov; 64(6): 409-12; 3. Hájek, et al. Am J Psychiatry. 2002 Feb; 159(2): 297-301; 4. Hájek, et al. Eur Psychiatry. 2006 Mar; 21(2): 118-22; 5. Greenberg, R. "Tick-borne infections and pediatric bipolar disorder." Neurology, Psychiatry and Brain Research 1.22 (2016): 11; 6. Zomer, et al. Clin Infect Dis. 2017 Oct 30;65(10):1689-1694; 7. Johnco, et al. Gen Hosp Psychiatry. 2018 Mar - Apr;51:85-89; 8. Zomer, et al. Eur J Intern Med. 2018 May;51:e19-e20.

Study name, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Population characteristics and Observed Psychiatric illnesses	Prevalence of Lyme in Patients with Illness _or Prevalence of Illness in Lyme patients_	Study Conclusions
Halperin, 1989 Stony Brook, NY	Prospective cohort study	5	Diagnosis was based on 1) history of well-documented erythema migrans or evidence of immunoreactivity against _B. burgdorferi_, using either (a) a specific ELISA or (b) a specific lymphocyte proliferation assay; and (2) prominent nervous system abnormalities	85 patients with serological evidence of _B. burgdorferi_ infection presenting with central nervous system manifestations (encephalopathy [41], neuropathy [27], meningitis [2], multiple sclerosis (MS) [6], and psychiatric disorders [3]).	3.5% (3 of 85) patients with Lyme presented with psychiatric disorders.	Patients presenting with psychiatric disorders, as well as patients with MS, lacked evidence of B. burgdorferi infection when cerebrospinal fluid antibody concentration was analyzed.
Nadelman, 1997 Westchester County, NY	Prospective cohort study	4	Blood was tested by Fluorescent immunoassay alone (90%) or Fluorescent immunoassay plus ELISA (10%). Positive samples were confirmed by Western Blot.	517 adults admitted to an acute psychiatric care facility.	0.2%* (1 of 517) were seropositive by ELISA. *This patient had a non-reactive Western Blot	The authors concluded that routine serologic testing was not warranted in psychiatric inpatients, regardless of residence in an endemic area.
Hájek, 2002/2006* Prague, Czech Republic	Case control study	7	ELISA for _B. afzelii_ was applied. Serum IgG >900 and serum IgM values >1000 were defined as positive. Circulating immune complexes were analyzed by ELISA for anti-_Borrelia_ antibody with patient's serum samples and with seven negative and eight positive control samples. Patients were considered	926 psychiatric patients admitted to Prague Psychiatric hospital. 28% of patients had schizophrenia or psychotic disorders; 31% had anxiety, somatoform, dissociative, and adjustment disorders; 29% had mood disorders.*	Seropositive psychiatric patients: 33% (166 out of 499)* Seropositive healthy controls: 19% (94 out of 499)* *Hájek, 2002.	Psychiatric patients exhibited serological signs of past _B. burgdorferi_ infection at a rate which was 1.7 times higher than healthy controls. This rate was unusually high compared to prior research, but the authors explained that the rate may be due to differences in presentation in European species of Borrelia.

			positive if they fit the parameters of at least one of these tests. Positive results were confirmed by Western Blot.	884 healthy controls were included. *Psychiatric symptom profiles found in Hájek, 2002.*	In a 2006 follow-up study, the authors found that seropositive status did not differ widely based on psychiatric diagnostic category.
Greenberg, 2016 Summit, NJ	Case series (27 cases)	NA	Serum samples were tested by ELISA and Western Blot.	27 children with bipolar disorder (mean age at diagnosis 7 years; range 5-12).	22% (6 of 27) of children presenting with Bipolar disorder were serologically diagnosed with Lyme Disease.	89% of the children observed (24 of 27) had serological evidence of one or more tick-borne pathogen. Of these patients, 83% (20 of 24) had a confirmed diagnosis of a tick-borne infection. The authors noted that the high rate of tick-borne infections in this cohort was provocative but were unable to make a conclusive statement about the relationship of tick-borne disease to pediatric bipolar disorder. They note that if further research showed similarly high rates of tick-borne infection, or of Lyme Disease in particular, in pediatric bipolar patient populations, serologic testing may be suggested in patients with pediatric mood disorders.
Zomer, et al., 2017 Apeldoorn, Netherlands	Retrospective study	7	Patients were classified based on clinical presentation of Lyme as well as serology results into one of four categories: (1) no clinical Lyme disease and negative serology; (2) no clinical Lyme disease and positive serology; (3) clinical Lyme disease and negative serology; and (4) clinical Lyme disease and positive serology. Serum samples were tested by IgG ELISA and IgG Immunoblot.	1,454 patients who were referred to the Lyme Center Apeldoorn (median age 51 years [range 18-87], 51% female). Of 1,454 patients, 30.1% (437) had no clinical Lyme disease and negative serology, 17.5% (255) had no clinical Lyme disease and positive serology, 30.0% (436) had clinical Lyme disease with negative serology, and 20.6% (300) had clinical Lyme disease with positive serology.	The overall median BDI-II score was 13. The median BDI-II score was 15 for patients with no clinical Lyme disease and negative serology; 11 for patients with no clinical Lyme disease and positive serology; 12 for patients with clinical Lyme disease and negative serology; and 12 for patients with clinical Lyme disease and positive serology.	The prevalence of depressive symptoms was lowest in patients with no clinical Lyme disease and positive serology (15.3%), and highest in patients with no clinical Lyme disease and negative serology (29.3%). The adjusted OR for depressive symptoms in patients with clinical Lyme disease and positive serology was 0.71 (95% CI, .50–1.03) compared to patients with no clinical Lyme disease and negative serology. Depressive symptoms were common among patients referred to a teriary Lyme center. However, the prevalence of depressive symptoms was similar among patients with confirmed Lyme disease and patients without Lyme disease. The authors conclude that depressive symptoms can not be used to discriminate for Lyme disease.

Johnco, et al., 2018	Cross-sectional study	5	Lyme disease was required to be confirmed by a physician.	147 adults between the ages of 18 and 82 years (mean 44 [SD 13]) who participated in online support groups for individuals with Lyme disease. Participants completed two different questionnaires related to obsessive compulsive disorder, with one questionnaire pertaining specifically to Lyme.	84.4% of participants (N= 124) exceeded the clinical cutoff for OCD according to the OCI-R instrument. Only 37% of participants (N= 55) acknowledged that they were experiencing symptoms of OCD. Of the 55 participants who were aware of their obsessive compulsive symptoms, 26% reported the onset of OCD within 6 months of contracting Lyme; 51% were unclear about when they contracted Lyme disease, but believed that their obsessive compulsive symptoms were temporally related to their Lyme diagnosis.	This study reported a high incidence of OCD, as measured by the self-report OCI-R scale. However, only 37% of participants were aware of their own symptoms. The authors concluded that symptoms of OCD are a common co-occurrence with Lyme disease. The study is limited by study design; all measures are patient self-report, which is prone to bias. The authors also acknowledge the potential for selection bias since participants were recruited from support groups which may be more appealing to individuals experiencing distress or dysfunction.
Zomer, et al., 2018 Apeldoorn, Netherlands	Retrospective study (subgroup of the above)	7	Patients were classified based on clinical presentation of Lyme as well as serology results into one of four categories: (1) no clinical Lyme disease and negative serology; (2) no clinical Lyme disease and positive serology; (3) clinical Lyme disease and negative serology; and (4) clinical Lyme disease and positive serology. Serum samples were tested by IgG ELISA and IgG Immunoblot.	This is a subgroup analysis of a retrospective cohort study that involved 1,454 patients who were referred to the Lyme Center Apeldoorn which compared individuals who were at least 65 years old to patients who were 18-64 years old. Of 252 patients who were ≥65 years old, 14.3% (36) had no clinical Lyme disease and negative serology, 23% (58) had no clinical Lyme disease and positive serology, 29% (73) had clinical Lyme disease with negative serology, and	Overall prevalence of depressive symptoms in older patients was 9.8% versus 24.7% in patients 18–64 years old (p<0.001).	Older patients were more likely to have clinical Lyme disease with positive serology and less likely to have depressive symptoms compared to younger patients. The prevalence of depressive symptoms observed in older patients at the Lyme center was not higher than the prevalence in the general older population.

			33.7% (85) had clinical Lyme disease with positive serology.		

*Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XIII. Should children with developmental, behavioral or psychiatric disorders be tested for Lyme disease?

A 2015 narrative review by Chang, et al. provided recommendations for various presentations of pediatric acute-onset neuropsychiatric syndrome (PANS). Lyme disease is discussed within this review as a possible infectious cause of PANS. The authors recommended testing for children exhibiting symptoms of PANS if they live in or have visited areas endemic for Lyme disease. A retrospective case series by Greenberg addressed the issue indirectly in children with pediatric bipolar disorder (shown below).

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Study name, Location	Study Design	Risk of bias	Lyme Disease Diagnosis method	Population characteristics and Observed Psychiatric illnesses	Prevalence of Lyme in Patients with Illness _or Prevalence of Illness in Lyme patients_	Study Conclusions
Greenberg, 2016 Summit, NJ	Case series (27 cases)	NA	Serum samples were tested by ELISA and Western Blot.	27 children with bipolar disorder (mean age at diagnosis 7 years; range 5-12).	22% (6 of 27) of children presenting with Bipolar disorder were serologically diagnosed with Lyme Disease.	89% of the children observed (24 of 27) had serological evidence of one or more tick-borne pathogen. Of these patients, 83% (20 of 24) had a confirmed diagnosis of a tick-borne infection. The authors noted that the high rate of tick-borne infections in this cohort was provocative but were unable to make a conclusive statement about the relationship of tick-borne disease to pediatric bipolar disorder. They note that if further research showed similarly high rates of tick-borne infection, or of Lyme Disease in particular, in pediatric bipolar patient populations, serologic testing may be suggested in patients with pediatric mood disorders.
Burbelo 2013	Case series	NA	Serum, luciferase immunoprecipitation system (LIPS) test for detecting B. burgdorferi antibodies, as well as C6 ELISA & Western blot	104 children w autism; 24 other developmental delay	C6: 101 negative, 3 BL C6, neg WB	82% from highly endemic states. No evidence for assoc autism, developmental delay w Lyme

| Ajamian, 2013 | Case series | NA | Serum 2-tier testing | Autism genetic research exchange: 37 w autism, 27 unaffected sibs; Weill Cornell Autism Research program 33 w autism; 8 unaffected sibs, 15 healthy controls | 70 autistic children – 1 ELISA +, 4 BL; all WB -; 50 controls 4+, 1 BL ELISA; all WB - | All from highly endemic states. No evidence for assoc autism, developmental delay w Lyme |

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XIV. What are the preferred antibiotic regimens for the treatment of acute neurologic manifestations of Lyme disease without parenchymal involvement of the brain or spinal cord?

IV ANTIBIOTICS vs. ORAL DOXYCYCLINE

In patients with Lyme neuroborreliosis, should oral doxycycline be used over IV antibiotics?

P: In patients with Lyme neuroborreliosis

I: Oral doxycycline

C: IV antibiotics (penicillin G or ceftriaxone)

Bibliography: 1. Karlsson, et al. Neurology. 1994 Jul; 44(7):1203-7; 2. Ljøstad, et al. Lancet Neurol. 2008 Aug; 7(8): 690-5.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Oral doxycycline	IV antibiotics (penicillin G or ceftriaxone)	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement of Neurologic Lyme Symptoms (at 4 to 6 months) *												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [c]	none	50/85 (58.8%)	32/69 (46.4%)	RR 1.27 (0.93 to 1.73)	127 more per 1,000 (from 32 fewer to 339 more)	⊕⊕◯◯ LOW	CRITICAL
Improvement of Cerebrospinal Fluid Pleocytosis (at 12 months)												
1	RCT [1]	serious [a]	not serious	serious [b]	very serious [d]	none	18/20 (90.0%)	8/9 (88.9%)	RR 1.01 (0.77 to 1.33)	11 more per 1,000 (from 204 fewer to 294 more)	⊕◯◯◯ VERY LOW	IMPORTANT
Treatment-Related Adverse Events												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [c]	none	25/88 (28.4%)	29/79 (36.7%)	RR 0.77 (0.50 to 1.20)	83 fewer per 1,000 (from 184 fewer to 74 more)	⊕⊕◯◯ LOW	CRITICAL
Withdrawals due to Adverse Events												

2	RCT [1,2]	serious [a]	not serious	not serious	serious [c]	none	0/57 (0.0%)	3/56 (5.4%)	NA [e]	54 fewer per 1, 000	⊕⊕◯◯ LOW	CRITICAL
Serious Adverse Events												
1	RCT [2]	not serious	not serious	not serious	serious [c]	none	1/57 (1.8%)	3/56 (5.4%)	RR 0.33 (0.03 to 3.05)	36 fewer per 1,000 (from 52 fewer to 110 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	2/31 (6.5%)	0/23 (0.0%)	NA [e]	65 more per 1,000	⊕⊕◯◯ LOW	CRITICAL
Allergic Reaction												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	2/31 (6.5%)	0/23 (0.0%)	NA [e]	65 more per 1,000	⊕⊕◯◯ LOW	CRITICAL

*Improvement of Neurologic Lyme Symptoms were defined as either "patients free of subjective and objective neurologic findings" in the Karlsson study, and as "clinical score of 0= no subjective or objective symptoms" in the Ljøstad study.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Karlson 1994 received a high risk of bias due to the high number of participants excluded from final analysis due to negative serology (16 out of 70 randomized patients) and to an unblinded study design.
b. Surrogate for improvement of neurological disease.
c. 95% CI is wide and crossing the null value.
d. 95% CI is wide and crossing the null value, OIS criteria not meant.
e. One arm has zero events; unable to estimate relative risk.

3rd GENERATION CEPHALOSPORINS vs. IV PENICILLIN

In patients with Lyme neuroborreliosis, should IV 3rd generation cephalosporins be used over IV penicillin G?

P: In patients with Lyme neuroborreliosis

I: IV 3rd generation cephalosporins (ceftriaxone or cefotaxime)

C: IV penicillin G

Bibliography: 1. Pfister, et al. Arch Neurol. 1989 Nov; 46(11): 1190-4; 2. Müllegger, et al. Infection. 1991 Jul-Aug; 19(4): 279-83 (PEDIATRIC).

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	3rd Generation Cephalosporins (ceftriaxone or cefotaxime)	IV Penicillin G	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement of Neurologic Lyme Symptoms (Follow-up at 10 day in Pfister, at 6 months in Müllegger)												
2	RCT [1,2]	serious [a]	not serious	not serious	serious [c]	none	21/23 (91.3%)	19/21 (90.5%)	RR 1.00 (0.86 to 1.16)	0 fewer per 1,000 (from 127 fewer to 145 more)	⊕⊕○○ LOW	CRITICAL
Cerebrospinal Fluid Lymphocytic Pleocytosis (Follow-up at a mean time of 7.2 months for cefotaxime and 8.2 months for penicillin)												
1	RCT [1]	not serious	not serious	serious [b]	very serious [d]	none	1/10 (10.0%)	0/10 (0.0%)	NA [e]	100 more per 1,000	⊕○○○ VERY LOW	IMPORTANT
Elevated Cerebrospinal Fluid Protein Count (Follow-up at a mean time of 7.2 months for cefotaxime and 8.2 months for penicillin)												
1	RCT [1]	not serious	not serious	serious [b]	very serious [d]	none	1/11 (9.1%)	2/10 (20.0%)	RR 0.45 (0.05 to 4.28)	110 fewer per 1,000 (from 190 fewer to 656 more)	⊕○○○ VERY LOW	IMPORTANT
Withdrawals due to Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	very serious [d]	none	0/11 (0.0%)	0/10 (0.0%)	NA [e]	0 per 1,000	⊕○○○ VERY LOW	CRITICAL
Treatment-related Adverse Events												

1	RCT [2]	serious [a]	not serious	not serious	very serious [d]	none	3/12 (25.0%)	0/11 (0.0%)	NA [e]	250 more per 1,000	⊕◯◯◯ VERY LOW	CRITICAL
Allergic Reaction												
1	RCT [2]	serious [a]	not serious	not serious	very serious [d]	none	2/12 (16.7%)	0/11 (0.0%)	NA [e]	167 more per 1,000	⊕◯◯◯ VERY LOW	CRITICAL

*Improvement of Neurologic Lyme Symptoms were defined as either "neurologic symptoms improve or subside" in the Pfister study, and as "complete clinical recovery = lack of neurological signs and symptoms" in the Müllegger study.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Both studies received a high risk of bias due to inadequate or compromised blinding.
b. Surrogate for improvement of neurological disease.
c. 95% CI is wide crossing the null value.
d. Low event rate and small sample size (not meeting the OIS criteria), 95% CI is wide and crossing the null value.
e. One or both arms has zero event; unable to estimate relative risk.

IV CEFTRIAXONE vs. IV CEFOTAXIME

In patients with Lyme neuroborreliosis, should IV ceftriaxone be used over IV cefotaxime?
P: In patients with Lyme neuroborreliosis
I: IV ceftriaxone
C: IV cefotaxime

Bibliography: 1. Pfister, et al. J Infect Dis. 1991 Feb; 163(2): 311-8.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	IV Ceftriaxone	IV Cefotaxime	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement of Neurologic Lyme Symptoms (Follow-up at a mean time of 7.4 months for ceftriaxone and 8.6 months for cefotaxime)												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	8/12 (66.7%)	9/15 (60.0%)	RR 1.11 (0.63 to 1.97)	66 more per 1,000 (from 222 fewer to 582 more)	⊕⊕○○ LOW	CRITICAL
Improvement of Cerebrospinal Fluid Pleocytosis (Follow-up at a mean time of 7.4 months for ceftriaxone and 8.6 months for cefotaxime)												
1	RCT [1]	not serious	not serious	serious [b]	serious [c]	none	12/12 (100.0%)	13/15 (86.7%)	RR 1.14 (0.90 to 1.44)	121 more per 1,000 (from 87 fewer to 381 more)	⊕⊕○○ LOW	IMPORTANT
Treatment-Related Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	very serious [c]	none	1/12 (8.3%)	3/15 (20.0%)	RR 0.42 (0.05 to 3.51)	116 fewer per 1,000 (from 190 fewer to 502 more)	⊕○○○ VERY LOW	CRITICAL
Withdrawals due to Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	very serious [c]	none	1/12 (8.3%)	1/15 (6.7%)	RR 1.25 (0.09 to 17.98)	17 more per 1,000 (from 61 fewer to 1,000 more)	⊕○○○ VERY LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	very serious [c]	none	1/12 (8.3%)	0/15 (0.0%)	NA [d]	83 more per 1,000	⊕○○○ VERY LOW	CRITICAL

Allergic Reaction											
1	RCT [1]	serious [a]	not serious	not serious	very serious [c]	none	0/12 (0.0%)	1/15 (6.7%)	NA [d]	67 fewer per 1,000	⊕○○○ VERY LOW	CRITICAL

*Improvement of Neurologic Lyme Symptoms was defined as "neurologic findings= normal, no complaints" in the Pfister study.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received a high risk of bias for an unblinded study design.

b. Surrogate for improvement of neurological disease.

c. Low event rate and small sample size (not meeting the OIS criteria), 95% CI is wide and crossing the null value.

d. One arm has zero event; unable to estimate relative risk.

IV DOXYCYCLINE vs. IV PENICILLIN

In patients with Lyme neuroborreliosis, should IV doxycycline be used over IV penicillin G?

P: In patients with Lyme neuroborreliosis

I: IV doxycycline

C: IV penicillin G

Bibliography: 1. Kohlhepp, et al. J Neurol. 1989 Dec; 236(8): 464-9.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	IV Doxycycline	IV Penicillin G	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement of Neurologic Lyme Symptoms (at 2 weeks) *												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	33/39 (84.6%)	29/36 (80.6%)	RR 1.05 (0.85 to 1.29)	40 more per 1,000 (from 121 fewer to 234 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Patients Requiring Retreatment due to Relapse (at mean time of 5 months)												
1	RCT [1]	not serious	not serious	not serious	serious [a,]	none	6/39 (15.4%)	4/36 (11.1%)	RR 1.38 (0.42 to 4.51)	42 more per 1,000 (from 64 fewer to 390 more)	⊕⊕⊕◯ MODERATE	CRITICAL

*Improvement of Neurologic Lyme Symptoms was defined as "treatment response" in the Kohlhepp study.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. 95% CI Is wide and crossing the null value.

3 WEEKS OF IV CEFTRIAXONE followed by prolonged ORAL AMOXICILLIN (100 days) vs. 3 WEEKS OF IV CEFTRIAXONE then PLACEBO

In patients with Lyme neuroborreliosis treated with 3 weeks of IV ceftriaxone, should prolonged oral amoxicillin be used rather than not?

P: In patients with Lyme neuroborreliosis

I: 3 weeks of IV ceftriaxone followed with 100 days of oral amoxicillin

C: 3 weeks of IV ceftriaxone

Bibliography: 1. Oksi, et al. Eur J Clin Microbiol Infect Dis. 2007 Aug; 26(8): 571-81.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	IV ceftriaxone followed by prolonged oral amoxicillin	IV Ceftriaxone alone	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement of Neurologic Lyme symptoms (at 12 months) *												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	59/73 (80.8%)	55/72 (76.4%)	RR 1.06 (0.89 to 1.25)	44 more per 1,000 (from 89 fewer to 178 more)	⊕⊕⊕◯ MODERATE	CRITICAL
							Definitive LB: 49/53 (92.5%)	Definitive LB: 47/54 (87.0%)	1.06 (0.93 to 1.21)	54 more per 1,000 (from 60 fewer to 169 more)		
							Possible LB: 10/20 (50.0%)	Possible LB: 8/18 (44.4%)	1.13 (0.57 to 2.21)	56 more per 1,000 (from 262 fewer to 373 more)		
Diarrhea												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	15/73 (20.5%)	4/72 (5.6%)	**RR 3.70 (1.29 to 10.61)**	**150 more per 1,000 (from 16 more to 534 more)**	⊕⊕⊕◯ MODERATE	CRITICAL

*Improvement of Neurologic Lyme Symptoms was defined according to the clinical status measured by VAS scale 1-100 (excellent or good clinical outcome=VAS <30).

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. CI crossing the null value.

b. Fragility due to low event rate.

2 WEEKS OF IV CEFTRIAXONE followed by prolonged ORAL AMOXICILLIN (100 days) vs. prolonged ORAL CEFIXIME (100 days)

In patients with Lyme neuroborreliosis, should extending initial IV Ceftriaxone with oral amoxicillin be used over prolonged oral Cefixime?

P: In patients with Lyme neuroborreliosis

I: 2 weeks of IV Ceftriaxone followed by oral amoxicillin (100 days)

C: prolonged oral Cefixime (100 days)

Bibliography: 1. Oksi, et al. Eur J Clin Microbiol Infect Dis. 1998 Oct; 17(10): 715-9.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	IV ceftriaxone followed by prolonged oral amoxicillin	Prolonged oral cefixime	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
			Certainty assessment				№ of events/№ of patients		Effect			
Improvement of Neurologic Lyme Symptoms (at 12 months) *												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	12/30 (40.0%)	12/30 (40.0%)	RR 1.00 (0.54 to 1.86)	0 fewer per 1,000 (from 184 fewer to 344 more)	⊕⊕○○ LOW	CRITICAL
Clinical Relapse after good clinical response OR no clinical response (at 12 months) *												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	2/30 (6.7%)	6/30 (20.0%)	RR 0.33 (0.07 to 1.52)	134 fewer per 1,000 (from 104 more to 186 fewer)	⊕⊕○○ LOW	CRITICAL
Treatment-Related Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	22/30 (73.3%)	14/30 (46.7%)	**RR 1.57 (1.01 to 2.44)**	**266 more per 1,000 (from 5 more to 672 more)**	⊕⊕○○ LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	4/30 (13.3%)	2/30 (6.7%)	RR 2.00 (0.40 to 10.11)	67 more per 1,000 (from 40 fewer to 607 more)	⊕⊕○○ LOW	CRITICAL
Diarrhea												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	3/30 (10.0%)	2/30 (6.7%)	RR 1.50 (0.27 to 8.34)	33 more per 1,000 (from 49 fewer to 489 more)	⊕⊕○○ LOW	CRITICAL

*Improvement of Neurologic Lyme Symptoms correspond to an "excellent clinical response= asymptomatic", while "good clinical response = markedly milder symptoms, or negligible residual symptoms".

