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Expectations and limitations of diagnostics in Lyme borreliosis

Veroniek Saegeman, 19 May 2016

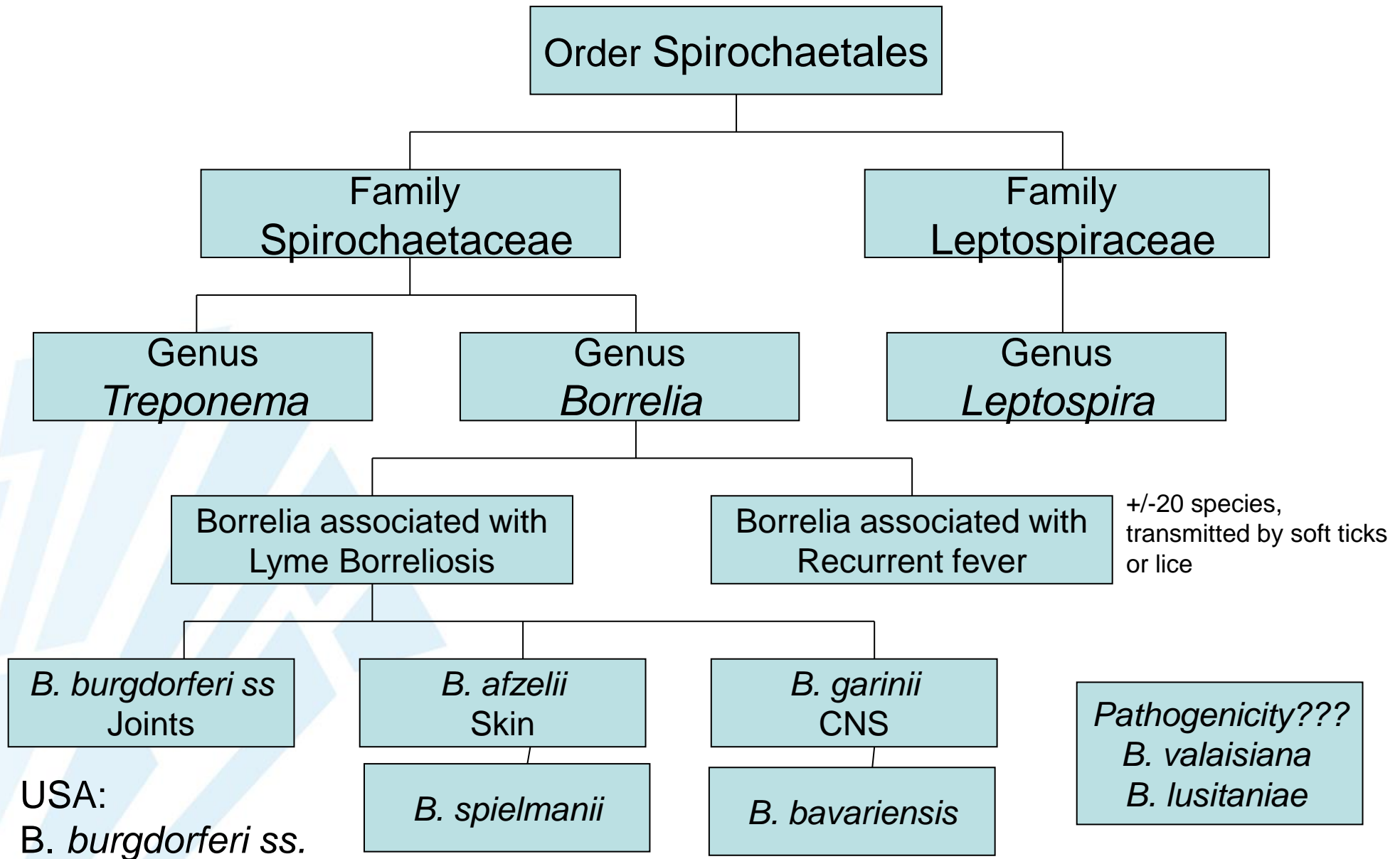
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Causative agent: *Borrelia burgdorferi sensu lato complex*