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received a high risk of bias due to inadequate or compromised blinding.

b. 95% CI is wide and crossing the null value.

c. Fragility due to low event rate and small sample size.

XVI. Should patients with Lyme disease and facial nerve palsy receive corticosteroids in addition to antimicrobial therapy?

Bibliography: 1. Clark, et al. Laryngoscope. 1985 Nov; 95(11): 1341-5; 2. Pfister, et al. Annals of the New York Academy of Sciences, 1988, 539(1): 485–487; 3. Hydén, et al. Am J Otolaryngol. 1993 May-Jun; 14(3): 179-86; 4. Jowett, et al. Laryngoscope, 2016 Sep 6; 5. Wormser, et al. Diagn Microbiol Infect Dis. 2018 Aug;91(4):336-338.

Study, Location	Study Design	Risk of Bias*	Population characteristics and Diagnostic method	Treatment(s) administered	% Taking Corticosteroid	Outcome: Resolution or improvement of facial palsy; Improvement of CSF pleocytosis; Prevention of subsequent neurologic events
Clark, et al., 1985 New Haven, CT	Prospective cohort study	4	Of 951 patients with Lyme disease, 101 presented with 124 facial nerve palsies. 23 patients (22.8%) had bilateral facial palsy. The diagnosis of Lyme disease was established on the basis of clinical and physical findings. 37.6% (38 of 101) of patients recalled a tick bite. 84.2% (85 of 101) of patients had documented erythema migrans. In 38 patients (37.6%), facial palsy was associated with other neurologic abnormalities, including radiculoneuritis or meningoencephalitis.	Antibiotics: 19 patients received oral penicillin; 4 patients received IV penicillin; 6 patients received oral tetracycline; 3 patients received oral erythromycin. 24 patients received no treatment.	33 patients (32.7%) received steroids with or without antibiotics.	33 patients who received steroids with or without antibiotics had initially reported 44 facial palsies. 41 of 44 (93.2%) facial palsies completely resolved. The median time to recovery was 24 days. One patient receiving steroids had a severe residual facial palsy and another had a recurrence of facial palsy after steroids were tapered.
Pfister, 1988 Germany	Randomized, double-blind placebo-controlled trial	Unclear Risk of Bias	21 patients with Bannwarth's syndrome who complained of intense radicular pain. 27% (3 of 11) of penicillin/corticosteroids patients and 20% (2 of 10) of penicillin/placebo patients presented with facial palsy. All patients had also presented with radicular pain.	10 days IV penicillin* + 7 days oral methylprednisolone vs. 10 days IV penicillin* + 7 days oral placebo *2 patients who were allergic to penicillin received doxycycline	52%	During the first two nights of treatment, improvement of radicular pain occurred faster with added CS. No patient in either group experienced relapse or functional motor deficits in the 3-18 month follow up. The authors suggest that additional corticosteroids should be taken into consideration only in selected cases in which intense radicular pain does not respond to analgesics and non-steroidal anti-inflammatory drugs since radicular pain improves very quickly by therapy with penicillin alone.

Hydén, 1993 Sweden	Prospective cohort study	6	142 Patients with acute peripheral facial palsy of primarily unknown origin within one week of onset. Only 11 of 16 patients with suspected Borreliosis were diagnosed with Borreliosis due to elevated titers by ELISA.	Patients with confirmed *Borrelia* diagnosis received 10 days of IV Penicillin (patients allergic to Penicillin received Cefuroxime), with or without cortisone.	44% (63/142) of total sample 55% (6/11) of patients classified with "*Borrelia* palsies"	Patients in the *Borrelia* group typically experienced better healing and shorter healing time with corticosteroid treatment, but the effect was not statistically significant. Patients receiving cortisone healed in a mean of 3.3 months, whereas patients not receiving the steroid healed in a mean of 6.4 months.
Jowett, 2016 Boston, MA	Retrospective cohort study	6	51 patients (mean age 39.6, range 6-72) with facial palsy associated with Lyme disease. The diagnostic criteria were facial palsy in addition to erythema migrans with known tick exposure, <u>or</u> facial palsy in addition to laboratory evidence of infection by CSF antibody titer or 2-tier serological testing.	18 patients received monotherapy with antibiotics. 17 patients received combination therapy with antibiotics and steroids. 16 patients received triple therapy with antibiotics, steroids, and antivirals (acyclovir or valacyclovir).	64.7% (33 of 51) patients were receiving corticosteroids in addition to antibiotic therapy.	The mean time of assessment following onset of facial palsy was 15.1 months (range 0.3-84 months). Significantly worse facial outcomes were seen among those who received double and triple therapy with steroids and antibiotics with/without antivirals, as compared to those who received mono-therapy with antibiotics alone. These effects were most pronounced among patients assessed ≥12 months following onset. The authors warned that clinicians should be cautious when differentiating viral or idiopathic facial palsy from facial palsy associated with Lyme disease, since combination therapy regimens can result in worse long-term facial function outcome.
Wormser, et al., 2018	Prospective cohort study	5	14 patients with facial palsy associated with Lyme disease were enrolled in a 12 month observational study. 11 of these participants received corticosteroids (mean age 46.5, range 25-70). Among these 11, seven (63.6%) had right-sided facial palsy, three (27.3%) had left-sided facial palsy and one (9.1%)	In addition to corticosteroids, all 11 participants received a 14–28-day course of doxycycline. Two participants were also treated with an oral beta lactam antibiotic.	100%	The 11 patients had been enrolled within 24 days of onset of the facial palsy (median 14 days, range 2–24 days). Corticosteroids had been prescribed prior to initiation of antibiotic therapy in 2 (18.2%) patients, coincident with the initiation of antibiotic therapy in 5 (45.5%) patients, and following the

| | | had bilateral facial palsy. None of the patients had been hospitalized.

100% of participants were seropositive for antibodies to *B. burgdorferi* by 2-tier testing. | | | initiation of antibiotic therapy in 4 (36.4%) patients.

Overall, 6 (54.5%, 95% C.I.: 28.0% to 78.7%) of the 11 had evidence of facial nerve dysfunction at the time of the last evaluation, which occurred at a mean of 13.1 months after the baseline visit (range 9.6 months to 19.6 months).

The authors conclude that though corticosteroids are commonly prescribed for facial palsy associated with Lyme disease, the use of corticosteroids is often associated with sequalae in these patients. |

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk". Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Lyme carditis

XVIII. Should all patients with early Lyme disease receive an electrocardiogram to screen for Lyme carditis?

Bibliography: 1. Woolf, et al. Pediatr Emerg Care. 1991 Dec;7(6):334-6; 2. Rubin, et al. Pacing Clin Electrophysiol. 1992 Mar;15(3):252-5; 3. Völzke, et al. Heart. 2005 Feb;91(2):235-6; 4. Mravljak, et al. Wien Klin Wochenschr. 2006 Nov;118(21-22):691-5; 5. Costello, et al. Pediatrics. 2009 May;123(5):e835-41; 6. Welsh, et al. J Pediatric Infect Dis Soc. 2012 Dec;1(4):293-8.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Early Lyme disease characteristics	Cardiac presentation description (if applicable)	% Lyme Patients with ECG abnormalities (N cases/N Lyme cases)	Study conclusions
Woolf, et al.,1991

Valhalla, NY | Prospective cohort study | 6 | 32 randomly selected pediatric (mean age 7.7 years; range 1-17) patients were classified as having "definite" Lyme Disease (N=14), "probable" Lyme Disease (N=10), "possible" Lyme Disease (N=6), or "unlikely" Lyme Disease (N=2), based on historical, physical examination, and positive Lyme serology.

Clinical signs and symptoms were weighted according to importance in making the diagnosis (e.g., objective symptoms were weighted more heavily).

All patients were treated with antibiotics for Lyme disease. | An electrocardiogram (ECG)was performed in 32 children with initial diagnosis of suspected Lyme Disease. No symptoms or signs of cardiac involvement were recorded in any patient at initial presentation.

Patients with "definite" Lyme Disease presented with atrioventricular (AV) block (N=2), left axis deviation (N=1), and ventricular ectopy (N=1); patients with "probable" Lyme presented with ST-T wave abnormalities (N=1), prominent sinus arrhythmia, sinus bradycardia, and wandering atrial pacemaker (N=1), and ectopic atrial bradycardia (N=1). No instances of complete heart block were detected. No patient required cardiac therapy. | 4 of 14 (28.6%) patients with "definite" Lyme, 3 of 10 (30%) patients with "probable" Lyme, and none of the patients reporting "possible" or "unlikely" Lyme presented with abnormal ECG findings.

The overall incidence of abnormal ECG findings was 21.9% (7 of 32) among patients with suspected Lyme Disease. | Many children with Lyme disease without cardiac symptoms may demonstrate ECG abnormalities.

The authors note that atrioventricular block is the most frequently reported ECG finding in Lyme Disease patients. They suggest that in cases of suspected Lyme disease, ECG is a useful tool for testing the possibility of cardiac involvement. |
| Rubin, et al., 1992

Valhalla, New York | Prospective cohort study | 6 | 61 patients (mean age 48 years old) were diagnosed with early Lyme Disease based on the presence of erythema migrans and residence in a county endemic for Lyme disease. Mean duration of | An electrocardiogram was performed in all patients at the time of presentation.

One patient presented with atrioventricular (AV) block at presentation. He was lightheaded, had a | 1.6% (1 of 61) of early Lyme Disease patients presented with an ECG abnormality. | None of the 54 treated patients without heart block at the initial presentation developed clinically overt carditis. The one patient who presented with AV |

			erythema migrans: 6.2 days; mean duration of symptoms: 11.1 days. Antibiotic treatment with a 20 day course of either oral doxycycline (300 mg/day) or oral cefuroxime (1000 mg/day) was initiated at presentation. One patient with high degree heart block was hospitalized and received IV ceftriaxone.	pulse rate of 40, and had a complete heart block with a wide left bundle branch block pattern QRS escape rhythm on ECG, which was later shown to be at the level of the AV node. The patient did not respond to IV atropine, but all abnormalities resolved within 2 weeks of IV ceftriaxone treatment. *Patients with known prior heart disease, preexisting abnormal ECG, or current antibiotic treatment were excluded from this study.*		block resolved with IV antibiotic treatment. The authors conclude that heart block complicating early Lyme Disease is rare and reversible. They were unable to determine if early oral antibiotic treatment played a preventative role in further cardiac abnormalities.
Völzke , et al., 2005 Northeast Germany	Cross-sectional study	7	A random sample of 4,310 subjects aged 20–79 years was drawn from the population. Patients' serum was assessed for anti-*Borrelia* IgG antibodies by ELISA. Patients were classified as having absence of *Borrelia* IgG, presence of elevated anti-*Borrelia* IgG, or positive anti-*Borrelia* IgG. 4,272 individuals provided serology data. 3.1% (131 of 4,272) of participants tested positive.	ECG data of 3,690 individuals were available. Echocardiography was performed in participants > 45 years. P, PR, and QRS duration and data on definite left bundle branch block (LBBB), right bundle branch block (RBBB), incomplete RBBB, left anterior fascicular block (LAFB), and left posterior fascicular block (LPFB) were analyzed.	The authors found no significant differences between seropositive and seronegative individuals with regard to ECG characteristics. Anti-*Borrelia* IgG was not associated with conduction abnormalities.	There was no significant difference between seronegative patients and those with low, elevated, or positive antibody titer with respect to left ventricular parameters and cardiac valve disorders. The authors concluded that there was no association between seropositivity for anti-*Borrelia* IgG and ECG changes or risk of cardiac disorder.
Mravljak, et al., 2006 Ljubljana, Slovenia	Case control study	7	<u>Lyme Disease cases</u>: 147 children (≤15 years old) were diagnosed with typical erythema migrans (EM) and had positive serum antibody titer for *B. burgdorferi* by IFA. 53.7% of patients had multiple EM, and 21.8% of patients had associated systemic symptoms. Median duration of EM: 4 and 5 days in solitary EM and multiple EM, respectively.	ECG was performed at the initial examination and prior to the start of therapy Early Lyme disease patients presented with 1st degree atrioventricular (AV) block (N=1), right heart axis (N=1), left heart axis (N=4), pathologic Q wave in V$_1$ (N=1) and prolonged QTc interval (N=1). None of the patients exhibited clinical signs or symptoms of heart involvement.	The overall incidence of ECG abnormality in early Lyme patients was 5% (8 of 147). Only one patient (0.07%) presented with transitory atrioventricular block indicative of Lyme carditis, which resolved within two weeks of starting antibiotic treatment.	In early Lyme disease patients, there was no difference between patients with normal ECG versus abnormal ECG with regard to bloodstream invasion of *B.burgdorferi* (p=0.2594) or presentation with associated systemic symptoms (p=0.6853). ECG abnormalities were more frequently found in healthy

			Controls: 148 age- and sex-matched healthy children	Control patients presented with 1st degree AV block (N=1), Mobitz II type 2nd degree AV block (N=1), right bundle-branch block (N=3), right heart axis (N=1), right bundle-branch block with right atrial hypertrophy (N=1), left heart axis (N=6), left ventricular hypertrophy (N=1), left heart axis and left anterior hemi-block (N=1), Lown-Ganong-Lewin syndrome (N=2), prolonged QTc (N=2), and wide QRS (N=1).	21% (31 of 148) of Control patients had ECG abnormalities.	children than in children with early Lyme disease (p=0.0303). The authors concluded that ECG abnormalities in children with early Lyme Disease are mild, nonspecific, and rare.
Costello, et al., 2009 Boston, MA	Case control study	7	207 pediatric (<21 years old) patients with early disseminated Lyme disease (multiple erythema migrans, meningitis, cranial neuritis, radiculoneuritis, ocular involvement, and/or carditis) with laboratory confirmation of *B. burgdorferi* infection. 98% of non-carditis patients and 100% of carditis patients had positive serology. Patients with carditis had a wide range of systemic involvement, including flu-like symptoms in 94%, meningitis in 48%, multiple erythema migrans in 48%, and erythema migrans in 24%.	ECG findings in patients with Lyme carditis were as follows: Normal (N=1),1st degree atrioventricular (AV) block (N=12),1st degree AV block+ ST-T changes (N=1), 1st degree AV block +prolonged corrected QT interval (N=1), 2nd degree AV block (N=7), complete heart block (N=5), prolonged corrected QT interval (N=4), and ST-T wave changes (N=2).	16% (33 of 207) of patients with early disseminated Lyme disease had carditis. 42% (14 of 33) of patients with Lyme carditis had advanced heart block, and 27% (9 of 33) of these patients had complete heart block.	Patients with Lyme carditis were significantly more likely to be >10 years old (p<0.001) and to present with arthralgia (p=0.02) or cardiopulmonary symptoms (p<0.001). The absence of any cardiopulmonary symptom had a 99% specificity for identifying those patients without carditis. All 9 patients with complete heart block and 3 of 5 patients with 2nd degree heart block received 21 to 28 days of IV ceftriaxone. 2 of 5 patients with 2nd degree heart block received ceftriaxone while hospitalized then received oral antibiotics for 21 to 28 days. Of 27 patients for whom follow-up data was available, 24 (88.9%) had complete resolution of their cardiac symptoms.

| Welsh 2012

Boston, MA; Philadelphia, PA; Wilmington, DE | Cross-sectional Study | 8 | 103 pediatric (median age 10.8 years; IQR 7.6-13.2) patients who were diagnosed with Lyme meningitis (CSF leukocyte count ≥10 cells/mm^3 in conjunction with positive acute Lyme serologic tests by Western blotting <u>or</u> physician-documented erythema migrans).

10 patients (10%) had physician-documented erythema migrans, 41 (40%) had both erythema migrans and positive serology, and 52 (50%) had positive Lyme serology without erythema migrans. Median duration of symptoms: 14 days (IQR: 5-21 days). | All patients had an ECG performed at presentation.

16 children had 1st degree AV block, 11 had prolonged QT interval, and 14 had ST-wave change. 7 children had multiple ECG abnormalities without evidence of depressed myocardial function: 4 had 1st degree AV block with ST-T wave changes; 2 had prolonged QTc interval with ST-T wave changes; and 1 with 1st degree AV block with prolonged QTc

ECG abnormalities resolved or improved by hospital discharge in 20 (95%) of the 21 children for whom follow-up ECGs were available. | 33% (25 of 70) of patients with Lyme meningitis had ECG abnormalities which were consistent with Lyme carditis. 7 children had multiple ECG abnormalities.

When the definition of carditis was restricted to those with atrioventricular (AV) block or prolonged QTc, 26 (25%) children met criteria for having carditis. | Children ≥13 years old were significantly more likely to experience ECG abnormalities than children <13 (p=0.01). Lyme meningitis patients with ECG abnormalities were significantly more likely to have experienced a fever for ≥5 days (p<0.01). Age and fever duration remained significantly associated with likelihood of carditis, even when the definition was restricted to AV block or prolonged QTc.

The authors concluded that ECG abnormalities were common in children with Lyme meningitis, suggesting a higher risk of cardiac involvement in patients with Lyme meningitis. The authors also pointed out specific demographic and clinical risk factors which may aid in the decision of whether or not to perform an ECG. |
|---|---|---|---|---|---|---|

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XIX. Which patients with Lyme carditis require hospitalization?

Bibliography: 1. Costello, et al. Pediatrics. 2009 May; 123(5): e835-41; 2. Steere, et al. Ann Intern Med. 1980 Jul; 93(1): 8-16; 3. Van der Linde, et al. Scand J Infect Dis Suppl. 1991; 77: 81-4; 4. Forrester, et al. Clin Infect Dis. 2014 Oct; 59 (7):996-1000; 5. Scheffold, et al. Dtsch Arztebl Int. 2015 Mar; 112(12): 202–8; 6. Meuhlenbachs, et al. Am J Pathol. 2016 May; 186(5):1195-205.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Lyme disease characteristics	Cardiac presentation description (if applicable)	% Lyme Carditis Patients Hospitalized, or Description of Fatal Lyme Carditis Event(s)	Study conclusions
Costello, et al., 2009 Boston, MA	Case control study	7	207 pediatric (<21 years old) patients with early disseminated Lyme disease (multiple erythema migrans, meningitis, cranial neuritis, radiculoneuritis, ocular involvement, and/or carditis with laboratory confirmation of *B. burgdorferi* infection. 98% of non-carditis patients and 100% of carditis patients had positive serology. Patients with carditis (33 of 207) had a wide range of systemic involvement, including flu-like symptoms in 94%, meningitis in 48%, multiple erythema migrans in 48%, and erythema migrans in 24%.	16% (33 of 207) of patients with early disseminated Lyme disease had carditis. 42% (14 of 33) of patients with Lyme carditis had advanced heart block, and 27% (9 of 33) of these patients had complete heart block. 12% (4 of 33) patients with carditis had depressed ventricular systolic function on echocardiogram, 9% (3 of 33) presented in cardiogenic shock. Two of these 3 children with severe Lyme carditis with complete heart block and severely depressed biventricular function were treated with a temporary dual-chamber pacing, of which one required cardiopulmonary resuscitation and extracorporeal membrane oxygenation (ECMO) for 5 days before complete recovery.	85% of Lyme carditis patients and 65% of Lyme patients without carditis were hospitalized (p=0.02). 32% of Lyme carditis patients and 0% of Lyme patients without carditis were admitted to intensive care (ICU) (p<0.001). 9 patients with carditis were admitted to the cardiac ICU for a median of 3 days (range 1-11). Patients with carditis were admitted to the hospital for a median of 4 days (range 1-13) compared to Lyme patients without carditis [3 days (range 1-20)] (p=0.03). There was no mortality in children with early disseminated Lyme disease.	Of 27 patients for whom follow-up data was available, 24 (88.9%) had complete resolution of their cardiac symptoms. Compared to Lyme patients without carditis, patients with carditis were more likely to be hospitalized, to remain in hospital longer, and to require intensive care. The authors recommended that children with Lyme carditis and myocardial dysfunction be hospitalized in an institution capable of providing pacemakers and rapid deployment ECMO for mechanical cardiac support.
Steere, 1980	Case series (20 cases)	NA	19 of 20 patients (age range: 6-58 years) were diagnosed with Lyme disease by erythema migrans. 14 had elevated serum IgM levels.	Eighteen of the 20 patients had AV block; 10 of them with high degree AV block developed symptoms of cardiac involvement (8 of these patients had a complete block). **Some**	Patients with first-degree block usually treated as outpatients.	Patients with high-degree AV block or first–degree block with PR interval longer than 0.30 seconds should be hospitalized because of risk to

New Haven, CT			At the time of presentation of cardiac symptoms, 15 patients had skin lesions, 10 were febrile, 7 patients had neurologic manifestations, and 13 patients had joint involvement.	**patients went from first-degree to complete block and back within minutes.** Duration of cardiac involvement typically ranged from 3 days to 6 weeks.		develop complete heart block. Patents with slightly prolonged PR intervals should restrict their activity, and should be followed as outpatients.
Van der Linde, et al., 1991 The Netherlands	Case series (105 cases; includes McAlister 1989)	NA	105 patients (39 North American and 66 European, mean age 39 years) with Lyme carditis. Diagnostic procedure for Lyme is not described in detail. Endomyocardial biopsies were obtained in 8 patients, in 5 of whom spirochetes were found.	77% (81 of 105) had AV block; 49% (52 of 105) had 3rd degree block; 12% (12 of 105) had 1st degree block.	6 of 105 patients had an incomplete recovery. In 41 patients, minor conduction disturbances remained. 1 of 105 patients died.	94% of all Lyme carditis patients had complete recovery, despite diverse treatment regimens. 10% of patients had spontaneous complete recovery. Follow-up ranged from 1 to 4 years. No relapse was reported.
Forrester, et al., 2014 Atlanta, Georgia	Case series (45 cases)	NA	45 patients (median age 32) with clinician-documented erythema migrans (EM) or laboratory confirmation of *B. burgdorferi* infection. 44% of patients presented with EM. Other associated presenting symptoms included syncope, fever, lightheadedness/dizziness, dyspnea, lethargy/weakness, palpitations, chest pain, headache, myalgia, and arthralgia.	Median age is 32 years. 84% males. 80% of patients had ECG documented 3rd-degree block when first evaluated. Of patients who developed third-degree heart block, median time from presentation to development was 3 hours (range 0.5-24 hours). 84% of patients who had 3rd degree heart block were male.	71% of patients presented with third-degree heart block from Lyme carditis in hospital, and 24% in outpatient clinic. Deaths due to Lyme carditis not included in this review.	Third-degree AV block can result in fatal arrhythmias if not managed and treated properly. With appropriate antibiotic therapy, AV block from Lyme carditis improves (third-degree blocks resolve within 1 week and lesser disturbances taking up to 6 weeks.)

Scheffold et al., 2015	Review of 9 Case reports	NA	Age range is 26-67 years. 7 males and 2 females. 4 of 9 cases present with positive *Borrelia* serology. 4 of 9 cases present with positive PCR. Identification of spirochetes in 6 of 9 cases (4 in the myocardium, 1 in skeletal muscle, and 1 in the thalamus).	Primary arrhythmogenic event (higher degree AV block) is immediate cause of death in these cases. 2 of 9 fatal cases were due to non-cardiac complications post Lyme disease.	Lyme disease mortality identified 9 documented deaths worldwide. 7 of 9 cases were sudden cardiac death as a result of acute lymphocytic myocarditis	Continuous ECG monitoring is required in patients who have experienced a syncope or present with a PR interval >300ms.
Muehlenbachs et al., 2016 (included 3 cases from Scheffold 2015) Atlanta, Georgia	Case series (5 cases)	NA	Autopsy samples evaluated by light microscopy, Warthin-Starry stain, immunohistochemistry, and PCR for *B. burgdorferi*, post mortem blood test by serology. 4 of 5 patients were seropositive by IgM but not IgG WB criteria. 2 of 5 cases presented with spirochetes in the leptomeninges. Spirochetes were not observed in other organs. 4 of 5 patients recalled past tick exposure.	5 cases of sudden cardiac deaths were associated with Lyme carditis. All cases had similar clinical and pathological presentations suggesting a disease mechanism of spirochete cardiac tropism during early disease dissemination, infiltration of cardiac tissue by inflammatory cells, and involvement of conduction system (which could mediate sudden death).	4 of 5 patients who died were male, at a median age of 28 years.	Underlying heart disease might be an additional risk factor for Lyme carditis. Healthcare professionals should evaluate all patients with suspected Lyme disease for cardiac signs and symptoms and obtain electrocardiogram promptly if carditis is suspected.

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XX. What pacing modality should be used for the management of Lyme carditis?

Bibliography: 1. Steere, et al. Ann Intern Med. 1980 Jul; 93(1): 8-16; 2.McAlister, et al. Ann Intern Med. 1989 Mar 1; 110(5): 339-45; 3. Van der Linde, et al. Scand J Infect Dis Suppl. 1991; 77: 81-4; 4. Costello, et al. Pediatrics. 2009 May; 123(5): e835-41; 5. Forrester, et al. Clin Infect Dis. 2014 Oct; 59 (7):996-1000.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Lyme disease characteristics	Cardiac presentation description (if applicable)	% Patients who received a pacemaker, % Temporary	Treatment course and Study conclusions
Steere, 1980 New Haven, CT	Case series (20 cases)	NA	19 of 20 patients (age range: 6-58 years) were diagnosed with Lyme disease by erythema migrans. 14 had elevated serum IgM levels. At the time of presentation of cardiac symptoms, 15 patients had skin lesions, 10 were febrile, 7 patients had neurologic manifestations, and 13 patients had joint involvement.	Eighteen of the 20 patients had AV block; 10 of them with high degree AV block developed symptoms of cardiac involvement (8 of these patients had a complete block). Some patients went from first-degree to complete block and back within minutes. Duration of cardiac involvement typically ranged from 3 days to 6 weeks.	Six of the 8 patients with complete heart block had temporary transvenous pacemakers inserted.	20 of 20 patients had improvement in degree of block within 24-48 hours. 19 of 20 patients had complete resolution of symptoms within 1-2 weeks. In all 6 patients who received pacemakers, the pacemakers were removed within 1 week. In patients with complete heart block, the authors suggested pacemaker and aspirin as the treatments of choice.
McAlister, 1989 New York City, NY	Case series (4 cases)	NA	4 serologically confirmed cases of Lyme carditis (all male, mean age 45). All patients lived in endemic regions. Patients were serologically confirmed for Lyme disease by ELISA. Only 1 patient recalled a tick bite and recalled a rash resembling erythema migrans. Another patient had noticed large welts on his back and had experienced some convulsive episodes in addition to cardiac symptoms. The other two patients noted no typical symptoms of Lyme, and reported headache, dyspnea, and lightheadedness.	100% of cases had severe symptomatic atrioventricular block, 75% (3 of 4) had episodes of prolonged ventricular asystole. 87% (45 of 52) of cases reported had documented atrioventricular block, 53% (28 of 52) were complete or high-grade atrioventricular block and most were symptomatic.	50% (2 of 4) had permanent pacemakers implanted (one later removed).	Only 1 of 4 patients did not completely resolve during the follow up period (persistent 2nd degree atrioventricular block 16 months post treatment). One patient experienced an adverse event due to use of a pacemaker (bacterial infection). The authors recommend hospitalization or continuous monitoring for patients with second degree or complete atrioventricular block or first-degree block if PR interval exceeds 0.30 seconds. Patients with high-grade or complete atrioventricular block may require temporary cardiac pacing.

Van der Linde, et al., 1991 The Netherlands	Case series (105 cases; includes McAlister 1989)	NA	105 patients (39 North American and 66 European, mean age 39 years) with Lyme carditis. Diagnostic procedure for Lyme is not described in detail. Endomyocardial biopsies were obtained in 8 patients, in 5 of whom spirochetes were found.	77% (81 of 105) had AV block; 49% (52 of 105) had 3rd degree block; 12% (12 of 105) had 1st degree block.	Thirteen European patients and 16 North American patients with AV block due to Lyme carditis had a temporary pacemaker inserted. In 5 patients with AV block, a pacemaker implantation was performed.	94% of all Lyme carditis patients had complete recovery, despite diverse treatment regimens. 10% of patients had spontaneous complete recovery. Follow-up ranged from 1 to 4 years. In 4 of 5 patients implanted with permanent pacemaker, conduction disturbances resolved completely, and in 2 patients, recovery led to removal of a pacemaker. Overall, 80% of patients who underwent pacemaker implantation were later pacemaker independent. Temporary pacemakers are often inserted, but the authors noted that permanent pacemakers are rarely indicated.
Costello, et al., 2009 Boston, MA	Case control study	7	207 pediatric (<21 years old) patients with early disseminated Lyme disease (multiple erythema migrans, meningitis, cranial neuritis, radiculoneuritis, ocular involvement, and/or carditis with laboratory confirmation of *B. burgdorferi* infection. 98% of non-carditis patients and 100% of carditis patients had positive serology. Patients with carditis (33 of 207) had a wide range of systemic involvement, including flu-like symptoms in 94%, meningitis in 48%, multiple erythema migrans in 48%, and erythema migrans in 24%.	16% (33 of 207) of patients with early disseminated Lyme disease had carditis. 42% (14 of 33) of patients with Lyme carditis had advanced heart block, and 27% (9 of 33) of these patients had complete heart block. 12% (4 of 33) patients with carditis had depressed ventricular systolic function on echocardiogram, 9% (3 of 33) presented in cardiogenic shock. Two of these 3 children with severe Lyme carditis with complete heart block and severely depressed biventricular function were treated with a temporary dual-chamber pacing, of which one required cardiopulmonary resuscitation and extracorporeal membrane	Temporary transvenous pacing was used for 1-7 days to treat complete heart block in 4 (12%) children. None of the patients with advanced heart block required a permanent pacemaker.	Of 27 patients for whom follow-up data was available, 24 (88.9%) had complete resolution of their cardiac symptoms. The authors recommended that children with Lyme carditis and myocardial dysfunction be hospitalized in an institution capable of providing pacemakers and rapid deployment ECMO for mechanical cardiac support. They commented on the fact that permanent pacemakers are rarely necessary for patients with Lyme carditis.

				oxygenation (ECMO) for 5 days before complete recovery.		
Forrester, et al., 2014

Atlanta, Georgia | Case series (45 cases) | NA | 45 patients (median age 32) with clinician-documented erythema migrans (EM) or laboratory confirmation of *B. burgdorferi* infection.

44% of patients presented with EM. Other associated presenting symptoms included syncope, fever, lightheadedness/dizziness, dyspnea, lethargy/weakness, palpitations, chest pain, headache, myalgia, and arthralgia. | 80% of patients had ECG documented 3rd-degree block when first evaluated. Of patients who developed third-degree heart block, median time from presentation to development was 3 hours (range 0.5-24 hours). 84% of patients who had 3rd degree heart block were male. | 40% (18 of 45) of patients required supportive transvenous pacing. 2 of 45 (4%) patients had permanent pacemaker placement. | The authors concluded that temporary transvenous pacing should be the modality of choice in Lyme carditis patients after appropriate antibiotic therapy has been administered. They noted that permanent pacing should be considered if symptoms do not resolve. |

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXI. What are the preferred antibiotics regimens for the treatment of Lyme carditis?

In patients with Lyme carditis, should oral doxycycline be used over IV antibiotics (ceftriaxone)?

P: In patients with Lyme carditis

I: Oral doxycycline

C: IV antibiotics (ceftriaxone)

Bibliography: 1. Dattwyler, et al. N Engl J Med. 1997 Jul 31; 337(5): 289-94

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Oral doxycycline	IV antibiotics (ceftriaxone)	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
			Certainty assessment				№ of patients		Effect			
Clinical cure (at last follow-up) *												
1	RTC [1]	not serious	not serious	serious [b]	serious [c]	none	63/72 (87.5%)	58/68 (85.3%)	RR 1.03 (0.90 to 1.17)	22 more per 1,000 (from 86 fewer to 145 more)	⊕⊕◯◯ LOW	CRITICAL
Treatment failure (at last follow-up) *												
1	RCT [1]	not serious	not serious	serious [b]	serious [c]	none	1/72 (1.4%)	1/68 (1.5%)	RR 0.94 (0.06 to 14.80)	1 fewer per 1,000 (from 14 fewer to 203 more)	⊕⊕◯◯ LOW	CRITICAL
Resolution of Erythema migrans (at 90 days)												
1	RCT [1]	not serious	not serious	serious [b]	serious [c]	none	63/67 (94.0%)	55/60 (91.7%)	RR 1.03 (0.93 to 1.13)	24 more per 1,000 (from 64 fewer to 120 more)	⊕⊕◯◯ LOW	CRITICAL
Withdrawals due to Adverse Events												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [c]	none	0/72 (0.0%)	4/68 (5.9%)	NA [d]	59 fewer per 1,000	⊕◯◯◯ VERY LOW	CRITICAL
Treatment-Related Adverse Events												

1	RCT [1]	serious [a]	not serious	serious [b]	serious [c]	none	31/72 (43.1%)	39/68 (57.4%)	RR 0.75 (0.54 to 1.05)	143 fewer per 1,000 (from 29 more to 266 fewer)	⊕◯◯◯ VERY LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [c]	none	18/72 (25.0%)	28/68 (41.2%)	**RR 0.61 (0.37 to 0.99)**	**162 fewer per 1,000 (from 3 fewer to 259 fewer)**	⊕◯◯◯ VERY LOW	CRITICAL
Diarrhea												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [c]	none	4/72 (5.6%)	25/68 (36.8%)	**RR 0.15 (0.06 to 0.41)**	**312 fewer per 1,000 (from 216 fewer 347 fewer)**	⊕◯◯◯ VERY LOW	CRITICAL
Allergic Reaction												
1	RCT [1]	serious [a]	not serious	serious [b]	serious [c]	none	9/72 (12.5%)	4/68 (5.9%)	RR 2.13 (0.69 to 6.58)	66 more per 1,000 (from 18 fewer to 328 more)	⊕◯◯◯ VERY LOW	CRITICAL

*Clinical response as a clinical cure (indicated by the resolution of objective clinical findings of Lyme disease), treatment failure (indicated by objective signs compatible with clinically active Lyme disease including arthritis or neurologic disease) or not assessable because of improper dose or length of treatment, concomitant antimicrobial therapy, failure to meet the entry criteria, withdrawal from the study because of severe adverse events or death.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Due to lack of blinding

b. Downgraded since only 6.4% of the cohort (9 out of 140 patients) presented with carditis (10% of patients taking ceftriaxone (7 of 68) and 3% of patients taking doxycycline (2 of 72)).

c. 95% CI is wide or crossing the null value

d. One arm has zero event; unable to estimate relative risk.

Transition from IV to oral antibiotics

Bibliography: 1. Oksi, et al. Eur J Clin Microbiol Infect Dis. 2007 Aug; 26(8): 571-81; 2. Costello, et al. Pediatrics. 2009 May; 123(5):e835-41.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Lyme disease characteristics	Treatment Received	Cardiac presentation description (if applicable)	Treatment course and Study conclusions
Oksi, 2007 Turku, Finland	Randomized Controlled trial	Low risk of bias	145 patients with possible (defined as uncommon manifestations, serum antibodies against *B. burgdorferi*, and exclusion of other causes) or definite (a classical objective manifestation of disseminated Lyme, inflammatory changes in the CSF or *Borrelia* DNA in the synovial fluid, and/or intrathecal antibodies against *B. burgdorferi*, along with exclusion of other causes) disseminated Lyme Disease. Mean age Ceftriaxone/amoxicillin: 52.3 years; 52% female; 72.6% definite diagnosis. Mean age Ceftriaxone/placebo: 50.5 years; 50% female; 75.4% definite diagnosis.	2g **IV** Ceftriaxone/day for 21 days, <u>followed by</u> 1 g **Ora**l Amoxicillin BID for 100 days (n=73) versus 2g IV Ceftriaxone/day for 21 days, followed by Oral Placebo BID for 100 days (n=72)	8 of 145 (5.5%) of patients presented with Lyme carditis at baseline. One patient had pericarditis, 4 presented with frequent ventricular extrasystoliae or supraventricular extrasystoliae, 3 presented with dissociation atrioventricular block grade I.	In patients with definite LB, the outcome was excellent or good in 49 (92.5%) amoxicillin treated patients and in 47 (87.0%) placebo treated patients; outcome was poor in 3 (5.7%) AMOX patients and in 6 (11.1%) PBO patients (p=0.49). No serious adverse effects of antibiotic treatment occurred in any of the 145 patients. Diarrhea was reported in 33 (22.8%) patients during IV ceftriaxone treatment and in 19 (13.1%) patients during oral treatment (15 in the amoxicillin group and 4 in the placebo group, p=0.012). No severe allergic reactions were observed. The authors concluded that there was no significant benefit to oral adjunct therapy in patients with disseminated Lyme disease who have received IV ceftriaxone.

| Costello, 2009

Boston, MA | Case control study | 7 | 207 pediatric (<21 years old) patients with early disseminated Lyme disease (multiple erythema migrans, meningitis, cranial neuritis, radiculoneuritis, ocular involvement, and/or carditis) with laboratory confirmation of *B. burgdorferi* infection.

16% (33 of 207) of patients with early disseminated Lyme disease had carditis. 98% of non-carditis patients and 100% of carditis patients had positive serology.

Patients with carditis had a wide range of systemic involvement, including flu-like symptoms in 94%, meningitis in 48%, multiple erythema migrans in 48%, and erythema migrans in 24%. | All 9 patients with complete heart block and 3 of 5 patients with 2nd degree heart block received 21 to 28 days of IV ceftriaxone. **2 of 5 patients with 2nd degree heart block received ceftriaxone while hospitalized then received oral antibiotics for 21 to 28 days.** | 42% (14 of 33) of patients with Lyme carditis had advanced heart block, and 27% (9 of 33) of these patients had complete heart block.

ECG findings in patients with Lyme carditis were as follows: Normal (N=1),1st degree atrioventricular (AV) block (N=12),1st degree AV block+ ST-T changes (N=1), 1st degree AV block +prolonged corrected QT interval (N=1), 2nd degree AV block (N=7), complete heart block (N=5), prolonged corrected QT interval (N=4), and ST-T wave changes (N=2). | Patients with Lyme carditis were significantly more likely to be >10 years old (p<0.001) and to present with arthralgia (p=0.02) or cardiopulmonary symptoms (p<0.001).

Of 27 patients for whom follow-up data was available, 24 (88.9%) had complete resolution of their cardiac symptoms. |

* Risk of Bias of Randomized Controlled Trial Data was assessed using the Cochrane Risk of Bias Tool and assigned an overall rating of "High risk" "Unclear Risk" or "Low Risk". Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXII. Should patients being evaluated for acute myocarditis/pericarditis or chronic cardiomyopathy of unknown cause be tested for Lyme disease?

Bibliography: 1. Stanek, et al. Scand J Infect Dis Suppl. 1991;77:85-7 ; 2. Rees, et al. Br Heart J. 1994 May;71(5):459-61; 3. Sonnesyn, et al. Am J Cardiol. 1995 Jul 1;76(1):97-100; 4. Gasser, et al. Cardiovasc Drugs Ther. 1996 Jul;10(3):351-60; 5. Kubánek, et al. Eur J Heart Fail. 2012 Jun;14(6):588-96 ; 6. Kuchynka, et al. Herz. 2015 Sep;40(6):892-7; 7. N'Guyen, et al. Infect Dis (Lond). 2016 Oct;48(10):763-4.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method	Cardiac presentation description (if applicable) and diagnostic criteria; baseline characteristics	% Cardiac Patients with Lyme disease (N Lyme cases/N cardiac cases)	Study conclusions
Stanek, et al., 1991 Vienna, Austria	Cross-sectional study	6	Patients were serologically tested by ELISA. Endomyocardial biopsy was performed for histological analysis and culture for *B. burgdorferi* was performed as well.	72 patients with chronic heart failure due to dilated cardiomyopathy (median age 51; range 18-78), 55 patients with coronary heart disease (median age 65; range 41-78), and 61 (median age 49; range 40-63) healthy blood donors were assessed.	26.4% (19 of 72) of patients with chronic heart failure due to dilated cardiomyopathy were positive by ELISA. In 2 patients with positive ELISA values, endomyocardial biopsy was performed and was culture positive. 12.7% (7 of 55) of patients with coronary heart disease and 8.2% (5 of 61) healthy blood donor samples were positive for antibodies for *B. burgdorferi* by ELISA.	The authors concluded that there was evidence of an association between Lyme Disease and chronic heart disease. They suggested that Lyme Disease be considered as a differential diagnosis and etiology of dilated cardiomyopathy.
Rees, et al., 1994 UK	Prospective cohort study	7	The patients' notes were reviewed for clinical evidence of Lyme disease. None of the patients were at occupational risk of tick bite exposure. None of the patients had either a documented rash consistent with erythema migrans or a previous illness compatible with Lyme disease.	97 patients with idiopathic dilated cardiomyopathy were observed (mean age 43 years; range 12-74). The mean duration of symptoms was 34 months. 30 (31%) patients had described an acute viral illness at disease onset, and 13 (14%) had either clinical (N= 7) or histological (N= 6) evidence of myocarditis.	Cases: 8.2% (8 of 97) of patients with dilated cardiomyopathy had raised antibodies to *B burgdorferi*. Controls: 5.3% (2 of 38) of geographically matched controls and 2.6% (1 of 39) of environmentally matched	There were no significant differences between patients with cardiomyopathy and either control group with regard to serum antibody titer (p=0.40). There were no significant differences between patients with cardiomyopathy and either control group with regard to serum antibody titer (p=0.40). Subsequent immunoblot analysis did

			Patients' serum was tested for antibodies to *B. burgdorferi* by ELISA. Serum samples from healthy blood donors from a non-susceptible population were obtained to determine the normal range for IgG antibodies.	Two matched control groups: Group 1 included age-, sex-, and geographically-matched patients who visited the same general practitioner. Group 2 comprised members of case patients' family (environmentally matched).	controls had raised antibodies to *B burgdorferi*.	not show antibody binding patterns consistent with B. burgdorferi infection in any of the samples. The authors stated that there was not sufficient evidence to link infection with *B. burgdorferi* to pathogenesis of idiopathic dilated cardiomyopathy.
Sonnesyn, et al.,1995 Minneapolis, MN	Cross-sectional study	5	Patients were evaluated for history of tick bites, rash, neurologic or rheumatologic complaints, unexplained febrile illnesses, and/or a prior diagnosis of Lyme disease. 57 of 175 (32.6%) patients reported histories of tick bite. 125 (71.4%) patients were residents of endemic areas, but no patient had reported a prior diagnosis of Lyme disease. IgG antibodies to *B. burgdorferi* were measured by EIA. Positive or borderline results were confirmed by Western Blot.	175 patients undergoing evaluation of heart failure and possible eligibility for cardiac transplantation were assessed. 44% (77 of 175) of patients had idiopathic cardiomyopathy, defined as indeterminable cause of ventricular failure. 41.7% (73 of 175) of patients had heart failure due to ischemic disease, defined as a history of myocardial infarction, bypass surgery, or an angiogram demonstrating >70% stenosis of an epicardial coronary artery. 14.3% (25 of 175) of patients had other etiologies for their heart failure. 101 blood donors served as healthy controls.	Cases: 4.6% (8 of 175) patients with cardiac failure were IgG positive. 14 of 175 (8%) of patients were seroreactive (positive or borderline). Controls: 2% (2 of 101) of healthy controls were IgG positive and 1%/ 3% (3 of 101) were seroreactive. All of these patients were negative by Western Blot.	Six seroreactive patients with severe cardiomyopathy were treated with antibiotics (3 with IV ceftriaxone for 14 days, and 3 with oral doxycycline for 1 month) due to clinical or exposure histories suggestive of Lyme disease. None of these patients had significant improvement in left ventricular ejection fraction. One patient noted a minimal change, but had improvement in chronic fatigue and myalgias with ceftriaxone. There was no correlation between serologic status and the etiology of heart failure, particularly between patients with "idiopathic" versus "ischemic" causes. There was no statistically significant difference between heart failure patients and healthy controls with regard to serologic status (p=0.07).
Gasser, et al., 1996 Graz, Austria	Prospective cohort study	5	Diagnosis of Lyme Disease depended up on either a history of a well-documented rash consistent with erythema migrans and secondary to a tick bite, or the objective involvement of two or more	46 patients with dilated cardiomyopathy diagnosed by the New York Heart Association (NYHA) and WHO/International Society and Federation of Cardiology (ISFC) criteria. Patients with concomitant cardiovascular	24% (11 of 46) of patients with dilated cardiomyopathy had clinical history of Lyme disease and positive ELISA to *B. burgdorferi*	All patients received standard care for dilated cardiomyopathy, as well as professional guidelines for behavioral factors. Patients with a typical history of Lyme disease and positive serology received treatment with IV ceftriaxone (2g BID for 14 days). 6 of 11 (55%)

			organ systems, with symptoms suggestive of disseminated Lyme, or both, as well as positive ELISA for *B. burgdorferi*. Patients lived in an endemic area. None of the patients had received treatment for *B. burgdorferi* infection prior to admission to the study. 9 of 11 patients who were positive by ELISA had a typical history of tick bite with erythema migrans and/or other organ involvement. 2 of 11 had no recollection of tick bite or erythema migrans, but had presented with neuropathy or oligoarthritis.	disease that could result in dilation and heart failure were excluded. All patients showed limitation in functional capacity per NYHA classification; duration of cardiac symptoms ranged from 0 to 16 months. Mean duration of cardiac symptoms: 3.9 months. NYHA class: I (N=6), II (N=14, III (N=18) and IV (N=8)		patients improved completely with ceftriaxone; 3 (37%) improved substantially, and only 2 (30%) patients did not improve at all. 50% of patients treated with ceftriaxone experienced diarrhea. No patients experienced an allergic reaction. In most patients with dilated cardiomyopathy associated with Lyme disease, symptoms can improve or be completely reversed following standard antimicrobial treatment.
Kubánek, et al., 2012 Prague, Czech Republic	Case control study	7	Endomyocardial biopsy was performed, and the samples were tested by PCR for *B. Burgdorferi* DNA. Patient sera were also tested by ELISA and PCR of peripheral blood, and positive results were confirmed by Western Blot. Clinical history of Lyme disease was investigated. All patients resided in a region endemic for Lyme disease. 7% (3 of 41) of patients with recent-onset dilated cardiomyopathy reported a history of treated Lyme disease.	41 patients (mean age 42 years) with dilated cardiomyopathy of <6 months duration (mean duration of symptoms: 2 months; range 1-3.25 months) participated in the study. Dilated cardiomyopathy was defined by the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of coronary artery disease, severe systemic arterial hypertension, and primary valve disease. NYHA class: I (N=1), II (N=23, III (N=10) and IV (N=7) A control group consisted of 15 patients with end-stage heart failure attributed to coronary artery disease.	10 of 41 (24%) biopsy samples of patients with recent-onset dilated cardiomyopathy were positive for *B. burgdorferi* DNA by PCR. None of the biopsy samples from control patients were positive for *B. burgdorferi* DNA. Patients with positive biopsies reported a similar rate of treated erythema migrans caused by Lyme disease: 1 of 10 positive patients (10%) vs. 2 of 31 negative patients (6.5%) (p=0.708).	The authors found a significantly higher prevalence of B. burgdorferi DNA in patients with recent-onset dilated cardiomyopathy as compared with controls (p=0.035). There was no significant difference between PCR-positive and PCR-negative individuals with regard to prior presentation of erythema migrans or treatment for Lyme disease. Positive biopsy samples showed a low prevalence of myocardial inflammation, and the authors noted modest improvement in left ventricular systolic function after antibiotic treatment. The authors suggest that this could be indicative of a late-stage or chronic infection. Although, endomyocardial

						biopsies were positive by PCR for B. burgdorferi in 10/41 (24%) patients with newly diagnosed dilated cardiomyopathy and no controls (patients with end stage coronary disease), these 10 patients also had a high incidence of positive blood PCRs for B. burgdorferi which would be very unusual in this setting. Lastly, treatment of these patients with IV ceftriaxone did not alter their outcome, compared with the other patients with dilated cardiomyopathy, calling these findings into question.
Kuchynka, et al., 2015 Prague, Czech Republic	Cross-sectional study	6	Serological testing of IgM and IgG antibodies against *B. burgdorferi* was performed by ELISA, and positive results were confirmed by Western Blot. Endomyocardial biopsy was performed for histological analysis. Serological analysis demonstrated IgG antibodies in eight (36%) seropositive patients; however, IgM antibodies were not detected in any subject. Immunohistochemical analysis revealed signs of myocardial inflammation in eight (36%) seropositive patients.	110 patients (mean age 53 years) who had experienced recent-onset, unexplained dilated cardiomyopathy for <12 months, and left ventricular systolic dysfunction persisting for ≥1 week after conventional heart failure therapy. Mean duration of symptoms: 2.4 months, mean NYHA class: 3.3. The diagnostic evaluation included physical examination, assessment of heart failure symptoms (NYHA classification), ECG, transthoracic echocardiography, analysis of blood for markers of inflammation, and markers of myocardial injury and overload.	25% (22 of 88) of cardiomyopathy patients were seropositive for *B. burgdorferi*. NYHA classifications of seropositive individuals were as follows: Class III (N= 10) and IV (N = 8), and Class II (N=2). Left bundle branch block occurred in 5 (23 %) seropositive patients and right bundle branch block occurred in 2 (9%) patients. ST-T changes were observed in 11 seropositive subjects (50 %) and 1st degree atrioventricular block in one (5%).	The authors concluded that Lyme disease may be a cause of recent-onset unexplained dilated cardiomyopathy in patients living in highly endemic regions. Since patients with Lyme-associated cardiomyopathy typically respond favorably to antibiotic treatment and concomitant heart failure medication, the authors recommend testing for *B. burgdorferi* in endemic regions, and particularly recommend assessment of endomyocardial biopsy specimens in the diagnosis of Lyme carditis.

| N'Guyen, 2016

Northeastern France | Prospective cohort study | 6 | Patients were serologically screened by ELISA. All sera with positive or borderline antibody results were confirmed by Western Blot. All patients resided in a region endemic for Lyme disease.