- **Direct methods:** causal agent is detected
 - Culture of *B. burgdorferi*
 - PCR detection of *B. burgdorferi*
- **Indirect methods:** detection of immune response against agent
 - = presence of antibodies against *B. burgdorferi*

- **Direct methods:** causal agent is detected
 - Culture of *B. burgdorferi*
 - Gold standard
 - Moderate sensitivity (skin > blood/CSF)
 - 2-6 weeks before result
 - Expertise

- **Direct methods:** causal agent is detected
 - PCR detection of *B. burgdorferi*
 - No standard primers
 - Problems:
 - Contamination → false positives
 - Inhibition of PCR reaction / low number of spirochaetes → false negatives

Direct methods: PCR

	<i>Erythème migrant</i>		<i>Méningoradiculites Formes compliquées précoces</i>		<i>Arthrites</i>		<i>Acrodermatite chronique atrophiante ACA</i>	
	<i>Serum</i>	<i>peau</i>	<i>Serum</i>	<i>LCR</i>	<i>Serum</i>	<i>Liquide* articulaire</i>	<i>Serum</i>	<i>Peau</i>
Culture	NR	38-80%	NR	≤10%	NR	<5%	NR	20-60%
PCR	NR	60-90%	NR	25%	NR	50-70%	NR	60-90%
ELISA	20-50%	NR	70-90%	50-90%	90-100%	NR	95-100%	NR

NR : non réalisable * : meilleurs résultats avec les biopsies synoviales

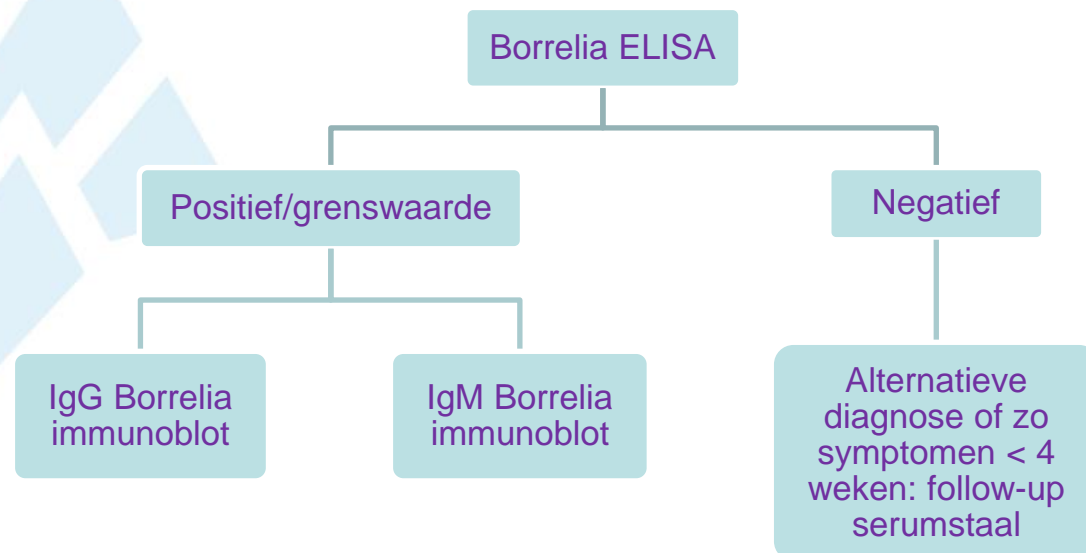
- Useful for skin and joint infection
- Not useful for neurological manifestations
 - Serological determination of intrathecal Ab synthesis is more sensitive
- Diagnostic value of PCR on blood/serum/plasma/urine:
not established → not recommended

- **Indirect methods**: detection of immune response against agent
= presence of antibodies against *B. burgdorferi*
Serology: 1° choice and highly available

Serological assays: compliant with



- European Concerted Action on Lyme Borreliosis (www.EUCALB.com)
- Two-tiered testing algorithm in USA and some European countries:
 - ELISA: a sensitive ELISA with $\geq 90\%$ **specificity**
 - Immunoblot confirmation with $\geq 95\%$ **specificity**
 - Local validation taking local epidemiology into account



Tired of Lyme borreliosis

MARCH 2011, VOL. 69, NO 3

Lyme borreliosis in the Netherlands

J. Coumou^{1,2}, T. van der Poll^{1,3}, P. Speelman³, J.W.R. Hovius^{1,3*}

Table 2. Testing for antibodies against *B. burgdorferi* in the normal population

Test	Lyme disease	No Lyme disease	Total
Positive	40	497	537
Negative	10	9453	9463
Total	50	9950	10,000

False positives = $(497/537) * 100 = 92.5\%$

False negatives = $(10/9463) * 100 = 0.1\%$

Sensitivity: 80%; specificity 95%, pre-test probability: 0.5%. Sensitivity and specificity are based on published literature (see text). The pre-test probability is an estimation based on recent RIVM data and the percentual distribution of symptoms of Lyme borreliosis.

Patients with a weak probability of Lyme borreliosis

- ELISA negative: Lyme borreliosis excluded
- ELISA positive: probably false positive

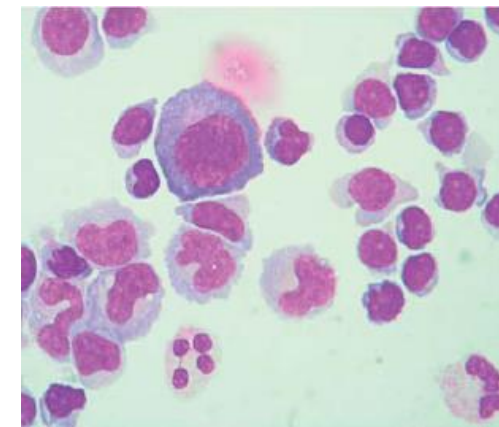
Serological assays: pitfalls

- Slow immune response
- Always interpret lab results together with duration of clinical symptoms
 - **6-8 weeks after onset of symptoms: no relevance of only IgM positivity**
 - **IgG development after >8 weeks of symptoms necessary to confirm Lyme borreliosis**
- Presence of specific Lyme antibodies \neq active Lyme disease
- Seronegativity does not exclude Lyme borreliosis (ECM)

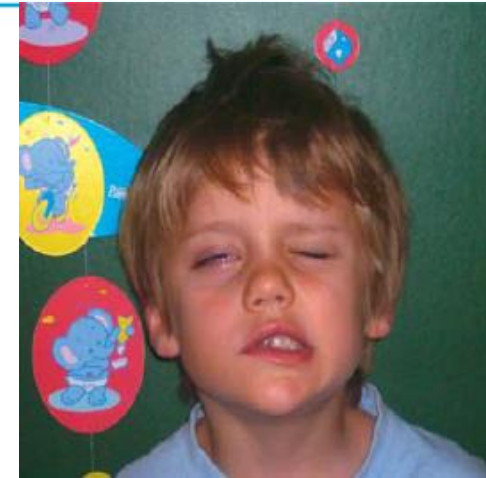
Serological assays: pitfalls

- Antimicrobial therapy at start of antibody response (beginning of infection)
 - can inhibit further immune response
 - or
 - persistence for years of *Borrelia* specific IgG/IgM production
- serological follow-up not useful for control of therapy

- Immunological marker: CXCL13
 - CXCL13: possible additional marker in case: antibody response, WBC count and clinical presentation cannot confirm Lyme neuroborreliosis (LNB)
 - Diagnosis of Lyme neuroborreliosis (EFNS)
 - Definite LNB:
 - Intrathecal antibody production
 - Pleiocytosis (lymphocytes)
 - Neurological symptoms compatible with LNB



- Immunological marker: CXCL13
 - Diagnosis of Lyme neuroborreliosis (LNB) (EFNS)
Definite LNB:
 - intrathecal antibody production
 - **May be absent in early cases of LNB**
 - Pleiocytosis (lymphocytes)
 - Neurological symptoms compatible with LNB
 - **DD: intrathecal synthesis or passive diffusion of antibodies through damaged blood brain barrier**
 - **No distinction between acute infection, convalescence or past infection**