10 of 15 patients underwent endomyocardial biopsy. In the event of a positive ELISA/Western Blot, the specimens were to be confirmed by PCR. | 15 patients with idiopathic dilated cardiomyopathy were prospectively evaluated. | None of the patients were serologically positive for antibodies against *B. burgdorferi sensu lato.* | The authors state that the non-existent seroprevalence in this cohort of patients living in an endemic region refutes the suggestion that *B. burgdorferi* might play an etiological role in dilated cardiomyopathy.

The authors advocate against systematic treatment of patients with idiopathic dilated cardiomyopathy with ceftriaxone in endemic regions. They suggest that antibiotic treatment should be limited to patients whose Lyme disease has been confirmed through patient history (tick bite exposure, etc.) and serology or to patients with positive *B. burgdorferi* serology who require a heart transplant. |
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* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Lyme arthritis

Background information: Distinction between Lyme arthritis from septic arthritis

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Study, Location	Study Design	Risk of bias *	Lyme Disease Diagnosis method	Population characteristics	Proportion of Patients with Lyme Arthritis among Patients with acute arthritis	Study Conclusions
Thompson, et al., 2009 Boston, MA	Cross-sectional study	8	Patients had positive Lyme serology results by ELISA, confirmed by positive IgG Western Blot.	179 pediatric (mean age 7.2 years; range 3-11) patients with acute mono-articular arthritis who had synovial fluid aspiration performed in an emergency department visit. The mean duration of arthritis symptoms was 2.5 days (range 1-7). Patients lived in an area endemic for Lyme disease. 48.6% of all patients recalled a recent fever; 83% of patients with septic arthritis recalled a recent fever. 7% of all patients recalled a recent tick bite; 16% of Lyme arthritis patients recalled a tick bite, whereas 2% and 3% of septic and non-septic non-Lyme patients recalled a tick bite, respectively.	46 (26%) patients were diagnosed with septic arthritis, 55 (31%) patients were diagnosed with Lyme arthritis, and 78 (43%) patients were diagnosed with non-septic non-Lyme arthritis.	Lyme arthritis patients were significantly more likely to have knee involvement (p<0.001, p<0.001) and a history of tick bite (p<0.02, p=0.03) than patients with septic arthritis or non-septic non-Lyme arthritis, respectively. Patients with Lyme arthritis had **lower** ESR levels (p<0.01), CRP levels (p<0.001), joint white blood cell counts (p=0.03), and percent joint neutrophils (p<0.001) **than patients with septic arthritis**. Patient with Lyme arthritis had **higher** ESR levels (p<0.001), CRP levels (p<0.001), joint white blood cell counts (p<0.001), and percent joint neutrophils

						(p<0.001) **than patients with non-septic non-Lyme arthritis.** Despite similar presentations, several key clinical factors can aid in differential diagnosis of acute arthritis.
Glotzbecker, et al., 2011 Boston, MA	Retrospective cohort study	3	All 8 patients had a positive IgG Western Blot for Lyme disease. ELISA testing was available for 6 of the 8 patients. 1 patient recalled tick exposure; none recalled a tick bite or rash.	8 pediatric patients (mean age 9.5 years; range 3-20) with Lyme arthritis isolated in the hip were included. All patients presented with hip pain (N=8), limp (N=3), or refusal to bear weight (N=5). One of 8 patients had a fever >38.5°C. Two of 8 patients had a peripheral white blood cell count >12,500/mm3 and 3 of 8 patients had an erythrocyte sedimentation rate>40mm/h. Aspiration was performed on 5 patients, with a median synovial fluid WBC of 41,500/mm³.	Of 138 patients who underwent incision and drainage for suspected septic arthritis, 8 patients (5.8%) were diagnosed with Lyme arthritis of the hip. Fluid or tissue culture was performed in 6 patients and was negative. *The authors used Kocher's criteria for evaluating the pediatric hip to differentiate transient synovitis from septic arthritis:* 1. *Fever >38.5°C* 2. *WBC count >12* 3. *ESR >40, and inability to bear weight on the affected leg.*	3 patients met 0 of 4 of Kocher's criteria, 1 patient met 1 of 4 criteria, 3 patients met 2 of 4 criteria, and 1 patient met all 4 criteria. Three of 8 patients were treated with irrigation and debridement. All patients were treated with antibiotics for 4 weeks and were asymptomatic at last follow-up. With the exception of one case, antibiotics were started within 2-10 days of presentation. The authors suggest that in endemic regions, Lyme arthritis should be considered in the differential diagnosis of atraumatic mono-articular hip pain associated with an effusion. Prompt diagnosis of Lyme arthritis could prevent unnecessary surgical

						intervention and lead to initiation of antibiotic therapy.
Milewski, et al., 2011

New Haven, CT | Retrospective cohort study | 8 | Patients had positive Lyme serology results by ELISA, confirmed by positive IgG Western Blot. | 391 pediatric (mean age 8.2 years; range 0-18) patients in an endemic area who had received synovial fluid aspiration due to joint effusion. 123 (31%) patients tested positive for Lyme disease, and 51 (13%) patients had septic arthritis. 3 patients had both Lyme disease and septic arthritis, and these patients are represented in both cohorts. 48.6% (190 of 391) patients did not have evidence of infection after aspiration. | Serological testing for Lyme disease was performed for 249 (64%) of 391 patients. The percentage of aspirations leading to Lyme disease testing ranged from 45% in 1992 to 87% in 1996, with no significant trend. The mean incidence of Lyme arthritis was seven cases per year.

Regardless of aspiration, the prevalence of Lyme arthritis among children with swollen knees was 45%. | "There were significantly more children with Lyme arthritis than septic arthritis (123 compared with fifty-one; p <0.001)."

Patients with septic arthritis were significantly more likely to present with fever and had significantly higher white blood cell counts in synovial fluid. A multiple regression analysis isolated "refusal to bear weight" as the strongest predictor for diagnosis of septic arthritis. Patients with Lyme arthritis were more likely to have knee involvement.

The authors concluded that serologic testing and synovial fluid analysis is essential in early diagnosis of Lyme arthritis in endemic areas. They note that prompt and accurate diagnosis of Lyme arthritis could prevent unnecessary surgical intervention in patients with joint effusion. |
| Deanehan, et al., 2013

Boston, MA | Retrospective cohort study | 8 | Patients had a history of a physician-documented erythema migrans rash or clinical manifestations | 474† pediatric (median age 7.4 years; IQR 4.6-11.3) patients with knee mono-arthritis who were evaluated for Lyme arthritis, septic knee arthritis, or another form of arthritis. Patients lived in a Lyme-endemic area. 35% (84 of 238) of Lyme arthritis patients versus 77% (10 of 13) of septic arthritis patients had a history of | 2.7% (13 of 474) of patients were diagnosed with septic arthritis. 50.4% (239 of 474) of patients were diagnosed with Lyme arthritis. 46.8% (222 of 474) of patients were diagnosed with another type of arthritis. | The proportion of patients undergoing synovial fluid aspiration differed by arthritis type. 100% of septic arthritis patients underwent synovial fluid aspiration, whereas only 64% and 32% of Lyme arthritis and "other" arthritis patients underwent the procedure, respectively (p<0.001). Patients with septic arthritis had |

			of Lyme disease with positive Lyme serology, defined as a positive IgG Western blot. .	fever; 19% (40 of 216) of patients with "other" cause of arthritis had a history of fever. 18% (30 of 168) of patients with Lyme arthritis recalled a tick bite. None of the patients with septic arthritis and 8% (10 of 125) of patients with "other" arthritis recalled a tick bite. The median duration of symptoms for all groups of patients was 3 days. †474 is the number of patients in the "derivation" cohort. The "validation" cohort (N=199) was made up of a portion of patients involved in the 2011 study by Milewski. Some were excluded due to diagnostic restrictions.		higher ESR and CRP levels than patients with Lyme arthritis or "other" arthritis. In Lyme endemic areas, a diagnosis of Lyme arthritis is much more likely than a diagnosis of septic arthritis. Based on a clinical prediction model designed by the authors, the conclusion was presented that children with absolute neutrophil count of <$10*10^3$ cells per mm^3 and ESR<40 mm/hour are at low risk for septic arthritis and may not require diagnostic synovial aspiration.

Aiyer, et al., 2014
 	
Hershey, PA
	Retrospective cohort study	3	Records of 155 patients with serologically confirmed Lyme arthritis, confirmed by antibody titers against *B. burgdorferi* by IgG Western Blot were reviewed. Some patients were also tested by ELISA.
			
			Six patients of 39 patients with
				98.7% (153 of 155) of patients reported involvement in more than one joint.
				
				39 of 155 pediatric patients (25.2%) with Lyme arthritis were diagnosed with Lyme arthritis of the ankle; only 2 of 39 (5.1%) patients reported isolated ankle involvement. 125 of 155 patients (80.6%) reported knee involvement. 26 patients (16.8%) reported hip involvement.
				
				Additional laboratory information gathered from patients included: ESR, white blood cell count (WBC), and blood and joint cultures (if available).
					All patients in this study were confirmed to have Lyme arthritis at study onset.
					
					The authors used Kocher's criteria for evaluating the pediatric hip to differentiate transient synovitis from septic arthritis:
					    4. *Fever >38.5°C*
					    5. *WBC count >12*
					    6. *ESR >40, and*
					    7. *Inability to bear weight on the affected leg.*
					
					(4 of 4 criteria meaning a nearly 99% chance of septic arthritis).
						Only three patients were found to have a fever >38.5°C at the time of presentation. Ten patients (10 of 39, 25.6%) had a WBC >12. Only15 patients (15 of 39, 38.5%) were found to have ESR values >40. Only 9 of 39 (22%) had pain with passive range of motion.
						
						All patients were treated with antibiotics. IV antibiotics were started in the event that differentiation between septic arthritis and Lyme arthritis was difficult (5 of 39 patients). One patient underwent a surgical irrigation and debridement procedure while waiting for Lyme serology results.
						
						The authors found that in endemic regions, poly-articular involvement is indicative of Lyme arthritis rather than septic arthritis. The

			Lyme arthritis of the ankle (15%) recalled a tick bite, and 11 (28%) recalled a rash.			sensitivity of poly-articular involvement related to Lyme disease was 97.4%. The authors suggest the following algorithm to differentiate Lyme arthritis from septic arthritis: ≤2 Kocher criteria, poly-articular disease, an ability to bear weight, and minimal pain with passive range of motion. They suggest that patients with these features should be treated with appropriate antibiotics while they await serologic confirmation.
Deanehan, et al., 2014

Boston, MA and New Haven, CT | Retrospective cohort study | 8 | Cases were identified in Lyme endemic areas. Lyme arthritis cases were confirmed by either serologic testing or positive synovial fluid culture. Lyme serology was not required for children with a positive synovial fluid culture. | 384 children from either Boston (54%) or New Haven (46%) who presented to the emergency room with knee monoarthritis who had synovial fluid culture obtained. The median age was 8.0 years (IQR 5.0-11.9 years). | Of the 384 study patients, 19 (5%) had septic arthritis, 257 (67%) had Lyme arthritis, and 108 (28%) had other inflammatory arthritis. | All of the 257 children with Lyme arthritis were IgG positive for Lyme antibodies and had a negative synovial fluid culture. 10 (4%) of these children also had record of a physician-diagnosed erythema migrans rash, and 23 (9%) were positive for *B. burgdorferi* by PCR testing of synovial fluid.

Children with other inflammatory arthritis had lower synovial white blood cell (WBC), absolute neutrophil counts (ANC), and % neutrophils than those with either Lyme or septic arthritis.

The authors assessed the relationship between arthritis type and synovial fluid WBC count, and after adjustment for age, peripheral WBC count, and hospital center, synovial fluid WBC did not differ by arthritis type (Lyme or septic).

In conclusive statements, the authors stress that septic arthritis is a rare cause of knee monoarthritis. They note that synovial fluid WBC, ANC, and % neutrophils are not useful discriminators between Lyme and septic |

						arthritis and that synovial fluid results should not serve as an early indicator for invasive management.
Cruz, et al., 2017 Lyme endemic area (unclear if in PA or RI)	Case-control study	7	Unclear (need full text)	93 pediatric patients who were residents of an area endemic for Lyme disease who underwent hip aspiration for the evaluation of hip pain were evaluated.	17 of 93 (18.3%) patients were diagnosed with Lyme arthritis. 40 of 93 (43.0%) patients were diagnosed with septic arthritis. 36 of 93 (38.7%) patients were diagnosed with transient synovitis.	Multivariable logistic regression revealed febrile history (OR: 16.3 [95% CI 2.35, 113.0]) and increased peripheral white blood cell (WBC) count (OR: 1.26 [95% CI 1.01, 1.58]) to be significantly associated with increased odds of being diagnosed with septic arthritis versus Lyme arthritis. Increased erythrocyte sedimentation rate (ESR) was significantly associated with increased odds of being diagnosed with Lyme arthritis versus transient synovitis (OR: 1.06 [95% CI 1.02, 1.10]), whereas febrile history (OR: 0.06 [95% CI 0.01, 0.49]) and increased peripheral WBC count (OR: 0.8 [95% CI 0.65, 0.98]) were associated with decreased odds of Lyme arthritis.
Baldwin, et al., 2016 Philadelphia, PA	Retrospective cohort study	8	Lyme disease diagnosis was confirmed by IgG Western Blot.	Medical records of 498 pediatric patients with knee effusions who had undergone arthrocentesis of the knee were retrospectively reviewed, and a total 189 patients with Lyme arthritis or septic arthritis were included in the cohort.	140 of 498 patients (28.1%) who underwent arthrocentesis of the knee were confirmed to have Lyme disease.	All patients with confirmed septic arthritis underwent emergent incision and drainage of the knee in the operating room. Of the 140 patients who were eventually diagnosed with Lyme disease, 46 (33%) underwent surgical

| | | | | | 23 patients (4.6%) had culture-positive septic arthritis (positive joint fluid culture <u>or</u> synovial WBC count of >60,000 white blood cells/mm^3 with negative Lyme titer). 26 patients (5.2%) had culture-negative septic arthritis. | incision and drainage based on a presumed diagnosis of septic arthritis. |
| | | | | *To avoid misclassification bias, undiagnosed knee effusions and joints with both a positive culture and positive Lyme titers were excluded.* | | On multivariate analysis, 4 independent factors for differentiating between septic arthritis and Lyme disease were identified: 1. history of fever reported (OR: 6.1 [95%CI 1.2, 31.7], p=0.032), severe pain with short arc motion (OR: 67.3 [95%CI 11.7, 389.1], p<0.001), CRP of ≥4 mg/L (OR: 1.2 [95%CI 1.0, 1.3], p=0.02), and age under 2 years (OR: 0.6 [95%CI 0.5, 0.8], p=0.009). The probability of septic arthritis with any one factor present was 18% compared with 100% with all 4 factors present, regardless of culture positivity.

In areas endemic for Lyme disease, the authors suggest that patients older than 2 years who do not fit any of the criteria above should be serologically tested for Lyme and observed until results are available. They suggest that patients with all 4 risk factors should be considered for urgent surgical intervention. |

Willis, 2003 New York, NY	Case series	NA	Patients had positive Lyme serology results, including EIA or ELISA and Western Blot. Clinical history of tick bite, fever, rash and other typical symptoms of Lyme was reviewed.	10 pediatric (mean age 6.2 years; range 2-12) patients presented with acute arthritis with joint swelling and impairment of movement. The duration of symptoms was a mean of 4 days (range 1-14). All patients denied a history of tick bite or rash. 6 patients had recalled a fever within a week of admission, and five patients had a fever upon admission.	All 10 patients were initially managed for acute septic arthritis, and surgical irrigation and debridement were planned.	EIA results were available within 1 hour for 3 of 10 patients. Positive Lyme titers prevented surgical management for these three patients. These patients immediately received IV ceftriaxone and responded within 2 days with resolution of pain, fever, and limp. 7 of 10 patients received operative joint irrigation and debridement due to delayed Lyme serology results. These patients responded favorably to surgery and had no complications. They were started on appropriate antibiotics within 3 to 5 days after initial presentation and responded to treatment with rapid resolution of arthritis. The authors suggested synovial fluid aspiration in any case of suspected bacterial sepsis. They suggested that serum white blood cell count, ESR, and CRP are useful distinguishing markers in septic arthritis and Lyme arthritis.
Daikh, 2013 Portland, ME	Case series	NA	Patients had positive Lyme serology results by ELISA, confirmed by positive Western Blot.	29 adults and 52 children with Lyme arthritis living in an endemic area. 32.4% (24 of 74) of patients recalled a tick bite. 21.4% (15 of 70) of patients had a history of erythema migrans. 8% (4 of 52) of children and 39% (11 of 28) of adults had a history of other arthritis (p=0.002).	The diagnosis of Lyme arthritis was initially suspected in children more often than in adults (p=0.04). Lyme arthritis was suspected at presentation in 39% (11 of 28) of adults versus 66% (33 of 50) of children.	Adults were more likely to have fluid obtained from their affected joint (p=0.002). Synovial fluid white blood cell counts were significantly higher in children (p<0.0001). There were no other significant differences between inflammatory markers in blood or synovial fluid between the two groups. "In endemic areas, Lyme disease should be considered in all patients with mono-articular and oligo-articular arthritis." The authors warned that an elevated synovial cell count

						should not be an automatic indication for surgical intervention.
Dart, et al., 2018

Boston, MA | Retrospective cohort study | 8 | Patients had positive 2-tiered Lyme serology results and negative synovial fluid bacterial culture results | Patients ≤21 years of age with hip monoarticular arthritis and a synovial fluid culture obtained who presented to 1 of 3 emergency departments located in Lyme disease endemic areas were included. Of the 238 eligible patients, 26 (11%) had septic arthritis, 32 (13%) had Lyme arthritis, and 180 (76%) had other arthritis. | Patients with septic arthritis had a higher median synovial fluid white blood cell (WBC) count than patients with Lyme arthritis. Eighteen patients (56%) with Lyme arthritis had synovial fluid WBC counts ≥50,000 cells per µL. Of the 94 patients who underwent surgical drainage, 13 were later diagnosed with Lyme arthritis. | In Lyme disease endemic areas, synovial fluid WBC counts cannot always help differentiate septic from Lyme arthritis. Rapid Lyme diagnostics could help avoid unnecessary operative procedures in patients with Lyme arthritis |

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXIII. What is the preferred diagnostic testing strategy for Lyme arthritis?

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		PCR, Culture, and Lymphocyte proliferation assay Data		
Study and Location	**Study Design**	**Sample Source(s) and Diagnosis description**	**Diagnosis Method, % positive**	**Study Conclusions**
Steere, et al., 1984

New Haven, CT | Cross-sectional study | Blood, skin biopsy specimens of erythema migrans, cerebrospinal fluid, lymph-node aspirates, and urine cultures were obtained from a total of 118 adults with recent onset Lyme disease.

Synovial fluid, synovial tissue or cartilage was obtained from patients with Lyme arthritis. | Specimens were cultured using Barbour Stoenner Kelly (BSK) medium. Cultures were incubated at 33°C and examined by dark-field microscopy.

Of 23 specimens of joint fluid, synovial tissue, and cartilage cultured, none were positive for *B. burgdorferi* spirochetes. | The authors noted that culture yields were in general low. None of the synovial fluid, synovial tissue, or cartilage specimens obtained from Lyme arthritis patients were positive.

The authors postulate that the number of organisms in affected tissues is small based on the difficulty in recovery of spirochetes and the possible need to concentrate specimens and to passage cultures. They noted that direct visualization of organisms in tissue may be even more problematic. |
| Johnston, et al., 1985

New Haven, CT | Cross-sectional study | Synovial tissue specimens were obtained from 17 patients (70.6% male; mean age 36, range 11-57 years) who met the clinical criteria for Lyme arthritis and underwent surgical excision or biopsy of synovial tissue. All patients had elevated IgG titers against *B. burgdorferi* as determined by ELISA.

Early in the illness, joint involvement was documented to be characteristically intermittent and oligoarticular but became chronic (duration >6 months) in the knees of 14 patients. Open surgical or arthroscopic synovectomies were performed as | Synovial samples were fixed in neutral buffered formalin and in some cases, Helly's fixative. All samples were stained with hematoxylin and eosin, and samples from synovectomies underwent additional staining. Samples were histologically examined by two separate observers and were compared with synovial tissue specimens from patients with other synovial diseases.

Lyme arthritis was correctly recognized by microscopy in 29% of Lyme arthritis patient specimens (5 of 17). This number was the same for both reviewers. For both reviewers, the | The authors noted histologic similarities in the synovial tissue of patients with severe Lyme arthritis to the synovial tissue of patients with rheumatoid arthritis; in both diseases, the synovial tissue shows villus hypertrophy, vascular proliferation, and a moderate to marked infiltrate of lymphocytes and plasma cells.

In 5 cases of Lyme disease, a specific microvascular lesion that was not found in the other synovial diseases was observed. This lesion manifests arteriolar muscle cell proliferation and concentric adventitial fibroplasia resembling the so-called |

| | | therapeutic measures in 8 (57.1%) of the patients with chronic arthritis. Closed biopsies were performed in the remaining 9 patients.

The mean duration from the onset of arthritis to the time of synovectomy or synovial biopsy was 27 months (range, 3-84 months). All patients were receiving non-steroidal anti-inflammatory agents (NSAIDs) at the time of excision or biopsy, but none had received intra-articular steroid injections (IACS) during the prior month. | correctly identified specimens had all originated from synovectomies.