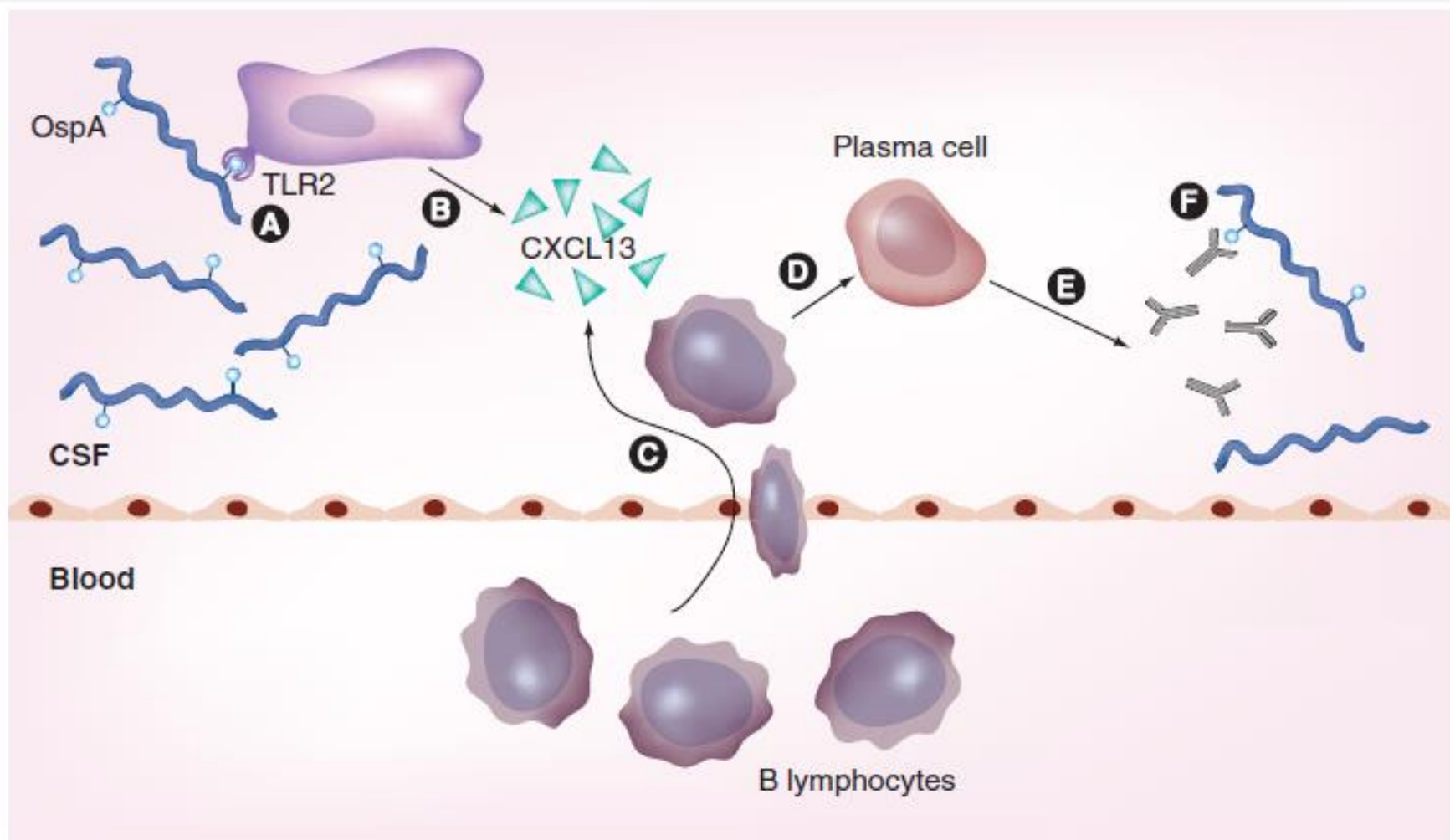
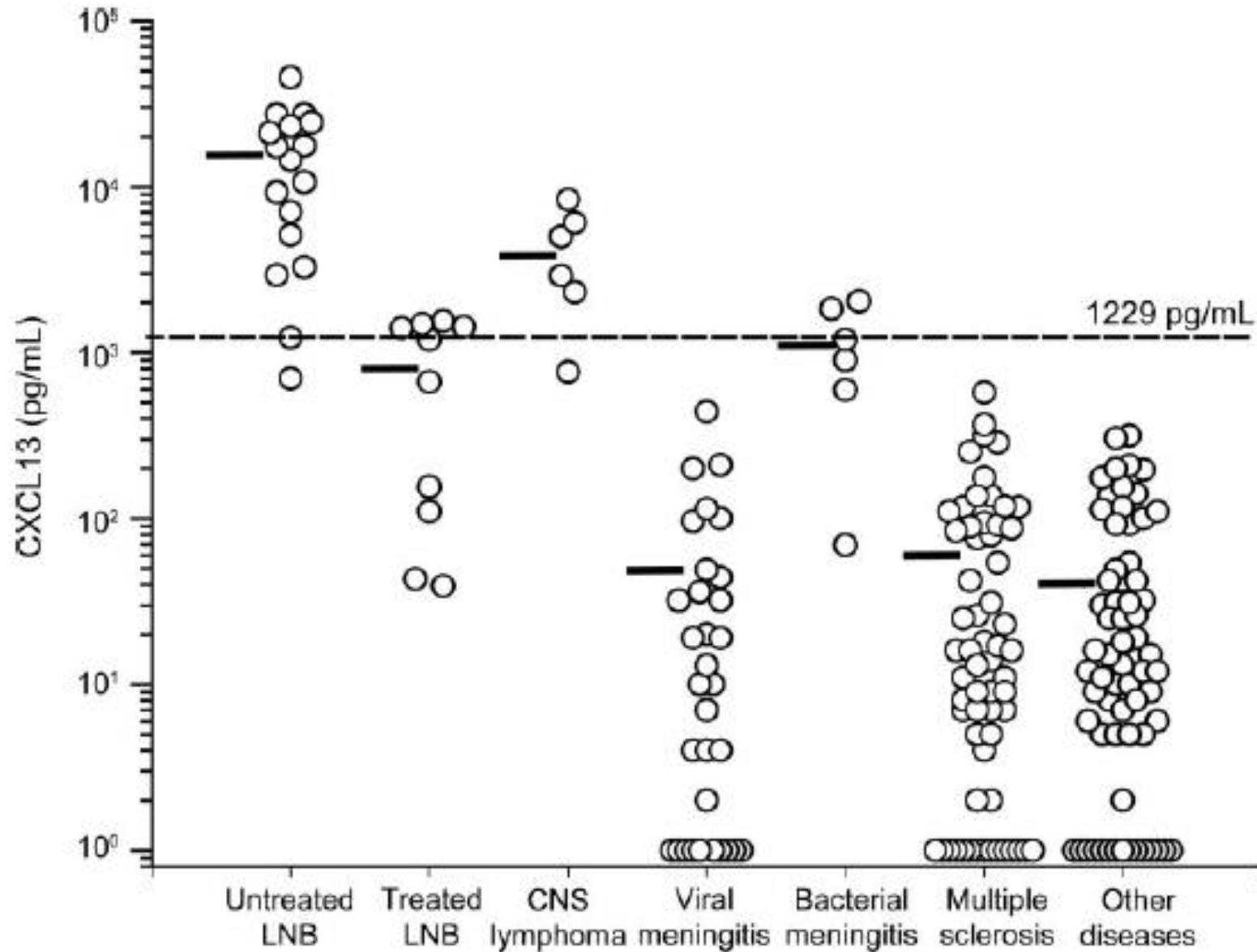