Spirochetes were observed both within the walls of thickened vessels and in the loose tissue in vascular beds in 2 of 8 synovectomy specimens. | "onion skin" vascular lesions seen in malignant hypertension, syphilis, and spleens of patients with systemic lupus erythematosus. As only rare inflammatory cells were observed, the authors note that this lesion did not seem to result by a direct immune attack on the vessel.
The authors concluded that the presence of spirochetes may be directly responsible for the unique microvascular lesion described above. |
|---|---|---|---|---|
| Liebling, et al., 1993

New Brunswick, NJ | Cross-sectional study | Ninety-nine specimens of blood, urine, cerebrospinal fluid (CSF), or synovial fluid were obtained from 44 patients living in an endemic area who had been diagnosed with Lyme disease by CDC criteria.

47 control specimens were also obtained (rheumatoid arthritis, N=5; SLE, N=4; juvenile polyarthritis, N=1; ankylosing spondylitis, N=1; mixed connective tissue disease, N=1; gouty arthritis, N=2; osteoarthritis, N=4; Reiter's syndrome, N=1; fibromyalgia, N=1; gonococcal arthritis, N=1; meningococcal arthritis, N=1; pneumonia, N=2; AIDS with toxoplasmosis, N=1; polycystic kidney disease, N=1; altered mental status, N=3; seizure disorder, N=3; cerebrovascular accident, N=3; disorders of unknown etiology not thought to be Lyme disease, N=11; normal healthy adult, N=1). | Specimens for which DNA was extracted from cultures of *B. burgdorferi* were tested by nested PCR (*MRL7* and *MRL8*; second set *MRL7* and *MRL11a*). Serum and CSF samples were also tested for presence of antibodies against *B. burgdorferi* by ELISA, confirmed by Western Blot.

In patients with Lyme arthritis, 5 of 11 (45.5%) serum samples were positive by PCR, whereas 4 of 5 (80%) synovial fluid samples were positive by PCR. Overall 9 of 16 (56.3%) samples obtained from Lyme arthritis patients were positive by PCR.

None of the synovial fluid or urine samples taken from patients with rheumatic diseases were positive by PCR. | The overall specificity of nested PCR for all body fluids was 96.4%, and overall sensitivity was 76.7%. The overall false-positive rate was 3.6%.

Nested PCR of synovial fluid showed 80% sensitivity and 95.7% specificity.

The authors noted that the only negative synovial fluid sample obtained from a patient with clinical and serologic evidence of Lyme arthritis was extracted prior to the use of hyaluronidase in the pretreatment process, and suggest that pretreatment with hyaluronidase may have mitigated the false negative.

Overall, the authors conclude that PCR of the synovial fluid is an effective diagnostic tool. |

Bradley, et al., 1994 Minneapolis, MN	Cross-sectional study	Synovial fluid samples were taken from patients and controls from Minnesota and Wisconsin, areas where the background seroprevalence of *B. burgdorferi* among healthy individuals is 1% to 2%. Lyme arthritis was suspected in patients with mono- or oligoarticular large joint involvement, seropositivity for *B. burgdorferi*, and no other known underlying disease. Controls presented with various arthritic processes (rheumatoid arthritis, spondyloarthropathy, gout, pseudogout, hemarthrosis, degenerative joint disease, lupus, papillary synovitis, and trauma) and were undergoing arthrocentesis of involved joints.	One half of the synovial fluid collected underwent nested PCR (initial primers *991* and *992*, confirmation by primer pairs *A2/A4* and *A149/A319*, as well as internal probes for the plasmid-encoded *B. burgdorferi ospA* gene) and the other half was cultured using Barbour Stoenner Kelly (BSK) medium. Synovial fluid from six of seven (86%; 95% CI: 42%-100%) patients thought to have Lyme arthritis was positive by PCR. All cultures for *B. burgdorferi* were negative. All 18 synovial fluid samples from patients with other rheumatological disorders were negative by PCR (p<0.001).	The results demonstrated that PCR of synovial fluid is an effective way to diagnose patients with Lyme arthritis. The authors propose that the discrepancy between PCR results and culture results could be attributed to organisms that are injured, dead, or otherwise inhibited from multiplication. The results show the intra-articular persistence of *B. burgdorferi* DNA in Lyme arthritis and suggest that persistent organisms and their components may be directly involved in ongoing immune and inflammatory processes, even in some antibiotic-treated patients.
Nocton, et al., 1994 New Haven, CT and Boston, MA	Prospective cohort study	Synovial fluid samples were collected from 117 patients with Lyme arthritis, which was defined by brief, intermittent attacks of oligoarthritis, elevated antibody titers for *B. burgdorferi* by ELISA, exposure to an endemic region, and exclusion of other forms of arthritis. Synovial fluid specimens were collected from 69 control patients with other forms of arthritis (rheumatoid arthritis, N=20; gout, N=7; osteoarthritis, N=7; degenerative joint disease, N=7; juvenile rheumatoid arthritis, N=5; pseudogout, N=2; psoriatic arthritis, N=2; Reiter's syndrome, N=2; scleroderma,	Samples were tested in a blinded manner in two different laboratories by PCR using four sets of primers and probes, three of which targeted plasmid DNA that encodes *ospA*, and one which targeted chromosomal DNA encoding *16S rRNA*. Sensitivity PCR (any set): 85.2% Specificity PCR (any set): 100% In both laboratories, the sensitivity of each of the three *ospA* primer-probe sets was high (75%-89%), with moderately concordant results, whereas the primer-probe set detecting chromosomal DNA was less sensitive.	Lyme arthritis patients with a positive PCR result had significantly higher white-cell counts in their synovial fluid (p<0.003), significantly shorter durations of illness (p<0.02) and arthritis symptoms (p<0.03), and a longer duration of arthritis after aspiration (p=0.03). 77.3% of patients with positive PCR had received no antibiotic treatment prior to synovial fluid aspiration, and 16% of patients with positive PCR had received less than 1 month of antibiotic treatment prior to aspiration; when these two groups were combined, the relationship to positive PCR results after treatment was statistically significant (p<0.001).

		N=2; spondyloarthropathy, N=2; other forms, N=13).		Of 12 patients for whom serial synovial fluid samples were available, 9 had positive PCR results for months to years later during subsequent episodes of arthritis. The authors conclude that PCR is a useful method for detecting *B. burgdorferi* DNA in synovial fluid in Lyme arthritis patients.
Persing, et al., 1994 New Haven, CT	Cross-sectional study	Synovial fluid samples were collected from 18 patients with Lyme arthritis who were negative by culture (19 synovial fluid specimens). The patients met the CDC case definition criteria for Lyme disease; all patients had intermittent episodes of arthritis, lived in areas endemic for Lyme disease, and had elevated antibody titers for *B. burgdorferi* by ELISA. Eleven control synovial fluid specimens were collected from patients with non-Lyme arthritic disorders (rheumatoid arthritis, chronic granulomatous disease, and systemic lupus erythematosus) and were processed and stored the same way as those from patients with Lyme arthritis.	PCR was performed using DNA sequences characteristic of plasmid-encoded genes *ospA* and *ospB*, as well as primers detecting the *16S* rRNA gene and the *B. burgdorferi* flagellin (*fla*) gene. All 19 samples were reactive with the *ospA* 149/319 primer pair and were even more strongly reactive with the *ospB* primer pair (100% sensitivity for both *ospA* and *ospB*). Only 8 of 19 samples (42.1% sensitivity) were reactive for *16S* rDNA and only 9 of 17 samples (52.9% sensitivity) were positive for the flagellin target.	In all 19 synovial fluid specimens, DNA sequences characteristic of plasmid-encoded genes *ospA* and *ospB* were easily detected. However, despite equivalent or even superior analytic sensitivity for detection of cultured organisms, the reactivity of two genomic DNA targets was often weak or absent altogether in the clinical specimens. This apparent overrepresentation of *B. burgdorferi* plasmid sequences was found exclusively in clinical specimens and not in cultured organisms. Based on the target imbalance observed, the authors suggest that the most sensitive and reliable targets for PCR detection of *B. burgdorferi* may lie on extra-chromosomal elements and not on the genome itself.
Jaulhac, et al., 1996 Northeastern France	Cross-sectional study	12 consecutive patients with clinically evident Lyme arthritis from whom synovial fluid or synovial tissue samples could be obtained were evaluated. A case of Lyme arthritis was defined as one that either strictly fulfilled the criteria of the CDC for Lyme arthritis or as a case of objective joint swelling in ≥1 large joint following a recent and well-documented erythema migrans. All patients had been	Synovial tissue and fluid samples were tested by PCR for a *B. burgdorferi* target from the flagellin (*fla*) gene. Blood samples were tested by EIA, confirmed by Western Blot. 5 of 12 patients were positive by PCR for *B. burgdorferi* DNA in the synovial fluid (42% sensitivity).	Among the 11 patients for whom both synovial fluid and synovial tissue samples were available and interpretable after PCR, *fla* sequences were statistically more often encountered in synovial tissue than in synovial fluid (p<0.05). 3 patients had Lyme arthritis despite antibiotic therapy: two had been previously treated with short courses of oral antibiotic therapy (doxycycline 100

		bitten by ticks before the onset of arthritis and had brief episodes of objective joint swelling in a few joints of the lower limbs, including at least 1 knee in all patients. All patients had a previous history of erythema migrans. Control synovial fluid/tissue samples were also taken from 11 patients with rheumatoid arthritis, 11 patients with osteoarthritis, 3 patients with septic arthritis, 3 patients with psoriatic arthritis, and 1 patient with Sjogren's syndrome.	10 of the 11 remaining patients were positive by PCR for *B. burgdorferi* DNA in the synovial tissue (91% sensitivity). The PCR results for synovial fluid tissue samples, taken together, showed that all 12 Lyme arthritis patients were positive (100% sensitivity). EIA findings were positive for 10 of the 12 patients with clinically evident Lyme arthritis. All sera which tested positive by EIA were confirmed as true-positives by Western blot analysis. None of the results in 29 control patients were significant by EIA and by Western blot.	mg BID for 3 weeks) and one had been previously treated with IV ceftriaxone (2 g/day for 3 weeks). All 3 patients' synovial tissue was PCR positive. The 2 patients previously treated with doxycycline were found to be PCR negative when testing synovial fluid, whereas the patient previously treated with IV ceftriaxone was found to be PCR positive in both synovial fluid and tissue. The authors suggested that negative PCR results with synovial fluid samples cannot exclude the presence of *B. burgdorferi* in the joint.
Priem, et al., 1997 Berlin, Germany	Case control study	Urine, cerebrospinal fluid, and synovial fluid specimens from 57 patients with different manifestations of Lyme disease were examined. Thirty-five patients with oligoarthritis, and in whom other rheumatic diseases (e.g.reactive arthritis, seronegative spondyloarthropathy, and rheumatoid arthritis) had been excluded, were diagnosed with Lyme arthritis, and 22 patients were diagnosed with neuroborreliosis. Seventeen patients had been treated with oral antibiotics prior to study onset. An additional group of 11 patients with histories and serologic results suggestive of Lyme disease who presented with nonspecific symptoms such as headache, subtle neurologic impairment, myalgias, or	Nested PCR was performed with primer sets targeting the *ospA* gene and a chromosomal gene segment encoding a 66-kDa protein (*p66*). Diagnostic sensitivity of PCR testing in synovial fluid samples was 85%. Diagnostic sensitivity of PCR testing in urine samples was 79%. Diagnostic sensitivity of PCR testing in those with paired synovial fluid and urine samples was 91% in patients with Lyme arthritis. One patient each with neuroborreliosis and with Lyme arthritis had PCR positive urine samples only.	In 17% of all PCR-positive cases, both primer sets yielded positive results, while the other patients were positive with only one primer set. Among these, more positive results were obtained with the *p66* gene primer than with the *ospA* primer. The specificity of the optimized PCR protocol exceeded 99%. The authors concluded that DNA from *B. burgdorferi* can sensitively and specifically be detected with the optimized PCR method described. They recommend use of at least two different primer sets, and whenever possible, recommend that urine and cerebrospinal fluid or synovial fluid should be analyzed in parallel to achieve maximum sensitivity of the test. However, it should be noted that nested PCR is prone to carryover contamination of amplicons.

		constitutional symptoms also underwent testing. All patients from both groups were from highly endemic regions and recalled a tick bite and/or erythema migrans rash. Lyme serology was performed by using full-antigen ELISA confirmed with Western Blot. The group of control subjects consisted of 37 patients with various rheumatic diseases including rheumatoid arthritis, reactive arthritis, systemic lupus erythematosus, and osteoarthritis and 21 patients with different inflammatory and non-inflammatory central nervous system diseases, including multiple sclerosis, myelitis, and meningitis. All control subjects were seronegative.	None of the control specimens was positive by nested PCR.	
Priem, et al., 1998 Berlin, Germany	Case series	Paired synovial fluid and synovial tissue specimens and urine samples from four patients with ongoing or recurring Lyme arthritis despite previous antibiotic therapy were tested. Patients were evaluated 8 to 10 weeks after antibiotic treatment had been completed. At the time of investigation, all four patients were still seropositive and had active arthritis despite previous antibiotic therapies. Arthritis completely resolved in all patients after additional antibiotic treatment.	PCR for the detection of *B. burgdorferi* DNA was carried out using primer sets specific for the *ospA* gene and a *p66* gene of *B. burgdorferi*. PCR with either primer set was negative in synovial fluid and urine, but was positive with at least one primer pair in the synovial tissue specimens. Synovial tissue samples of patient 1 and 2 showed positive PCR results with the *ospA* primer while the synovial tissue specimen of patient 3 was PCR positive with the *p66* primer only. Synovial tissue samples from patient 4 were PCR positive with both primer sets.	The authors concluded that in patients with treatment-resistant (now called post-antibiotic) Lyme arthritis, negative PCR results in synovial fluid or urine after antibiotic therapy do not rule out the intra-articular persistence of *B. burgdorferi* DNA. They recommend PCR analysis of both synovial tissue and synovial fluid for borrelial DNA, because positive results in synovial tissue are strongly suggestive of ongoing infection. However, it should be noted that nested PCR is prone to carryover contamination of amplicons.

Carlson, et al., 1999 New Haven, CT	Retrospective study	Synovial tissue samples were obtained from 26 patients (50%male; mean age 33 years (range 12–60 years) with antibiotic treatment-resistant now called post-antibiotic) Lyme arthritis who underwent arthroscopic synovectomies. The mean disease duration from onset to beginning of arthritis symptoms was 12 months (range 3-25 months). Thirteen patients had early manifestations of the infection. The remaining 13 patients presented with knee swelling. In all 26 patients, 1 or both knees were chronically affected. All patients met the CDC criteria for Lyme disease and had brief, intermittent attacks of oligoarthritis followed by chronic arthritis in a knee, exposure in an area endemic for the disease, and a positive antibody response to *B burgdorferi* by ELISA and Western blot. Synovial tissue specimens were also obtained from 10 control subjects (osteoarthritis, N=3; rheumatoid arthritis, N=2; juvenile rheumatoid arthritis, N=1; reactive arthritis, N=2; sarcoid arthritis, N=1; undifferentiated arthritis, N=1).	Samples were tested by PCR using 3 different primer-probe sets, which targeted plasmid DNA encoding *B. burgdorferi ospA* or *ospB* or a chromosomal encoded flagellin protein, *P41* *B. burgdorferi* DNA was not detected with the 3 primer-probe sets in any of the synovial samples from the 26 patients with Lyme arthritis or from the 10 control subjects.	All 26 Lyme arthritis patients had been treated for arthritis with antibiotic therapy prior to testing. Three patients received only intravenous ceftriaxone or penicillin for 10–21 days, and 2 patients were given only oral doxycycline or amoxicillin for 6 or 9 weeks. The remaining 21 patients received both oral and intravenous therapy, usually oral doxycycline and intravenous ceftriaxone. Overall, the mean duration of antibiotic therapy in the 26 patients was 8 weeks (range 1.5–14 weeks). The mean duration from the completion of therapy to synovectomy was 7 months (range 0.1–35 months). In this study, *B. burgdorferi* DNA was not detected In the synovial tissue of any of the 26 Lyme arthritis patients who underwent arthroscopic synovectomies following prolonged courses of antibiotic treatment. The authors state that their results indicate that synovial inflammation may persist in some patients with Lyme arthritis even after the eradication of the spirochete from the joint with antibiotic therapy.
Schnarr, et al., 2001 Hannover, Germany	Prospective cohort study	52 patients who were residents of a region highly endemic for Lyme disease who had been diagnosed with undifferentiated oligoarthritis for <1 year and were experiencing joint effusion were enrolled.	Synovial fluid samples were tested by nested PCR for presence of *Chlamydia trachomatis* and *Borrelia burgdorferi* DNA. Fifteen of 52 UOA patients (29%) had a positive PCR result for either pathogen.	The authors identified 6 individuals without significant anti-*Borrelia* antibodies according to the CDC criteria, but with intra-articular *B. burgdorferi* DNA detection. Among these 6 patients, 1 had a history of tick bite and erythema migrans. This patient did not fulfill the CDC criteria for Lyme disease because he had a *B. burgdorferi* titer of 1:160

| | | Undifferentiated oligoarthritis (UOA) was defined as the presence of synovitis in 1-4 joints, without psoriatic skin lesions, in patients whose symptoms did not meet the criteria for any other defined rheumatic disease.

For comparison of the obtained PCR data among UOA patients and patients with other definite arthritides, samples were obtained from cohorts of patients with rheumatoid arthritis (N=31), patients with Lyme arthritis (N=16), and patients with *Chlamydia*-induced arthritis (N=14). | Synovial fluid specimens of 9 patients (17%) were positive for *Chlamydia trachomatis* DNA.

Synovial fluid specimens of 6 patients (12%) had *B. burgdorferi* DNA.

DNA from neither pathogen was found in the synovial fluid from any of the 31 rheumatoid arthritis patients. | but only a single, very weak *ospC* band in IgG Western blot. Another patient with a *B. burgdorferi* titer of 1:160 had no visible bands in IgG and IgM Western blot. Prior episodes of tick bite and erythema migrans were not valuable predictors of the presence of *B. burgdorferi* DNA in synovial fluid (p=0.79 and p=0.09, respectively).

The authors suggest that a possible explanation for the striking seronegativity in this cohort is that the serologic test performed may be insufficient in some European regions highly endemic for other genospecies such as *B. afzelii*. The authors concluded that optimized PCR protocols can lead to the identification of a considerable frequency of *Borrelia*- and *Chlamydia*-specific DNA in the synovial fluid of patients with UOA. However, it should be noted that nested PCR is prone to carryover contamination of amplicons. |
| Lipowsky, et al., 2003

Zurich, Switzerland | Case control study | Synovial fluid and tissue samples were obtained from 11 Lyme arthritis patients and from 15 controls with diagnosis of rheumatoid arthritis (N=10) or calcium pyrophosphate dehydrate deposition disease (n=5)

9 patients had clinical symptoms of knee arthritis, 2 had oligoarthritis involving the knee. All patients tested positive for *B. burgdorferi* by ELISA, confirmed by Western Blot. | Synovial fluid and synovial tissue samples were analyzed by species-specific and broad-range PCRs sequencing of *16s* rRNA gene and *B. burgdorferi* flagellin (*fla*) gene.

B. burgdorferi DNA was detected in 9 of 11 patients (82% synovial fluid, 18% synovial tissue) by species-specific PCR. Only one patient tested positive by broad-range PCR. | The authors did not observe the persistence of *B. burgdorferi* DNA after antibiotic treatment. They concluded that synovial fluid was the preferable specimen to detect DNA by PCR. They noted that antibiotic treatment with ceftriaxone or doxycycline usually resulted in complete cure of Lyme arthritis and that species-specific PCR was a useful tool in monitoring treatment success. |

| Kannian, et al., 2007 Boston, MA | Prospective cohort study | At least 3 serial serum samples from 41 patients with antibiotic-refractory arthritis (65.9% male; median age 41, range 13-64 years) and 23 patients with antibiotic-responsive arthritis (65.2% male; median age 38, range 13-67 years), and samples from 10 non–antibiotic-treated, historical control patients were tested. Median disease duration from the start of the first course of antibiotics to the first sample date was 1 month in antibiotic-responsive patients (range, 0-2 months) and 2.5 months in antibiotic-refractory patients (range 0-7 months).

The 23 patients with antibiotic-responsive arthritis were usually treated with oral doxycycline for 1 month, and arthritis often resolved during that time. In contrast, the 41 patients with antibiotic refractory arthritis frequently received oral doxycycline for 2 months or IV ceftriaxone for 1 month or in many circumstances, received both medications, but their arthritis persisted for a median duration of 10 months after starting therapy. | IgG antibody responses to *B. burgdorferi* sonicate, rDbpA, MaBP-OspA, GST-Arp, or the VlsE C6 peptide were determined by ELISA.

Sonicate ELISA:
• Antibiotic-responsive: % positive= 100
• Antibiotic-refractory: % positive= 100

DbpA ELISA:
• Antibiotic-responsive: % positive= 96
• Antibiotic-refractory: % positive= 83

VlsE C6 peptide ELISA:
• Antibiotic-responsive: % positive= 100
• Antibiotic-refractory: % positive= 90

OspA ELISA:
• Antibiotic-responsive: % positive= 74
• Antibiotic-refractory: % positive= 71 | With the exception of 2 patients with antibiotic-refractory arthritis in whom antibody titers increased long after antibiotic therapy, the similar decline in antibody titers after the initiation of therapy in both antibiotic-treated groups suggested that synovial inflammation persists after the period of infection in most patients with antibiotic-refractory arthritis.

The authors note that in this study, antibody titers with the VlsE peptide ELISA declined earlier and the change in titer was greater than those of the other spirochetal proteins tested, which supports the suggestion that this test may be valuable for monitoring treatment progress. However, by 4–6 months, patients usually had only a 1–2-fold change in titer, and a longer period was required to observe a 4-fold change in titer. The authors concluded that the rate of decline of antibodies to the VlsE peptide or other spirochetal proteins is usually too slow to be useful when decisions about further antibiotic therapy need to be made in patients with Lyme arthritis. The authors suggest that patients with Lyme arthritis who have a sustained, gradual decline in antibody reactivity most likely have nearly complete or total eradication of spirochetes from the joint as a result of antibiotic therapy, even if joint inflammation persists after the period of infection. |

Babady, et al., 2008 Rochester, MN	Retrospective study	23,777 cerebrospinal fluid (N=15,939), blood (N=5,703), synovial fluid (N=1,976), tissue (N=92), and other sample sources (N=67) were tested by a reference laboratory. For assay validation, results of the real-time PCR assay were compared with those obtained by conventional PCR using blood, cerebrospinal fluid, synovial fluid, and tissue.	Samples were tested by a real-time PCR encoding for the borrelial plasminogen binding protein. Synovial fluid: 6.4% positive (127 of 1,976) Tissue: 6.5% positive (6 of 92) Blood: 0.1% positive (6 of 5,703) Cerebrospinal fluid: 0.09% (14 of 15,939) Among patients with a positive PCR in synovial fluid or tissue, 8 also had blood or CSF submitted for testing by PCR with negative results.	Though cerebrospinal fluid and blood are most frequently submitted for Lyme PCR, they demonstrate the lowest positivity rates. Detection of *B. burgdorferi* was highest in synovial fluid (6.4%) and tissue (6.5%), even though these specimen types making up a small percentage (8.3% and 0.4%, respectively) of the total number of specimens submitted for testing by PCR. The authors note that their data are in agreement with results reported in previous studies using either real-time or conventional PCR, which have shown a low positivity rate for blood and cerebrospinal fluid, even in patients with known Lyme disease. The authors state that blood submitted for Lyme PCR is rarely useful and recommend synovial fluid PCR in the context of a positive serologic test.
Li, et al., 2011 Boston, MA	Case control study	115 joint fluid samples were collected from 63 patients with Lyme arthritis. All patients had strong IgG antibody responses to *B. burgdorferi*. Antibiotic-responsive arthritis was defined as arthritis that resolved within 3 months after the start of a 4-week course of IV antibiotics or an 8-week course of oral antibiotics. Antibiotic-refractory (now called post-antibiotic) arthritis was defined by persistent joint swelling for 3 months after the start of 4 weeks of IV antibiotics or 8 weeks of oral antibiotics, or both. For comparison, control synovial fluid or synovial tissue samples from patients with rheumatoid arthritis or undifferentiated mono-arthritis were tested.	Among 23 patients with antibiotic-responsive arthritis, 10 of the 14 (71%) samples collected prior to treatment were PCR-positive for *recA* DNA. Only 2 of 14 (14%) samples obtained after the start of therapy had positive PCR results (p<0.001). Among the 40 patients with post-antibiotic Lyme arthritis, 2 of 5 pretreatment samples (40%) had positive PCR results for *recA* DNA, and 16 of 24 samples (67%) obtained during the first 3 months after the start of antibiotics had positive PCR results, but the percentages were not significantly different.	In the majority of patients with Lyme arthritis, PCR results in pretreatment synovial fluid samples were positive. In patients with post-antibiotic arthritis, positive PCR results persisted for as long as 11 months, but positive results in samples obtained during the post-antibiotic period did not correlate with relapse or with the subsequent duration of arthritis, and at the time of synovectomy, all results of PCR of synovial tissue were negative. The authors make note that *B. burgdorferi* mRNA, a marker of spirochetal viability, was not detected in any of the 11 synovial fluid samples from Lyme arthritis patients, even when obtained prior to antibiotic administration. The median ratio of spirochetal rRNA to DNA, which is a measure of

205

		115 joint fluid samples were analyzed by standard PCR targeting the *recA* gene and quantitative PCR targeting *B. burgdorferi flaB* gene. For samples from which both RNA and DNA were extracted, the concomitant DNA and cDNA samples were analyzed by qPCR, targeting *B. burgdorferi flaB* and 16S sequences as well as eukaryotic 18S sequence. Quantitative PCR determinations of *B. burgdorferi* DNA, messenger RNA (mRNA), and ribosomal RNA (rRNA) were made in 10 skin samples from 11 synovial fluid samples from Lyme arthritis patients.	During the first 3 months of treatment, the antibiotic-responsive arthritis and post-antibiotic Lyme arthritis groups were significantly different with regard to the proportion of positive PCR results (14% versus 67%) (p=0.003). Four to 6 months after the start of antibiotic therapy, when all but 2 patients with post-antibiotic arthritis were treated with DMARDs, 8 of 26 samples (31%) had positive *recA* PCR results. The results of the *recA* PCR and *flaB* qPCR assays were highly concordant (p<0.001), but the *flaB* qPCR was slightly more sensitive than the *recA* PCR.	ribosomal activity, was only 0.15 in the 3 Lyme arthritis samples with positive results, compared to 160 in 10 erythema migrans skin samples with positive results. Noting an 83% culture positive rate in erythema migrans skin biopsy samples and that they had never successfully cultured spirochetes from synovial fluid samples, the authors suggest that detection of *flaB* mRNA appears to correlate with culture results in Lyme disease. The authors concluded that PCR testing of the synovial fluid may help to confirm a diagnosis of Lyme arthritis and may be beneficial in diagnosis if a patient has recurrent arthritis after a negative PCR result and a prolonged period of remission, but that this test is not a reliable indicator of active infection in patients with persistent arthritis after antibiotic therapy.
Maraspin, et al., 2011 Ljubljana, Slovenia	Retrospective study	The medical files of patients <15 years old who were diagnosed with borrelial lymphocytoma (N=53), Lyme neuroborreliosis (N=176), Lyme arthritis (N=13), or acrodermatitis chronica atrophicans (N=200) were retrospectively reviewed. Lyme arthritis diagnosis required joint swelling in at least one large joint, presence of *B. burgdorferi*-specific IgG in serum, and the exclusion of alternative explanations for the arthritis.	All serum samples were cultured for presence of spirochetes using Kelly Pettenkofer medium. Samples were incubated at 33°C and examined weekly for up to 12 weeks by darkfield microscopy for the presence of spirochetes. *Borrelia burgdorferi sensu lato* was isolated from the blood in 1 of 13 (7.7%) patients with Lyme arthritis	At the time of the blood culture, erythema migrans was present in 1 of 11 (36.4%) patients with positive blood cultures: in 1 patient with borrelial lymphocytoma, 1 patient with Lyme arthritis, and in 2 patients with Lyme neuroborreliosis. The authors note that in patients with manifestations of Lyme disease other than erythema migrans, the isolation rate of *B. burgdorferi* from blood by culture is low (11 of 442 overall, 2.5%). Successful culture is nearly three times more likely when concomitant erythema migrans is present (36.4% vs.13.2%, p=0.0513), and it is associated with a relatively short duration of clinical manifestations.
			Serologic Testing Data	

Study and Location	Study Design	Population Characteristics	Meta-analysis details	Results and Study Conclusions

Waddell, et al., 2016	Systematic Review and Meta-analysis	Searched from 1995 – Sep. 2013
		Included 48 North American diagnostic test studies that compared results of one test using a validated test panel, results of clinical diagnosis, or a gold standard test result or investigated inter-test agreement. No studies were excluded based on their quality assessment. Studies evaluating in-house tests were included; however, heterogeneity analyses on the impact of the non-commercial tests were performed, where applicable.
		The following disease stages were addressed: Early/acute (*Stage 1*; <30 days; includes EM); Early disseminated (*Stage 2*) (neurologic/cardiac/multiple EM); Late (*Stage 3*) (late neuroborreliosis/arthritis).
			The included tests were evaluated in the context of clinical diagnosis or compared with one another. No studies addressed serologic testing of cerebrospinal fluid (CSF).
			Meta-analysis was conducted using hierarchical logistic regression and bivariate models that account for the correlation between sensitivity and specificity.
			Due to broad inclusion criteria, many studies received downgraded risk of bias ratings in the selection, performance (inadequate blinding), reporting, and/or funding domains.
				1. **Two-tier test vs. clinical diagnosis**
				Stage 3 (Late neurologic or arthritis) (N=8 studies):
				  ● Sensitivity 99.4% (95% CI: 95.7%-99.9%)
				  ● Specificity 99.3% (95% CI: 98.5%-99.7%)
				Convalescent Lyme (treated at stage 2 or 3) (N=7 studies):
				  ● Sensitivity 80.0% (95% CI: 70.8%-86.8%)
				  ● Specificity 98.3% (95% CI: 96.6%- 99.2%)
				
				2. **EIA (1st tier tests, including ELISA) vs. clinical diagnosis**
				Stage 3 (Late neurologic or arthritis) (N=8 studies):
				  ● Sensitivity 94.7% (95%CI: 86.0%-98.2%)
				  ● Specificity 96.1% (95% CI: 94.2%-97.4%)
				Across all studies, the sensitivity for C6 ELISA was highest, with the lowest variability over other tests and test protocols.

Cook and Puri, 2016	Meta-analysis	Search dates unclear: 1995 - unknown (Latest included article was published in 2015; Epub in Jul 2014).
		Included any studies (N=18 studies, 12 from US) evaluating commercially available serologic tests.
		The included studies did not evaluate the tests in clinical settings, where the use of antibiotics or other factors may influence the antibody response. The review did not evaluate microscopy, culture, PCR, or novel technologies (LTT etc.).
			Samples were proved positive based on records of erythema migrans, positive serology and/or culture, or CDC-certified panels.
			Only studies in which test specificity was reported to be at least 85% were included, to avoid overinflated sensitivity at the cost of lowered thresholds and too many false-positives.
			Sensitivities of each test were not evaluated within every stage of borreliosis due to the lack of standard definitions of disease stages and the possibility of retrospective selection bias.
				Weighed Sensitivities for Lyme Arthritis (all test types)
				  ● Lyme Arthritis: 95.8% (95%CI: 81.8%-100%)
				  ● Neurologic/arthritis: 92.2% (78.4%-100.0%)

Leeflang, et al., 2016	Systematic Review and Meta-analysis	Last search date: Feb. 2014. The oldest included study was published in 1987. Only European studies evaluating the diagnostic accuracy of serologic assays for Lyme borreliosis against a reference standard for clinical criteria (sometimes combined with positive serology) in "possible" or "suspected" Lyme patients were included (N=75 studies); these patients counted as "cases". Indirect fluorescent antibody assays were not evaluated because of the rare use in practice.	Meta-analysis was performed using Hierarchical Summary ROC (HSROC) model, a hierarchical meta-regression method incorporating both sensitivity and specificity while taking into account the correlation between the two. The authors noted that the included studies had high levels of heterogeneity and bias and did not represent the tests in true clinical settings.	Eight case-control studies compared diagnostic methods on Lyme arthritis patients versus healthy controls, but meta-analysis of these studies was not possible. The median sensitivity for all ELISA tests was 96% (IQR 93%-100%) and the median specificity was 94% (IQR 91%-97%). No data on synovial fluid analysis were available.
Study; Location	**Study Design**	**Population Characteristics**	**Diagnosis Method, % Positive**	**Study Conclusions**
Molins, et al., 2017	Blind laboratory study	Serum samples from 471 well-characterized Lyme patients and controls from the CDC Lyme Serum Repository were used.	bioMérieux Vidas Lyme IgM II (LYM) EIA (1 tier) ● Late Lyme, Lyme Arthritis, Sensitivity: 63% ● All Negative Controls, Specificity: 87% bioMérieux Vidas Lyme IgG II (LYG) EIA (1 tier) ● Late Lyme, Lyme Arthritis, Sensitivity: 100% ● All Negative Controls, Specificity: 97% bioMérieux Vidas Lyme IgG II (LYM/LYG) EIA (2 tier) ● Late Lyme, Lyme Arthritis, Sensitivity: 97% ● All Negative Controls, Specificity: 97% bioMérieux Vidas Lyme combined (LYT) EIA (1 tier) ● Late Lyme, Lyme Arthritis, Sensitivity: 100% ● All Negative Controls, Specificity: 85% bioMérieux Vidas Lyme combined (LYT) EIA (2 tier)	The overall sensitivities and specificities for standard two-tiered testing (STTT) were similar between the two testing strategies (LYT versus LYM/LYG), although differences in first-tier test results between the two were observed. A modified two-tiered (MTTT) algorithm that uses the Vidas EIAs (LYT or LYM/LYG) as the first-tier test followed by the C6 EIA as the second-tier test also gave similar sensitivities and specificities when the dissociated or combined assays were tested but resulted in significantly higher overall sensitivities than and specificities similar to those of STTT.

| | | | Late Lyme, Lyme Arthritis, Sensitivity: 97% All Negative Controls, Specificity: 97%
C6 EIA (1 tier)
Late Lyme, Lyme Arthritis, Sensitivity: 100%
All Negative Controls, Specificity: 97%
C6 EIA (2 tier)
Late Lyme, Lyme Arthritis, Sensitivity: 97%
All Negative Controls, Specificity: 99%
LYT-C6 (MTTT)
Late Lyme, Lyme Arthritis, Sensitivity: 100%
All Negative Controls, Specificity: 98%
LYM/LYG-C6 (MTTT) Late Lyme, Lyme Arthritis, Sensitivity: 100% All Negative Controls, Specificity: 99% | |

XXIV. What are the preferred antibiotic regimens for the initial treatment of Lyme arthritis?

ANTIBIOTICS vs. PLACEBO

In patients with Lyme arthritis, should antibiotic therapy be used over no antibiotic therapy?

P: In patients with Lyme arthritis

I: Antibiotic therapy

C: No antibiotic therapy

Bibliography: 1. Steere, et al. N Engl J Med. 1985 Apr 4; 312(14): 869-74.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Antibiotics (benzathine penicillin)	Placebo	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of patients/№ of events**		**Effect**			
Complete Resolution of Arthritis (at one month)												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	7/20 (35.0%)	0/20 (0%)	NA [c]	**350 more per 1,000 (from 559 more to 141 more)**	⊕⊕⊕◯ MODERATE	CRITICAL
Withdrawals due to Adverse Events (at 10 days)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	4/20 (20.0%)	0/20 (0.0%)	NA [c]	200 more per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT
Allergic Reaction (at 10 days)												
1	RCT [1]	not serious	not serious	not serious	serious [b]	none	0/20 (0.0%)	0/20 (0.0%)	NA [c]	0 per 1,000	⊕⊕⊕◯ MODERATE	IMPORTANT

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Fragility due to low event rate and small sample size

b. Low event rate and small sample size

c. One or both arms has zero event; unable to estimate relative risk.

CEPHALOSPORIN vs. PENICILLIN

In patients with Lyme arthritis, should 3rd generation cephalosporins be used over penicillin?

P: In patients with Lyme arthritis

I: 3rd generation cephalosporins (ceftriaxone or cefotaxime)

C: Penicillin

Bibliography: 1. Dattwyler, et al. Lancet. 1988 May 28; 1(8596): 1191-4 ; 2. Hassler, et al. Infection. 1990 Jan-Feb; 18(1): 16-20.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	3rd generation Cephalosporins	Penicillin	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of patients/№ of events**		**Effect**			
Improvement of arthritis after treatment (at 10 to 14 days) *												
1	RCT [1]	serious [a]	not serious	not serious	very serious [b]	none	9/9 (100.0%)	2/7 (28.6%)	**RR 3.04 (1.08 to 8.58)**	**583 more per 1,000 (from 23 more to 1,000 more)**	⊕◯◯◯ VERY LOW	CRITICAL
Resolution of arthritis (within 3-6 months)												
2	RCT [1,2]	serious [a]	serious [c]	not serious	serious [b]	none	30/52 (57.7%)	13/44 (29.5%)	RR 1.97 (0.24 to 16.25)	287 more per 1,000 (from 225 fewer to 1,000 more)	⊕◯◯◯ VERY LOW	CRITICAL
Improvement of arthritis (within 3-6 months) *												
1	RCT [2]	not serious	not serious	not serious	serious [b]	none	31/39 (79.5%)	17/34 (50.0%)	**RR 1.59 (1.10 to 2.31)**	**295 more per 1,000 (from 50 more to 655 more)**	⊕⊕⊕◯ MODERATE	CRITICAL
Relapse (after 12-24 months)												
1	RCT [2]	not serious	not serious	not serious	serious [b]	none	3/69 (4.3%)	1/66 (1.5%)	RR 2.87 (0.31 to 26.90)	28 more per 1,000 (from 10 fewer to 392 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Withdrawal due to Adverse Events												
1	RCT [2]	not serious	not serious	not serious	serious [b]	none	3/69 (4.3%)	4/66 (6.1%)	RR 0.72 (0.17 to 3.08)	17 fewer per 1,000 (from 50 fewer to 126 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Serious Adverse Events												

1	RCT [2]	not serious	not serious	not serious	serious [b]	none	2/69 (2.9%)	2/66 (3.0%)	RR 0.96 (0.14 to 6.59)	1 fewer per 1,000 (from 26 fewer to 169 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Diarrhea												
1	RCT [2]	not serious	not serious	not serious	serious [b]	none	9/69 (13.0%)	6/66 (9.1%)	RR 1.43 (0.54 to 3.81)	39 more per 1,000 (from 42 fewer to 255 more)	⊕⊕⊕◯ MODERATE	CRITICAL

* Improvement of arthritis was defined as "full remission" or "partial remission" (presented with arthritis, but after treatment continued to have persistent arthralgia without signs of inflammation) (Hassler study) and as absence of objective evidence of arthritis (either relapsing or remitting) (Dattwyler study).

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. The entire randomized sample was not used for this outcome in Dattwyler study

b. Fragility due to low event rate and small sample size

c. Inconsistency as measured by I^2= 94%

DOXYCYCLINE vs. AMOXICILLIN

In patients with Lyme arthritis, should doxycycline be used over amoxicillin?
P: In patients with Lyme arthritis
I: Doxycycline
C: Amoxicillin

Bibliography: 1. Steere, et al. Arthritis Rheum. 1994 Jun; 37(6): 878-88.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Doxycycline	Amoxicillin	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Resolution of Arthritis (within 3 months)												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	18/20 (90.0%)	16/18 (88.9%)	RR 1.01 (0.81 to 1.26)	9 more per 1,000 (from 169 fewer to 231 more)	⊕⊕◯◯ LOW	CRITICAL
Objective Findings of Dissemination of Lyme Disease (Development of neuroborreliosis)												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	1/18 (5.6%)	4/16 (25.0%)	RR 0.22 (0.03 to 1.79)	195 fewer per 1,000 (from 198 more to 243 fewer)	⊕⊕◯◯ LOW	CRITICAL
Allergic Reaction												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/20 (0.0%)	4/20 (20.0%)	NA [c]	200 fewer per 1,000	⊕⊕◯◯ LOW	CRITICAL
Gastrointestinal Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	0/20 (0.0%)	3/20 (15.0%)	NA [c]	150 fewer per 1,000	⊕⊕◯◯ LOW	CRITICAL

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received a High risk of bias rating due to the high number of participants excluded from final analysis due to inability to assess the outcome of interest and to lost to follow-up (8 out of 48 randomized patients).
b. Small number of events.
c. One arm has zero events; unable to estimate relative risks.

CEFTRIAXONE 14-days vs. 28-days

In patients with Lyme arthritis, should 14 days of IV ceftriaxone be used rather than 28 days of IV ceftriaxone?

P: In patients with Lyme arthritis

I: 14 days of ceftriaxone

C: 28 days of ceftriaxone

Bibliography: 1. Dattwyler, et al. Wien Klin Wochenschr. 2005 Jun; 117(11-12): 393-7.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	14 days IV Ceftriaxone	28 days IV Ceftriaxone	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of patients/№ of events**		**Effect**			
Resolution of Arthritis (at 3 months) *												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	45/76 (59.2%)	38/53 (71.7%)	RR 0.83 (0.64 to 1.06)	122 fewer per 1,000 (from 43 more to 258 fewer)	⊕⊕◯◯ LOW	CRITICAL
Resolution of Arthritis (at 12 months) *												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	54/65 (83.1%)	34/43 (79.1%)	RR 1.05 (0.87 to 1.27)	40 more per 1,000 (from 103 fewer to 213 more)	⊕⊕◯◯ LOW	CRITICAL
Joint Pain and Swelling (at last evaluation)												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	5/80 (6.3%)	2/63 (3.2%)	RR 1.97 (0.40 to 9.81)	31 more per 1,000 (from 19 fewer to 280 more)	⊕⊕◯◯ LOW	CRITICAL
Total Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	42/80 (52.5%)	37/63 (58.7%)	RR 0.89 (0.67 to 1.20)	65 fewer per 1,000 (from 117 more to 194 fewer)	⊕⊕◯◯ LOW	CRITICAL
Withdrawal due to Adverse Event												

1	RCT [1]	serious [a]	not serious	not serious	serious [c]	none	3/80 (3.8%)	10/63 (15.9%)	**RR 0.24 (0.07 to 0.82)**	**121 fewer per 1,000 (from 29 fewer to 148 fewer)**	⊕⊕◯◯ LOW	CRITICAL
Serious Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	1/80 (1.3%)	0/63 (0.0%)	NA [d]	13 more per 1,000	⊕⊕◯◯ LOW	CRITICAL
Dermatologic Adverse Events												
1	RCT [1]	serious [a]	not serious	not serious	serious [b]	none	6/80 (7.5%)	8/63 (12.7%)	RR 0.59 (0.22 to 1.61)	52 fewer per 1,000 (from 77 more to 99 fewer)	⊕⊕◯◯ LOW	CRITICAL

* Clinical response was defined as "cure" (resolution of all signs and symptoms), improvement (clinical abnormalities subsiding but with incomplete resolution), failure (no apparent response to therapy). Last evaluation includes all randomized patients who had received treatment, regardless of their discontinuation date.

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Study received high risk of bias rating due to the high number of participants excluded from final analysis due to negative serology (58 out of 201 randomized patients) and to lost to follow-up (14 patients at 3 months and 35 at 12 months) and lack of blinding.

b. 95% CI is wide and crossing the null value.

c. Fragility present, OIS criteria not meant.

d. One arm has zero events; unable to estimate relative risk.

XXV. What are the approaches to patients in whom Lyme arthritis has not completely resolved?

Bibliography: 1. Steere, et al. Arthritis Rheum. 1994 Jun 1; 37(6): 878-88; 2. Steere, et al. Arthritis Rheum. 2006 Oct; 54(10): 3079-86; 3. Gaude, et al. Paediatr Child Health. 2015 Oct; 20(7): 377-80.

Study, Location	Study Design	Risk of bias*	Lyme Disease Diagnostic method	Population characteristics	Treatment(s) administered	Treatment Outcome and Study Conclusions
Steere, et al., 1994 Boston, MA	Prospective cohort study† †2 of 16 patients had been enrolled in a previous RCT comparing oral doxycycline to oral amoxicillin	6	Patients were seropositive by ELISA for antibodies to *B. burgdorferi*. Patients with features of neurologic Lyme were excluded.	16 patients ≥ 13 years old who had an initial attack or intermittent episodes of arthritis in ≥ 1 joints and had at ≥ 1 inflamed joint at the time of study entry. Patients had continuous joint swelling without improvement for ≥3 months after treatment with other antibiotics, including tetracycline, doxycycline, or amoxicillin for at least 30 days, intramuscular benzathine penicillin for 3 wk, or IV penicillin G for ≥2 wk.	IV ceftriaxone (2g/day for 14 days)	None of the 16 patients experienced resolution of arthritis within 1-3 months of ceftriaxone treatment. 7 patients (1of whom received intra-articular corticosteroids 2 months after treatment) experienced resolution of joint swelling within 4-12 months without further antibiotic treatment. Seven other patients were re-treated with IV ceftriaxone (2g/day for 1 month) ≥3 months after initial treatment. Three of 7 patients experienced resolution of arthritis within 3-36 months of re-treatment and required no further antibiotic treatment. In total, 62.5% (10 of 16) patients experienced resolution of arthritis within 3 years and did not require surgical or DMARD intervention. Six of 16 patients (4 of whom had been re-treated with 1 month of ceftriaxone) underwent synovectomy due to persistent inflammation. Five of these patients resolved after the procedure, but one patient experienced persistent synovitis for 2 years.

Steere, et al, 2006 Boston, MA	Retrospective cohort study	7	Patients were serologically confirmed by ELISA and positive Western Blot. In case of joint puncture, presence of *Borrelia* DNA was assessed by PCR.	117 patients with monoarticular or oligoarticular arthritis. Fifty patients had antibiotic-responsive arthritis (arthritis resolved after ≤4 weeks of IV antibiotic or ≤8 weeks of oral antibiotics). 88% (44 of 50) of antibiotic –responsive patients had responded to initial treatment with oral doxycycline (100 mg BID) or oral amoxicillin (500 mg TID) for a median of 4 weeks (range 4-8 weeks).	IV ceftriaxone (2g/day for ≤4 weeks)	12% (6 of 50) of antibiotic-responsive patients had received either initial IV ceftriaxone or, more commonly, IV ceftriaxone following treatment with oral antibiotics. 61% (41 of 67) of antibiotic-refractory patients had received IV treatment, with or without oral therapy. The authors recommended retreatment with oral antibiotics for 30 days in the event of mild residual swelling after initial oral antibiotic treatment. They recommended IV ceftriaxone as a second line treatment in patients who experienced moderate to severe joint swelling despite a one-month course of oral antibiotics.
Glaude, et al., 2015 Nova Scotia, Canada	Retrospective cohort study	4	Patients had clinical evidence of Lyme disease and were serologically confirmed by ELISA and positive Western Blot. 1 patient was serologically negative but had probable Lyme disease (physician-observed rash,	Seventeen pediatric patients (94.1% male; median age 11.5 years, range 2-15 years) with Lyme arthritis were observed. All patients received antibiotic treatment for Lyme arthritis. 10 of 17 (58.8%) of patients presented with mono-articular arthritis, 23.5% (N=4) presented with arthritis affecting 2-4 joints, and 17.6% (N=3) presented with ≥5 joints affected (range 5-13).	14 of 17 (82.4%) received initial oral antibiotics 3 of 17 (17.6%) received initial IV ceftriaxone, all of whom were experiencing concurrent neurologic symptoms.	<u>Arthritis resolved in 10 patients after a single course of antibiotics.</u> Seven patients received a second course of 14-28 days of antibiotics. Three patients received a third course of antibiotics for continued joint effusion or pain. Patients were followed for a median of six months (1- 53 months) after initiation of antibiotic therapy. <u>At the last follow-up, 15 patients (88.2%) had complete resolution of Lyme arthritis.</u> Two patients experienced antibiotic-refractory arthritis with persistent synovitis and functional limitations. One of these two patients showed evidence of joint damage despite intra-articular steroid injection and synthetic and biologic DMARDs.

			tick exposure). All patients were residents of an area endemic for Lyme disease.		