Figure 2. The inflammatory B-cell response in the cerebrospinal fluid in response to the CNS infection. *Borrelia* are recognized by monocytic cells (A), which produce the B-cell-attracting chemokine CXCL13 (B). B cells immigrate into the CSF (C) and mature into plasma cells (D). These plasma cells can produce *Borrelia burgdorferi*-specific antibodies (E) that can eventually destroy the invading spirochetes (F).

CSF: Cerebrospinal fluid; Osp: Outer surface protein; TLR: Toll-like receptor.
Adapted with permission from [62].

- Immunological marker
 - In early Lyme neuroborreliosis
 - 88-100% of patients had an increased level of CXCL13
 - CXCL13 is not specific for LNB
 - Increased in infections with spirochaetes, neurological diseases
 - CXCL13 decreases fast after initiation of antimicrobial treatment
 - Not clear whether CXCL13 can be used for late neuroborreliosis
 - not recommended
 - Insufficient evidence for use of other immunological markers (CD57, C3a, C4a)



Black line indicates mean; dashed line indicates cutoff. LNB = Lyme neuroborreliosis.

- Retrospective study UZ Leuven (n=82)
 - Selection based on serum/CSF immunoblot result
 - Intrathecal Ab synthesis (n=30)
 - Passive diffusion (n=24)
 - No intrathecal Ab synthesis (n=18)
 - Other CNS infection (n=8)
- Prospective study (n=47)

- Results retrospective study UZ Leuven (n=82)

reference ranges CXCL13 (EuroImmun)

Standard range:	< 20pg/ml - Exclusion of neuroborreliosis
Borderline range:	20 to 30pg/ml
Increased:	30 to 100pg/ml
Strongly increased:	> 100pg/ml - Suspected acute neuroborreliosis if corresponding symptoms are present.

- Results retrospective study UZ Leuven (n=82)

EFNS LNB	Result CXCL13 Euroimmun				total
	normal	borderline	increased	strongly increased	
dLNB	0	0	1	24	25
pLNB	3	0	1	1	5
no LNB	35	2	3	2	42
other CNS infection	5	0	2	3	10

dLNB: 100% patients: increased CXCL13 (sensitivity)

No LNB / other CNS infection: 81% CXCL13 negative (specificity)

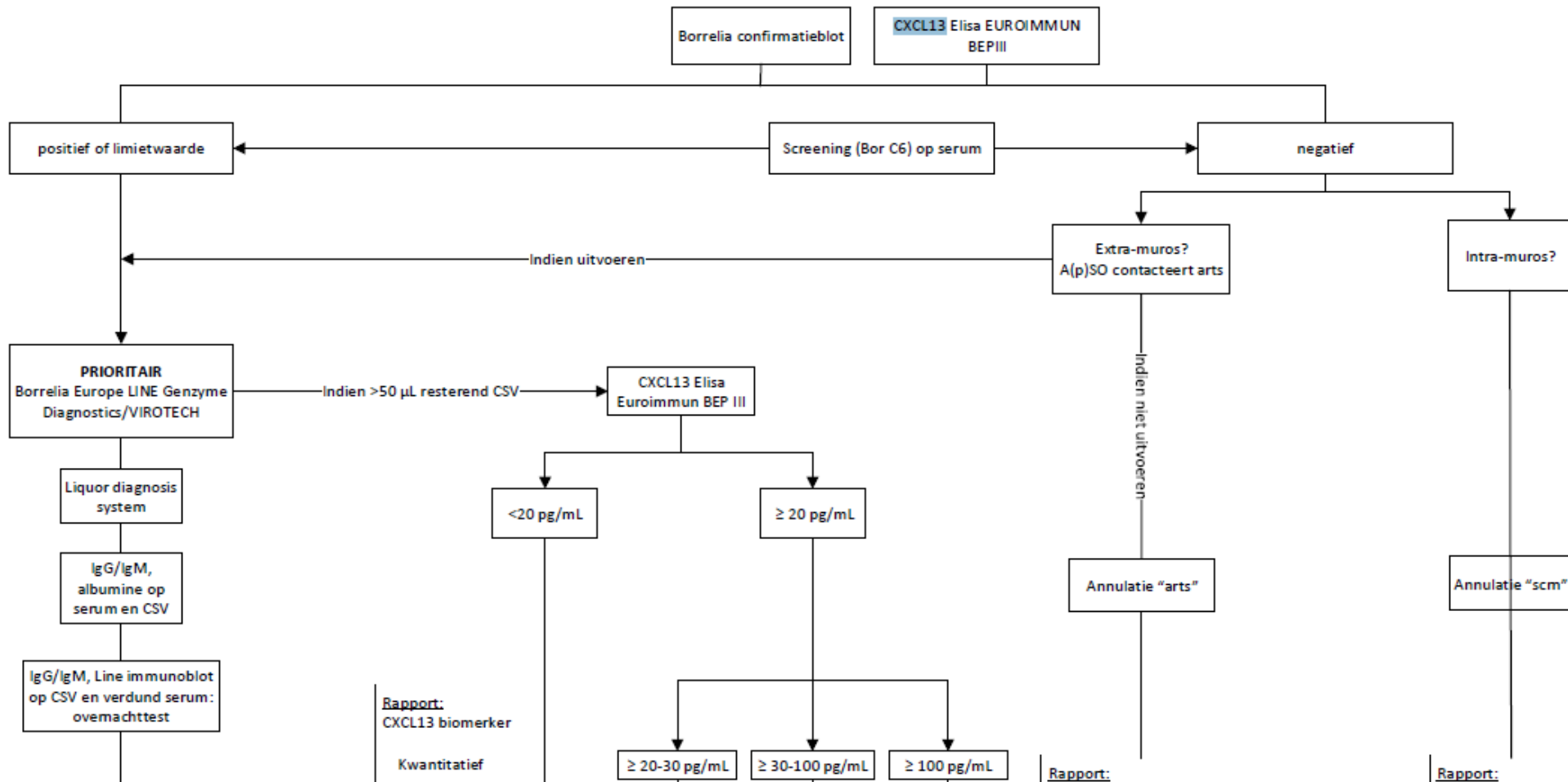
- Results retrospective study UZ Leuven (n=82)