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXVI. How should post-antibiotic (previously termed antibiotic-refractory) Lyme arthritis be treated?

Bibliography: 1. Schoen, et al. Arthritis Rheum. 1991 Aug; 34(8): 1056-60; 2. Steere and Angelis, Arthritis Rheum. 2006 Oct; 54(10): 3079-86; 3. Tory, et al. J Rheumatol. 2010 May; 37(5): 1049-55; 4. Nimmrich, et al. Rheumatol Int. 2014 Jul; 34(7): 987-94.

Study, Location	Study Design	Risk of bias *	Diagnosis method	Population characteristics	Initial Antibiotic regimen(s) and %Antibiotic Refractory	Outcome
Schoen, 1991 New Haven, CT	Prospective cohort study	5	Patients were serologically confirmed by ELISA.	20 patients with chronic, antibiotic-refractory Lyme arthritis (defined as continual joint pain and swelling in 1 or more joints for at least 1 year, preceded by brief attacks of oligoarticular arthritis and accompanied by an elevated IgG antibody response to *B. burgdorferi*) living in a Lyme endemic area who received synovectomy. The median duration of arthritis symptoms prior to synovectomy was 38 months (range 5-84).	65% (13 of 20) of patients had received oral tetracyclines or penicillin, and 65% (13 of 20) of patients had received IV penicillin or ceftriaxone (either alone or in addition to oral antibiotics) during the course of their arthritis. 75% of patients (15 of 20) had received NSAIDS, and 50% had received intra-articular corticosteroids. The median time from completion of antibiotic therapy to surgery was 5 months (range 0-36).	65% (13 of 20) of patients had complete resolution of joint inflammation within 1 month after synovectomy. At follow-up assessment 2-3 years later, all of these patients had normal findings on joint examination, or only minimal decreases in joint range of motion. 15% (3 of 20) of patients experienced reduction in inflammation within one month, but were more functionally disabled after their operation due to muscular atrophy or due to meniscal or ligament tears. 20% (4 of 20) of patients experienced persistent or recurrent synovitis despite synovectomy. None of the 20 patients subsequently experienced extra-articular manifestations of Lyme disease.

						The authors concluded that synovectomy is an effective treatment for chronic, antibiotic-refractory Lyme arthritis.
Steere, 2006 Boston, MA	Retrospective cohort study	7	Patients were serologically confirmed by ELISA and positive Western Blot. In case of joint puncture, presence of *Borrelia* DNA was assessed by PCR.	117 patients with monarticular or oligoarticular arthritis. Fifty patients had antibiotic-responsive arthritis (arthritis resolved after ≤4 weeks of IV antibiotic or ≤8 weeks of oral antibiotics), and 67 patients had antibiotic-refractory arthritis (persistent joint swelling for 3 months after ≥ 4 weeks of IV antibiotic or ≥8 weeks of oral antibiotic, or both).	57.2% (67 of 117) of patients were antibiotic refractory. 39% (26 of 67) of antibiotic-refractory patients had received either oral doxycycline (100 mg BID) or amoxicillin (500 mg TID) alone. 61% (41 of 67) of antibiotic-refractory patients had received oral doxycycline or amoxicillin followed by IV ceftriaxone (2 g/day) or had received IV antibiotics alone.	62 of 67 antibiotic-refractory patients were evaluable at study follow-up. 72.6% (45 of 62) of these patients responded to post-antibiotic treatment with **NSAIDs, with or without IA corticosteroids (IACS)**. Of the 27.4% of patients who failed treatment with NSAIDs/IACS, 70.6 % (12 of 17) received a **synovectomy** immediately after this treatment failure. The other 5 patients received **DMARDs**. 2 patients who experienced treatment failure post-synovectomy went on to receive DMARDs as well. 7 of 12 patients who received a **synovectomy** resolved after the procedure. In 1 patient who initially failed, arthritis eventually resolved. 85.7% (6 of 7) patients who received **DMARDs** responded to treatment. The one patient who failed DMARD treatment received a synovectomy and eventually responded.

						Overall, only 3.2% (2 of 62) of antibiotic-refractory patients experienced total treatment failure.
Tory, 2010 Boston, MA	Retrospective cohort study	7	Patients were serologically confirmed by ELISA and positive Western Blot.	99 pediatric (mean age 9.6; range 2-18) patients living in an endemic area who were diagnosed with Lyme arthritis. 91% (90 of 99) of patients had presented with arthritis as the main manifestation of Lyme Disease. 6% (6 of 99) and 28% (28 of 99) of patients recalled erythema migrans and/or flu-like illness, respectively. Mean disease duration prior to treatment was 2 weeks (range 0-52).	Initial treatment in 92% of patients was a 28-day course of doxycycline (100 mg BID) or amoxicillin (50 mg/kg/day, divided into 3 doses). Of the remaining 8 patients, 3 were initially treated with IV ceftriaxone (75–100 mg/kg/day) due to an initial diagnosis of septic arthritis (2 cases) or due to prolongation of symptoms for nearly 1 year (1 case). 23 (23.2%) patients had ongoing evidence of synovitis 3 months after completion of oral antibiotic therapy (N=8) or IV antibiotic therapy (N=4) or both (N=11). Patients received MTX, HCQ, SSZ, NSAIDs, IA corticosteroids, or DMARDs.	Of three antibiotic-refractory patients who had continued synovitis despite prolonged antibiotic therapy, two achieved remission at follow-ups of 9 and 12 months, after having received **MTX and HCQ+SSZ**, respectively. One patient who received SSZ was lost to follow-up. 54.5% (6 of 11) of antibiotic-refractory patients who received **NSAIDs alone** achieved remission within 6 months. 66.7% (4 of 6) of patients who received **IA corticosteroids** achieved remission, and 2 of these 6 patients were subsequently treated with DMARDs. Of these two patients, one had been treated with MTX and was in complete remission at a follow-up one year later.
Nimmrich, 2014	Prospective cohort study	7	Patients were serologically confirmed by ELISA and positive Western Blot for IgG antibodies against *B. burgdorferi*. In case of	31 pediatric (mean age 9.6 years; range 3-15) patients with Lyme arthritis, defined as clinically detected joint swelling and/or joint effusion in the presence of	In 26 cases, initial treatment was with IV antibiotic for 2-8 weeks; the other 5 patients had received prior oral antibiotics. 22.6% (7 of 31) of all patients had received at least 2	All patients with refractory arthritis who received **IA triamcinolone** initially showed notable clinical improvement. 25% (2 of 8) of these patients required additional IA steroid injections due to relapse.

| Sankt Augustin, Germany | | | joint puncture, presence of *Borrelia* DNA was assessed by PCR. | serologically confirmed Lyme disease. Mean duration of arthritis upon study entry was 1.9 months. | antibiotic courses.

8 patients (25.8%) with refractory arthritis received one course of intra-articular (IA) triamcinolone. | Four patients who received only one IA steroid injection remained asymptomatic until the last follow-up; a mean of 18.1 months. |

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Prolonged symptoms following treatment of Lyme disease

XXVII. Should patients with persistent symptoms following standard treatment of Lyme disease receive additional antibiotics?

RETREATMENT WITH ANTIBIOTICS vs NO ADDITIONAL ANTIBIOTICS

In patients with persistent symptoms following standard treatment of Lyme disease, should retreatment with antibiotic therapy be used over no additional antibiotics?

P: In patients with persistent symptoms following standard treatment of Lyme disease

I: Retreatment with additional antibiotic therapy

C: No additional antibiotics

Bibliography: 1. Klempner, et al. N Engl J Med. 2001 Jul 12; 345(2): 85-92; Klempner, MS. Vector Borne Zoonotic Dis. 2002; 2(4):255-63; Kaplan, et al. Neurology. 2003 Jun 24; 60(12): 1916-22; (these 3 articles describe the same cohort of patients included in 2 RCTs but report different complementary data); 2. Fallon, et al. Neurology. 2008 Mar 25; 70(13): 992-1003; 3. Krupp, et al. Neurology. 2003 Jun 24; 60(12):1923-30; 4. Sjöwall, et al. BMC Infect Dis. 2012 Aug10; 12:186; 5. Sriskandarjah, et al. International Journal of Clinical Practice, 2018, e13216.

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Retreatment with additional antibiotics	No additional antibiotics	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Improvement in Quality of Life (SF-36)) (at 6 months)												
2	RCT [1]	not serious	not serious	not serious	serious [a]	none	23/57 (40.4%)	21/58 (36.2%)	RR 1.11 (0.70 to 1.77)	41 more per 1,000 (from 136 less to 219 more)	⊕⊕⊕◯ MODERATE	CRITICAL
Improvement in Quality of Life (Physical component of SF-36) (at 6 months)												
2	RCT [1]	not serious	not serious	not serious	serious [a]	none	20/57 (38.6%)	15/58 (25.9%)	RR 1.36 (0.77 to 2.38)	90 more per 1,000 (from 75 less to 260 more)	⊕⊕⊕◯ MODERATE	CRITICAL

Mean SF-36 Physical Component Score (Higher scores indicate better quality of life) (at 6 months)

1	RCT [2]	not serious	not serious	not serious	serious [a]	none	N= 23	N= 14	MD 5.2 higher in patients treated with antibiotics (1.68 lower to 12.08 higher)		⊕⊕⊕◯ MODERATE	CRITICAL

Mean FSS-11 Score (Lower scores indicate less fatigue severity) (at 6 months)

2	RCTs [2,3]	serious [b]	not serious	not serious	serious [c]	none	N= 49	N= 36	**MD 0.83 lower in patients treated with antibiotics (1.57 lower to 0.09 lower)**		⊕⊕◯◯ LOW	CRITICAL

Improvement in Quality of Life (Mental component of SF-36) (at 6 months)

2	RCT [1]	not serious	not serious	not serious	serious [a]	none	19/57 (33.3%)	22/58 (57.9%)	RR 0.88 (from 0.54 to 1.44)	46 less per 1,000 (from 221 less to 129 more)	⊕⊕⊕◯ MODERATE	CRITICAL

Mean SF-36 Mental Component Score (Higher score indicate better quality of life) (at 6 months)

1	RCT [2]	not serious	not serious	not serious	serious [b]	none	N= 23	N= 14	**MD 8.6 lower in patients treated with antibiotics (16.4 lower to 0.8 lower)**		⊕⊕⊕◯ MODERATE	CRITICAL

Mean neurocognitive performance (cognitive "index" score) (at 6 months)

1	RCT [2]	not serious	not serious	not serious	serious [a]	none	N= 20	N= 12	MD 0.20 lower in patients treated with antibiotics (0.65 lower to 0.25 higher)		⊕⊕⊕◯ MODERATE	CRITICAL

Mean Improvement in Subjective Pain Score (Higher scores indicate more pain) (at 6 months)

3	RCTs [1,2]	not serious	not serious	not serious	serious [a]	none	N= 87	N= 79	MD 5.11 lower in patients treated with antibiotics (14.35 lower to 4.13 higher)		⊕⊕⊕◯ MODERATE	IMPORTANT

Patients Withdrawing Due to Adverse Events (Ranging from 3 to 6 months)

5	RCTs [1,2,3,4]	serious [d]	not serious	not serious	serious [a]	none	9/130 (6.9%)	8/118 (6.8%)	RR 0.92 (0.37 to 2.29)	5 fewer per 1,000 (from 43 fewer to 87 more)	⊕⊕◯◯ LOW	CRITICAL

Adverse Event Related to Route of Administration (at 6 months)

| 2 | RCTs [2,3] | serious [b] | not serious | not serious | serious [a] | none | 2/51 (3.9%) | 4/41 (9.8%) | RR 0.49 (0.06 to 4.24) | 50 fewer per 1,000 (from 92 fewer to 316 more) | ⊕⊕◯◯ LOW | CRITICAL |

Serious Adverse Events (Ranging from 3 to 6 months)

| 5 | RCTs [1,2,3,4] | serious [d] | not serious | not serious | serious [a] | none | 4/130 (3.1%) | 3/118 (2.5%) | RR 1.05 (0.18 to 6.15) | 1 more per 1,000 (from 21 fewer to 131 more) | ⊕⊕◯◯ LOW | CRITICAL |

Gastrointestinal Adverse Events (Ranging from 3 to 6 months)

| 4 | RCTs [1,3,4] | serious [d] | not serious | not serious | not serious | none | 21/107 (19.6%) | 8/104 (7.7%) | **RR 2.14 (1.05 to 4.36)** | **88 more per 1,000 (from 4 more to 258 more)** | ⊕⊕⊕◯ MODERATE | CRITICAL |

Vascular access device related adverse events

| 21 | Single-arm observational[e] studies (meta-analysis) [5] | not serious | not serious [f] | not serious [g] | not serious | Large magnitude of effect [h] | 441/ 12,147 (4.1%) | NA | NA [i] | **41 more per 1,000 (26 more to 64 more)** | ⊕⊕⊕◯ MODERATE | CRITICAL |

Drug-related adverse events

| 23 | Single-arm observational[e] studies (meta-analysis) [5] | not serious | not serious [f] | not serious [g] | not serious | Large magnitude of effect [h] | 929/ 14,543 (6.7%) | NA | NA [i] | **67 more per 1,000 (47 more to 95 more)** | ⊕⊕⊕◯ MODERATE | CRITICAL |

CI: Confidence interval; **MD:** Mean difference; **RR:** Risk ratio

* Fallon 2008: thrombus [n=2] and staphylococcal infection [n=1], Krupp: IV sepsis [n=3]

Summary of regimens used in each study:

    -Fallon: 10 weeks of IV ATB

    -Krupp: 4 weeks of IV ATB

    -Kaplan/Klemper (both studies): 4 weeks of IV ATB followed by 8 weeks of doxy

    -Sjowall: 3 weeks of doxy

Explanations

a. 95% CI is wide and crosses the null value, OIS criteria not met (sample size less than 200).

b. Krupp study received high risk of bias ratings due to potentially compromised blinding (self-reported outcomes) and potential attrition bias (asymmetrical lost-to-follow-up at the 6-month follow-up visit (more frequent in the placebo group).

c. Due to the following concerns, rating down for indirectness (uncertainty due to lack of validity of FSS-11 in Lyme disease, i.e. FSS was validated in other diseases and modified for Lyme disease) and imprecision (sample size less than 200 and uncertainty related to minimal important difference).

d. Krupp study received high risk of bias ratings due to potentially compromised blinding (self-reported outcomes) and potential attrition bias (asymmetrical lost-to-follow-up at the 6-month follow-up visit (more frequent in the placebo group)). Sjöwall study received two high risk of bias ratings due to potential attrition bias and inappropriate study design.

e. According to the authors of the meta-analysis, only 4 out of the 42 studies were considered at high risk of bias thus it was not rated down for risk of bias.

f. Not rated down for heterogeneity despite high I square values. Reasons: 1) statistical heterogeneity was mainly driven by some outlier studies that showed high adverse events rates that were likely by chance; 2) pooling of absolute rates substantially increases statistical heterogeneity due to differences in baseline risks of populations.

g. No serious concern regarding indirectness as only rates for "OPAT" intervention (Outpatient parenteral antibiotic therapy) were included.

h. Assuming that patients in the control group would not receive i.v. access and not receive antibiotics, the events in the control group would be zero; therefore, the magnitude of effect is at least expected to be large, which increases the confidence in the estimate of effect.

i. The risk for i.v. access or drug related adverse events in the control group was assumed to be zero, as those patients would not receive i.v. access nor antibiotics. Due to zero events in the control group, a RR could not be calculated.

RETREATMENT WITH 2 WEEKS OF PARENTERAL ANTIBIOTICS FOLLOWED BY PROLONGED ORAL ANTIBIOTICS vs 2 WEEKS OF PARENTERAL ANTIBIOTICS

In patients with persistent symptoms following standard treatment of Lyme disease, should 2 weeks of parenteral antibiotic therapy followed by 12 weeks of oral antibiotic therapy be used over 2 weeks of parenteral antibiotic therapy?

P: In patients with persistent symptoms treated with 2 weeks of parenteral antibiotic therapy

I: 2 weeks of IV Ceftriaxone followed by 12 weeks of oral antibiotic therapy

C: 2 weeks of IV Ceftriaxone

Bibliography: 1. Berende, et al. N Engl J Med. 2016 Mar 31; 374(13): 1209-20 (PLEASE); 2. Berende, et al., PLoS One. 2018 Apr 2;13(4):e0195260 (PLEASE).

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	2-week of IV ceftriaxone followed by 12 weeks of oral antibiotics†	2 weeks of IV ceftriaxone	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of events/№ of patients**		**Effect**			
Mean SF-36 Physical Component Score (Higher scores indicate better quality of life) (at 14 weeks)												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	N= 182	N= 98		MD 0.51 higher (1.21 lower to 2.22 higher)	⊕⊕⊕◯ MODERATE	CRITICAL
Mean SF-36 Mental Component Score (Higher scores indicate better quality of life) (at 14 weeks)												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	N= 182	N= 98		MD 0.24 higher (1.68 lower to 2.16 higher)	⊕⊕⊕◯ MODERATE	CRITICAL
Mean Fatigue Score (Checklist Individual Strength; range 8-56; Lower scores indicate less fatigue severity) (at 14 weeks)												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	N= 96	N= 49		MD 0.3 higher (3.12 lower to 3.72 higher)	⊕⊕⊕◯ MODERATE	CRITICAL
Mean QALYs per patient (within 1-year)												
1	RCT [1]	not serious	not serious	not serious	serious [a]	none	N= 175	N= 96		MD 0.00 higher (0.06 lower to 0.07 higher)	⊕⊕⊕◯ MODERATE	CRITICAL

Patients Withdrawing Due to Adverse Events (within 14 weeks)

1	RCT [1]	not serious	not serious	not serious	serious [a]	none	10/182 (5.5%)	4/98 (4.1%)	RR 1.30 (0.41 to 4.12)	12 more per 1,000 (from 24 fewer to 127 more)	⊕⊕⊕◯ MODERATE	CRITICAL

Serious Adverse Events (within 14 weeks)

1	RCT [1]	not serious	not serious	not serious	serious [a]	none	4/182 (2.2%)	0/98 (0.0%)	NA [b]	22 more per 1,000	⊕⊕⊕◯ MODERATE	CRITICAL

Gastrointestinal Adverse Events (within 14 weeks)

1	RCT [1]	not serious	not serious	not serious	serious [a]	none	32/182 (17.6%)	12/98 (12.2%)	RR 1.42 (0.77 to 2.64)	51 more per 1,000 (from 28 fewer to 201 more)	⊕⊕⊕◯ MODERATE	CRITICAL

Allergic Reactions (within 14 weeks)

1	RCT [1]	not serious	not serious	not serious	serious [a]	none	9/182 (4.9%)	1/98 (1.0%)	RR 3.17 (0.57 to 17.77)	22 more per 1,000 (from 4 fewer to 171 more)	⊕⊕⊕◯ MODERATE	CRITICAL

*All patients in Berende 2016 received 2 weeks of IV ceftriaxone before initiating the randomized controlled trial. During the 2-week period of open-label IV ceftriaxone treatment, none of the patients in either group experienced a catheter-associated infection.

†Patients receiving antibiotics in Berende 2016 received either Oral Doxycycline (100 mg BID) for 12 weeks or Oral Clarithromycin (500 mg BID) with Oral Hydroxychloroquine (200 mg BID) for 12 weeks. These groups were combined for the purpose of this analysis.

CI: Confidence interval; **MD:** Mean difference; **RR:** Risk ratio

Explanations

a. 95% CI is wide and/or crossing the null value
b. Comparator arm had zero events; unable to estimate relative risk.

Cutaneous manifestations of Eurasian Lyme disease

XXVIII. What is the preferred antibiotic regimen for the treatment of borrelial lymphocytoma?

Bibliography: 1. Arnez et al. Pediatr Infect Dis J. 2015 Dec; 34 (12):1319-22; 2. Glatz et al. Acta Derm Venereol. 2015 May 95(5): 565-71; 3. Maraspin, et al. Clin Infect Dis. 2016 Oct 1; 63(7): 914-21.

Study, Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Lyme disease characteristics	Presentation of borrelial lymphocytoma	Treatment Received	Treatment course and Study conclusions
Arnez et al., 2015 Slovenia	Prospective study	5	Thirty-three patients (22 male, 11 female), median age 5.5 (range 2-13). Borrelia lymphocytoma (BL) clinically diagnosed. Positive antibody titer by IFA or indirect chemiluminescence immunoassay (LIASON). 9% (3 of 33) patients had concomitant BL and EM. 3% (1 of 33) of patients had history of skin lesion (possibly EM) before inclusion into the study.	Interval between tick bite and time when skin lesion was first noticed by patient was significantly shorter in patients with tick bite at site of later BL than those with BL elsewhere. In 91% (30 of 33) of patients with BL the disease began with skin lesions, in 6% (2 of 33) with systemic symptoms and in 3% (1 of 33) with history of untreated solitary erythema migrans. 27% (9 of 33) of patients with BL had other clinical signs of infections: regional lymphadenopathy in 24% (8 of 33) and 3% (1 of 33) had conjunctivitis.	Antibiotic treatment according to Slovenian recommendations for early Lyme borrelia (LB) in children. 43% of patients treated with azithromycin, 39% treated with penicillin VK, 15% amoxicillin, and 3% ceftriaxone (patient with concomitant meningitis).	Positive borrelial serum antibody titers identified in 40% (12 of 30) patients with BL. 79% (26 of 33) of patients with BL were followed up. Median duration of BL (in 24 patients) was 16 days (range, 2-46 days), and the median duration of systemic symptoms (in 5 patients) was 15 days (range, 3-40). In 15% (4 of 33) of patients the symptoms appeared during or after treatment. 4% (1 of 33) patients had a Jarisch-Herxheimer reaction. BL can be treated with the same antibiotic regimen as used for treatment of erythema migrans. All patients had no signs or symptoms of Lyme disease 3 months after inclusion into the study.
Glatz et al., 2015 Austria	Retrospective study	7	Borrelial lymphocytoma (BL) infection and erythema migrans (EM) was diagnosed by faculty dermatologists based on clinical case definitions in 204 pediatric patients. Median age 6 (range 2-12) for BL only and median age 9	BL is a non-tender, soft, well circumscribed, bluish-red nodule or plaque of 1-5 cm. Patients whom BL was surrounded by solitary EM represented a combination of skin manifestations (solitary erythema migrans is an	At least 50% of patients in all treatment groups were treated with amoxicillin and about one-third of patients were treated with penicillin VK.	Median time of 2 months until resolution of BL was significantly longer than resolution time (1-2 weeks) for EM (solitary and multiple). No significant difference in resolution of BL between patients who had BL and patients who had concomitant BL and EM.