Virotech CSV immunoblot result	EFNS LNB	Result CXCL13 Euroimmun				total
		normal	borderline	increased	strongly increased	
Intrathecal synthesis (n=30)	dLNB	0	0	1	24	25
	pLNB	2	0	1	0	3
	neg	2	0	0	0	2
Passive diffusion (n=16)	pLNB	1	0	0	0	1
	neg	13	0	1	1	15
Passive diffusion/intrathecal synthesis (n=8)	neg	8	0	0	0	8
NEG	pLNB	0	0	0	1	1
	neg	12	2	2	1	17
HIV		1	0	0	2	3
HS		4	0	2	1	7

- Retrospective study UZ Leuven (n=82)
- Results prospective study (n=47)
 - All consecutive samples with request for neuroborreliosis
 - No LNB present (EFNS criteria)
 - 4 patients with increased CXCL13: neurosarcoidosis, B-cell lymphoma, AML
 - 3 patients with positive C6 ELISA and negative immunoblot
 - CXCL13 was negative

Result CXCL13 Euroimmun			
normal	borderline	increased	strongly increased
43	0	2	2

- Lyme borreliosis serology
 - To interpret together with clinical symptoms
 - Pre-test probability
 - No activity test
 - Positive serology \neq illness
 - Negative serology \neq absence of illness
 - Introduction of CXCL13 to better diagnose acute neuroborreliosis



Rapport:
 Borrelia IgG immunoblot:
 Borrelia IgM immunoblot:
 negatief/positief/onbeslist
 Borrelia serologie besluit:
 interpretatie door A(p)SO of medisch verantwoordelijke

Rapport:
 CXCL13 biomerker
 Kwantitatief
 kwalitatief

<20 pg/mL	negatief
≥ 20-30 pg/mL	limiet
≥ 30-100 pg/mL	positief
≥ 100 pg/mL	sterk positief

Neuroborreliose besluit: interpretatie door A(p)SO of medisch verantwoordelijke
 Commentaar: Chemokine CXCL13 is verhoogd in lumbaal vocht bij acute neuroborreliose en is een aanvullende parameter bij niet conclusieve antistofrespons en liquor biochemie. Ook andere infecties en inflammatie in het CZS kunnen gepaard

Rapport:
 Borrelia IgG immunoblot:
 Borrelia IgM immunoblot:
 Borrelia serologie besluit:
 CXCL13 biomerker:
 Neuroborreliose besluit:
 Niet uitgevoerd; na overleg met de behandelende arts

Rapport:
 Borrelia IgG immunoblot:
 Borrelia IgM immunoblot:
 Borrelia serologie besluit:
 CXCL13 biomerker:
 Neuroborreliose besluit:
 Niet uitgevoerd; screeningtest op serum is negatief
 Indien toch uitvoering gewenst, contacteer ons.