			(range 5-14) for concomitant BL and EM. 21.6% (44 of 204 patients) of patients had BL. 6% (12 of 204) of patients had concomitant BL and EM.	expanding round to oval, sharply demarcated, red to bluish-red erythema of at least 5 cm in diameter with or without central clearing).	Antibiotics administered orally in body-weight adjusted doses for 2-4 weeks for 203 of 204 patients.	Extracutaneous symptoms disappeared within a few days after starting therapy in most children without significant difference between groups; extracutaneous symptoms in children with BL were particularly short-lived. None of the patients developed any later sequelae during a follow-up period of 6-24 months.
Maraspin, et al., 2016 Ljubljana, Slovenia	Prospective cohort study	7	*Borrelial* infection was indicated by a reliable history of erythema migrans ≤6 weeks before borrelial lymphocytoma and presence of borrelial antibodies in serum and/or isolation of *Borrelia* from tissue, erythema migrans, blood, or cerebrospinal fluid. 72.2% (104 of 144) of patients experienced erythema migrans lesions concomitant with borrelial lymphocytoma. 11 of 144 (7.2%) patients presented with other objective symptoms of Lyme disease, such as arthritis, meningitis, facial palsy, or cardiac involvement. 56.2% (81 of 144) of patients recalled a recent tick bite.	144 adults (median age 49, range 35-60) were diagnosed with borrelial lymphocytoma at a single outpatient clinic over a 29-year period. The characteristic skin lesion was most commonly found on the breast or the ear lobe. All patients had typical clinical appearance of the skin lesion or clinical presentation with positive histological findings, and evidence of borrelial infection. Most patients had been experiencing symptoms associated with borrelial lymphocytoma <30 days (median 27 days, IQR 9, 68).	79.2% (114 of 144) of patients were treated with oral antibiotics-including doxycycline amoxicillin, penicillin, cefuroxime and azithromycin. 20.8% (30 of 144) of patients received IV ceftriaxone.	Borrelial lymphocytoma disappeared within a median of 21 days (IQR 10, 30) after treatment initiation. Symptoms typically resolved significantly more rapidly in younger patients and in patients who had a shorter duration of borrelial lymphocytoma prior to receiving treatment. 9.7% (14 of 144) of patients experienced treatment failure. Patients who had presented with signs of disseminated Lyme borreliosis experienced four times higher odds of treatment failure than those without (95%CI: 1.22, 13.07), emphasizing the importance of early diagnosis and treatment. Fourteen-day antibiotic treatment used for erythema migrans is recommended for patients presenting with borrelial lymphocytoma.

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXIX. What is the preferred antibiotic regimen for the treatment of acrodermatitis chronica atrophicans?

Bibliography: 1. Weber, et al. Ann N Y Acad Sci. 1988; 539: 324-45; 2. Aberer, et al. Infection. 1996 Jan-Feb; 24(1): 85-7; 3.Kindstrand, et al. Acta Neurol Scand. 2002 Nov; 106(5): 253-7; 4.Lenormand, et al. J Am Acad Dermatol. 2016 Apr; 74(4): 685-92.

Study and Location	Study Design	Risk of bias*	Lyme Disease Diagnosis method; Lyme disease characteristics	Presentation of acrodermatitis chronicum atrophicans	Treatment Received	Treatment course and Study conclusions
Weber, et al., 1988 Germany	Prospective cohort study	6	IgG antibody titers against *B. burgdorferi* of ≥64 were required for diagnosis of acrodermatitis chronica atrophicans.	34 patients with acrodermatitis chronica atrophicans were observed. Only patients with the typical bluish-red discoloration present at least on one extremity, in addition to presence of IgG antibody titers, were included.	Oral penicillin (N=14) (8 pts rec'd penicillin VK, usually 4.5 M IU; 6 pts rec'd propicillin, 3.0 M IU, 2-3 doses for 2-4 wk) vs tetracyclines (N=6) (1 pt rec'd tetracycline, 500 mg twice/day for 3 wks; 3 pts rec'd doxycycline, 100 mg twice/day for 10-21 days; 2 pts rec'd minocycline, 100 mg twice/day for 7-14 days); IV vs. Oral: 9 patients receiving parenteral penicillin were compared with 20 patients receiving either oral penicillin or tetracyclines.	6 of 9 patients receiving IV treatment and 9 of 20 patients receiving oral treatment experienced complete resolution of acrodermatitis chronica atrophicans (ACA). 2 of 6 patients treated with tetracycline and 7 of 14 patients treated with oral penicillin experienced complete resolution. Resolution of ACA occurred in a median of 18 months (range 1-73) with oral penicillin treatment and in a median of 11 months (range 5-34) with parenteral penicillin treatment. Resolution of ACA occurred in a median of 8 months (range 2-38) with tetracycline treatment. 17 of 34 patients (50%) experienced ACA remnants which resolved within a median of 14 months (range 2-73). 24 of 34 (70.6%) patients experienced other later manifestations of ACA, including musculoskeletal manifestations, peripheral neuropathy, lymphadenopathy, or relapse. Jarisch-Herxheimer reactions occurred in 1 of 6 patients treated with tetracycline, in 1 of 9 patients treated with IV penicillin, and in none of the patients treated with oral penicillin.

Aberer, et al., 1996 Austria	Prospective cohort study	6	Patients were serologically tested for antibodies against *B. burgdorferi* by ELISA. Presence of *Borrelia* DNA in urine was investigated by PCR in 26 patients before treatment. 6 of 26 patients were positive by PCR. 3 of the initially negative patients were positive upon reinvestigation after 2 months.	46 patients with clinically and histologically diagnosed ACA (67% female, 33% male, mean age=73) were observed. Other manifestations included neuropathy, juxtaarticular nodules and hypertonia, juxtaarticular nodules, IgM hyperimmunoglobulinemia, arthralgia, and pseudoscleroderma, depressive syndrome, and anetoderma.	Oral penicillin (14 pt rec'd 1.5 M IU t.i.d. for 30 days; 5 rec'd 1.5 M IU for 20 days; 19 pts total) vs.tetracyclines (13 pts rec'd oral doxycycline 100 mg for 20 or 30 days); IV vs. Oral: 14 patients receiving IV ceftriaxone (2 g/day for 15 days) are compared with 32 patients receiving either oral penicillin or oral tetracyclines	10 of 14 (71.4%) patients receiving IV treatment and 28 of 32 (87.5%) patients receiving oral treatment experienced complete resolution of ACA. 10 of 13 (76.9%) patients treated with tetracycline and 18 of 19 (94.7%) patients treated with oral penicillin experienced complete resolution. Persistence of ACA or associated symptoms for longer than 6 months was seen in 11 of 46 patients. All 11 of these patients were successfully retreated. The authors concluded that a 4-week course of oral antibiotic treatment is effective in treating ACA patients.
Kindstrand et al., 2002 Sweden	Prospective cohort study	6	Forty-seven patients included (66% female), mean age 67 (range, 31-89, median 71). Diagnosis clinical, serological, and histological means. Positive antibody titer by ELISA. Neurological follow-up was performed in 43 of 47 patients.	Patients included were previously untreated and had clinically, serologically, and histologically verified ACA with objective neurological and/or neurophysiological neuropathy signs with no other disease or predisposition for neuropathy. Duration of ACA before diagnosis (based on cutaneous discoloration) range from 0.3-20 years (mean 3.3 years, median 1.5 years).	Twenty-one patients received intravenous antibiotic treatment for 2 weeks with benzyl penicillin (11 patients) or cefuroxime (10 patients), followed by oral doxycycline (200 mg daily) for 2 weeks. Twenty-six patients were treated with oral doxycycline (200 mg daily) for 3 weeks. 3 patients were treated with oral doxycycline for 6-12 months after intravenous antibiotic treatment because of persistent arthralgia.	Improvement of erythema and edematous swelling occurred within 2 months. Inflammatory lesions disappeared in 85% (40 of 47) of patients within 6 months. Neuropathy symptoms (except numbness) disappeared or improved by 6-month follow-up. Allodynia disappeared in all patients. Pain in extremities disappeared in 24 of 28 patients. Paresthesia disappeared within 6 months. Muscle weakness was unchanged after treatment. In 14 of 15 patients, local dysalgesia in ACA areas disappeared after treatment. In patients with polyneuropathy or mono-neuropathy/regional neuropathy, clinical evaluation remained unchanged at 6 months. No difference in clinical response between initial intravenous antibiotic therapy followed by oral antibiotics and oral antibiotic treatment only.

Lenormand, et al., 2016 France	Prospective cohort study	5	Diagnosis of (ACA was confirmed by clinical observation, serological data review, positive culture and/or PCR of skin biopsy sample. Tick bite was recalled by patient in 4 of 20 (20%) cases and prior episode of erythema migrans was noted in 5 of 20 (25%) cases.	20 patients (12 female, 8 male age 33-86 years) with confirmed ACA were observed. Lesions had been present for a mean of 2 years (range 2 months to 10 years). Lesions were typical of ACA in all but 1 case. Unusual manifestations, such as numerous small violaceous patches and equidistant small spinous papules with background faint erythema, were observed in 2 patients.	18 of 20 patients received doxycycline for 21- 28 days. 2 of 20 patients received ceftriaxone for 14-21 days.	Partial or complete improvement of lesions was observed in all patients after antibiotic treatment with doxycycline or ceftriaxone.

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Lyme disease coinfections

Background information: *Bartonella* coinfection

Bibliography: 1. Podsiadły, et al. Ann N Y Acad Sci. 2003 Jun; 990: 404-6; 2. Podsiadły, et al. Vector Borne Zoonotic Dis. 2011 Jul; 11(7): 985-9; 3. Chmielewski-Badora, et al. Ann Agric Environ Med. 2012; 19(2): 271-4; 4. Eskow, et al. Arch Neurol. 2001 Sep; 58(9):1357-63; 5. Gupta, et al. Eye (Lond). 2009 Jul; 23(7): 1607; 6. Rigaud, et al. Clin Microbiol Infect. 2016 Aug;22(8):735.e1-9.

Study, Location	Study Design	Risk of bias*	Population characteristics, Disease presentation	Bartonella Diagnosis Method, Lyme Disease Diagnosis method (if applicable)	% Co-infected (N cases/N Lyme cases)	Treatment(s) received and Study Conclusions
Podsiadły, et al., 2003 Warsaw, Poland	Cross-sectional study	5	17 patients with clinical symptoms suggesting neuroborreliosis were serologically surveyed. Symptoms included meningitis (N=9), multiple sclerosis (N=2), headache (N=2), double vision with difficulty walking (N=1), mediastinal lymphadenitis with pulmonary interstitial changes (N=1), depression with paresis of face muscles (N=1), and bilateral facial nerve palsy (N=1).	Patients were tested for antibody titers against *B. henselae* by IFA. Patients were tested for antibodies against *B. burgdorferi* by ELISA.	1 of 17 (0.06%) patients was positive for *B. henselae* alone. This patient had received ceftriaxone for assumed neuroborreliosis 2 months previously. 12 of 17 patients (70.6%) were positive for *B. burgdorferi infection*. 2 of 12 (16.7%) patients with neuroborreliosis were positive for both *B. burgdorferi* and *B. henselae*.	The authors concluded that *B. henselae* can be an etiological agent of a central nervous system infection. They suggest that mixed infections with Lyme and *Bartonella* should be taken into account in patients with neurologic symptoms, particularly in the event of incomplete resolution of neuroborreliosis symptoms after administration of appropriate antibiotic treatment for Lyme disease.
Podsiadły, et al, 2011 Warsaw, Poland	Cross-sectional study	5	129 occupationally exposed foresters (80% male; mean age 29 years) working in an endemic region who were at high exposure to tick bites.	Indirect immunofluorescence assay (IFA) was used to measure titers of anti-*A. phagocytophilum, B. microti* and *Bartonella* IgG.	10% of forest workers reported hand antibodies to both *B. burgdorferi* and *Bartonella* spp. 34% (44/129) of workers were seropositive to *B. burgdorferi*.	The most frequent findings of seropositivity in occupationally exposed forest workers in Poland were with *B. burgdorferi* and *Bartonella.* This information is unable to discern when seropositive statuses were acquired.

				Anti-*B. burgdorferi* IgM and IgG were measured by ELISA.	*Bartonella* spp. antibodies were found in about 30% (38/129) of the individuals.	
Chmiele wski-Badora, et al., 2012 Lublin, Poland	Cross-sectional study	6	Group1: 39 occupationally exposed farmers living in an endemic region. Group 2: 119 occupationally exposed foresters (mean age 29 years). All of these patients had reported at least one tick bite. Group 3: 32 healthy blood donors (mean age 29 years).	Semi-quantitative indirect immunofluorescence test (IFT) was used to measure titers of anti-*A. phagocytophilum, B. microti* and *Bartonella* IgG. Anti-*B. burgdorferi* IgM and IgG were measured by ELISA.	27.7% of farmers, 23.1% of forestry workers, and 37.5% of the control group were seropositive for *Bartonella.* *38.5% of farmers, 47.9% of forestry workers, and 12.5% of the control group were seropositive for B. burgdorferi*	Occupationally exposed participants in Poland were frequently seropositive for *B. burgdorferi* and *Bartonella.* 7.7% of farmers and 9.2% of forestry workers were seropositive for these two pathogens
Rigaud, et al., 2016 Alsace, Lorraine, Champag ne-Ardenne, Franche-Comte and Bourgog ne regions of France	Cross-sectional study	6	Anonymous blood samples were collected from 2,975 forestry workers (2,916 men and 59 women). *Borrelia burgdorferi sl.* and TBEV serology was available for all participants (2,975) and *A. phagocytophilum, F. tularensis,* and *Bartonella henselae* serology was available for 2,908 participants.	Screening for anti-*A. phagocytophilum* IgG antibodies was performed by ELISA using a recombinant protein P44 antigen. Doubtful or positive sera were retested by immunofluorescence assay (IFA). Anti-*B. burgdorferi* IgG was measured by ELISA and confirmed by Western Blot.	14.1% (419/2975) seropositive for *Borrelia burgdorferi sl*, 1.7% (50/2908) for *Anaplasma phagocytophilum* and 1.7% (48/2908) for *Bartonella henselae.* The seroprevalences of *Babesia divergens* and *Babesia microti* studied in a subgroup of participants seropositive for at least one of these latter pathogens were 0.1% (1/810) and 2.5% (20/810), respectively.	Seropositivity for a single pathogen was recorded 21.5% (627/2908), and for two pathogens (other than Babeisa) in 2.0% (57/2908). The most common multiple seropositive pairings were B. burgdorferi sl together with F. tularensis (22/57), Tick-borne encephalitis virus (11/57), Bartonella henselae (9/57) or A. phagocytophilum (7/57).

Eskow, et al., 2001 Flemington, NJ and Mt. Laurel, NJ	Case series (4 cases)	NA	4 patients residing in an area endemic for Lyme disease who were experiencing ongoing symptoms attributed to chronic Lyme disease were evaluated for co-infection with *Bartonella*. The most common symptoms before therapy were cognitive dysfunction, headache, and fatigue. All subjects had clinical presentation consistent with mild encephalopathy.	Patients were tested for antibody titers against *B. henselae* by IFA. Blood samples were tested for *B. henselae*-specific DNA by PCR. CSF was analyzed, and 3 of 4 patients had the presence of both *B. henselae* and *B. burgdorferi* in their CSF. *B. henselae* DNA was detected in live deer ticks near the houses of 2 infected patients.	100% (all patients were co-infected with Lyme disease and *Bartonella*)	Patient 1: The patient received empirical treatment for Lyme disease with doxycycline due to clinical presentation and tick bite history. The patient experienced no improvement. After *Bartonella* infection was diagnosed, the patient received IV cefotaxime (6 g/day for 6 weeks) and experienced prompt resolution. Patient 2: The patient presented with a history of late-stage Lyme disease. He was symptomatic despite an 8-week course of IV ceftriaxone (2 g/day). After diagnosis with *Bartonella* infection, the patient was started on IV cefotaxime (8g/day for 28 days) and experienced consistent, but gradual improvement. Patient 3: The patient had been previously treated for Lyme disease with doxycycline, but symptoms had persistence after completion of therapy. After analysis of CSF revealed neuroborreliosis, the patient received IV ceftriaxone for 28 days. *B. burgdorferi* DNA was no longer detectable after ceftriaxone treatment, but *B. henselae* DNA persisted. The patient was put on IV doxycycline but noticed a return of symptoms shortly after initiation of this treatment. Antibiotic therapy was switched to azithromycin (500 mg/day for 14 days), and the patient experienced complete resolution. Patient 4: The patient was negative for evidence of *B. burgdorferi* by PCR, Western Blot, and CSF analysis. Ticks removed from this patient were positive for *B. henselae* only. The patient resolved fully with a 28-day course of doxycycline (300 mg/day).
Gupta, et al., 2009 Durham, NC	Case report	NA	51-year-old woman experienced sudden loss in visual acuity in the right eye. Ophthalmological examination revealed disc edema and macular star, prompting a diagnosis of neuroretinitis.	Patient tested positive for IgM and IgG antibody titers against *B. henselae* by IFA. Patient was positive for *B. burgdorferi* by Western Blot (2 of 3 bands for IgM, 2 of 10 bands for IgG).	100% (only one patient)	The patient initially received azithromycin and rifampin for *B. henselae* infection. Symptoms persisted, and one week later, the patient was confirmed as positive for antibodies against *B. burgdorferi* in CSF. She was then started on 1 month of IV ceftriaxone, in addition to azithromycin and rifampin. The patient experienced marked improvement within 8 days. Disc edema and macular star resolved over the course of 4 months, and visual acuity improved.

		The patient had experienced "constitutional symptoms of an undetermined etiology" several months before visual symptoms began.	The patient's CSF was positive for IgM antibodies one week after the initial Western Blot.		

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

XXX. Under what circumstances should a patient with Lyme disease be evaluated for co-infection with *A. phagocytophilum* or *B. microti*?

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Study and Location	Study Design	Risk of bias*	Diagnosis method	Population characteristics and Co-infection type	% Co-infected (N cases/N Lyme cases)
Krause, et al. 1996 Block Island, RI	Prospective cohort study	7	<u>Lyme:</u> Physician diagnosis of erythema migrans, and/or Laboratory confirmation through serology or PCR of blood <u>Babesiosis:</u> Presence of symptoms with Laboratory confirmation through thin blood smear, serology, or PCR	Residents with Lyme disease and/or babesiosis who had lived on Block Island for ≥1-2 months during the peak transmission season	Babesiosis: 11% (26/240)
Mitchell, et al. 1996 MN and WI	Cross-sectional study	6	<u>Lyme:</u> Physician diagnosis of erythema migrans with culture-confirmed infection with *B. burgdorferi* <u>Babesiosis:</u> Presence of symptoms and Laboratory confirmation through serology and blood smear <u>Anaplasmosis:</u> Presence of symptoms with Laboratory confirmation through PCR, and with/without confirmation by blood smear and/or serology	Patients living in the upper Midwest (Minnesota and Wisconsin) with culture-confirmed erythema migrans.	Babesiosis: 2.1% (2/96) Anaplasmosis: 5.2% (5/96) Babesiosis and Anaplasmosis: 2.1% (2/96)
Belongia, et al. 1999 Marshfield, WI	Case-control study	7	<u>Lyme:</u> Physician diagnosis of erythema migrans, and laboratory evidence of acute *B. burgdorferi* infection, through serologic testing or positive culture <u>Anaplasmosis:</u> Presence of symptoms with Laboratory confirmation through PCR and serology	Patients who were residents of Wisconsin who were diagnosed with erythema migrans and/or suspected Anaplasmosis. *Control group was assessed against cases to identify potential residential and behavioral risk factors for acquiring infection.*	Anaplasmosis: confirmed: 3% (8/283); probable: 4% (11/283)

Wang, et al. 2000 Nantucket Island, MA	Retrospective cohort study	6	<u>Lyme:</u> CDC criteria; History of erythema migrans, or serological confirmation with ≥1 late manifestation of the disease <u>Babesiosis:</u> History of Laboratory confirmation through blood smear or serology; <u>acute co-infection:</u> defined as Babesiosis and Lyme definitively occurring together, rather than presence of antibodies to both	Permanent residents of Nantucket Island who reported a clinician's diagnosis of Lyme disease or a history of a positive serologic test for Lyme.	<u>Babesiosis:</u> 2% (4/200) (patients reporting acute co-infection)
Krause, et al. 2002 Block Island, RI, Nantucket, MA, and southeastern CT	Prospective cohort study	7	<u>Lyme:</u> Physician diagnosis of erythema migrans, and/or Laboratory confirmation through serology or PCR of blood <u>Babesiosis:</u> Presence of symptoms with Laboratory confirmation through serology, thin blood smear, or PCR <u>Anaplasmosis:</u> Presence of symptoms with Laboratory confirmation through serology, thin blood smear, or PCR	Patients who experienced an erythema migrans rash or flu-like illness suggestive of Lyme disease, babesiosis, and/or anaplasmosis living in highly endemic areas	<u>Babesiosis:</u> 38% (61/161) <u>Anaplasmosis:</u> 4% (7/161) <u>Babesiosis and Anaplasmosis:</u> 3% (4/161)
Steere, et al. 2003 East Lyme, CT, and Wakefield, RI	Prospective cohort study	8	<u>Lyme:</u> Physician diagnosis of erythema migrans, and Laboratory confirmation through serology and culture <u>Babesiosis:</u> Presence of symptoms with Laboratory confirmation through PCR and serology <u>Anaplasmosis:</u> Presence of symptoms with Laboratory confirmation through PCR and serology	Patients with erythema migrans and culture-confirmed Lyme disease living in highly endemic areas.	<u>Babesiosis:</u> 2% (2/93) <u>Anaplasmosis:</u> 2% (2/93)
Horowitz, et al. 2013 Valhalla, NY	Prospective cohort study	8	<u>Lyme:</u> CDC criteria; History of erythema migrans, or serological confirmation <u>Anaplasmosis:</u> Four separate case definitions were assessed: A= Positive blood culture for *A. phagocytophilum* required; B= Positive blood culture and/or a 4x rise in antibody to *A. phagocytophilum* to ≥ 1:640; C= Either of the criteria required for definition B and/or an antibody titer of 1:2560, irrespective of a 4x rise in antibody titer; D= Either positive culture or an antibody titer of 1:640, irrespective of a 4x rise.	Patients living in an area endemic for Lyme disease and Anaplasmosis with potential tick exposure that had an erythema migrans lesion or reported a viral infection-like illness without features suggestive of upper respiratory tract infection or gastroenteritis.	<u>Anaplasmosis:</u> ● Definition A: 2.3% (7/302) ● Definition B: 5.0% (15/310) ● Definition C: 7.7% (24/311) ● Definition D: 10.0% (31/311)

* Risk of Bias of Observational Data was rated on a scale from 0 (worst) to 9 (best) using the Newcastle-Ottawa Quality Assessment Scale for Observational Studies.

Study Conclusions about the Clinical presentation of Co-infection with Babesia and/or Anaplasma	
Krause, et al. 1996	Patients who were co-infected with *B. microti* and Lyme disease reported 1.5 times as many different symptoms and signs of disease as subjects with Lyme disease alone (P<.001). Co-infected subjects presented with a more diverse array of symptoms and experienced statistically significantly longer disease duration than patients with Lyme disease alone.
Belongia, et al. 1999	The authors found that patients with laboratory evidence of Anaplasmosis with concurrent erythema migrans and laboratory evidence of *B. burgdorferi* were significantly less likely to report fever, chills, and fatigue than patients who presented with Anaplasmosis alone.
Krause, et al. 2002	The study reported that a combination of fever, chills, and headache was noted in 44% of Lyme disease patients with a co-infection, whereas this clinical presentation was found in only 13% of patients with Lyme disease alone. Patients with Lyme disease alone tended to report fewer symptoms than co-infected patients, and the duration of symptoms was statistically significantly longer (mean duration Lyme: 3.9 weeks; Lyme and Babesia: 5.5 weeks, p<0.055; Lyme and *Anaplasma*: 11.0 weeks, p<0.001) in patients with co-infections than in patients with Lyme disease alone.
Steere, et al. 2003	Of four patients co-infected with Lyme disease and either *A. phagocytophilum* or *B. microti*, three experienced 8-11 diverse symptoms, including fever, chills, headache, fatigue. One co-infected patient had persistent fatigue after treatment. The number of co-infected cases was too small to come to any statistically significant conclusion.
Horowitz, et al. 2013	Study found that co-infected patients in the group that includes those with a positive culture for *A. phagocytophilum* and/or with a 4-fold rise in antibody titer to ≥1:640 presented with significantly more symptoms and were more likely to report fever, sweats, rigor, and/or headache than patients with early Lyme disease alone.