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REGION

Diagnosis of meningitis and other CNS infections

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Methodological considerations
Study design

Studies of laboratory origin

- Selected patient groups (selection bias)
  - Include only patients with a confirmed diagnosis (e.g. positive cultures)
- Risk of incorrect grouping of patients
  - Contamination of the CSF sample
    - Also blood contamination of the CSF may influence the microbiological testing (10% of all lumbar punctures are traumatic)

Studies of clinical origin

- Risk of incorrect or inappropriate grouping of patients
  - Incorrect interpretation of laboratory results
  - Heterogeneous patient groups
    - Includes patients with unknown and known microbiological aetiology
    - Early vs. late in the course of the disease
      - > CSF PMN predominance initially in EV meningitis in 2/3 of cases
        - » Rice et al. *CID* 1995
    - Treatment with antibiotics at time of CSF sampling
      - > in 20% with bacterial meningitis
    - Does sepsis and immunosuppressive therapy influence CSF analysis?

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No clinical feature can distinguish between different CNS infections

	Bacterial meningitis N=54	Viral meningitis N=63	Encephalitis N=12
Known etiology	61%	32%	17%
Women/men	50%	75%	50%
Age in years	20 (1-87)	25 (3-66)	53 (13-87)
Underlying illness	13%[b]	3	0
Back rigidity	85%[c]	87%[c]	36%
Decreased consciousness	51%[b]	0%	92%
Assisted ventilation	26% [b]	0%	18% [b]
Days in hospital	10 (7-53)[b]	4 (1-17)	12 (11-65)
Fatal outcome	15%[b]	0%	17%

b) vs. viral meningitis c) vs. encephalitis, P<0.05

Østergaard et al *SJID* 2004

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Lumbar puncture is the essential and acute procedure to diagnose CNS infections

CSF opening pressure

CSF WBC and differential count, CSF/blood glucose ratio, CSF protein levels

CSF Gram stain and culture (+blood culture)

CSF PCR/antigen/antibody evaluation

Few serious complications with lumbar puncture

- Brain herniation in 1% of 418 patients with papilledema
  - Korein et al. *Neurology* 1959
- Suspicion of intracranial mass lesion → CT scan before lumbar puncture (blood culture before lumbar puncture)
  - Advanced age (>60 years), immunocompromised, previous CNS disease, altered consciousness, focal neurological deficits, seizures
    - Hasbun et al. *NEJM* 2001

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No CSF biochemical finding can distinguish between different CNS infections

	Bacterial meningitis N=54	Viral meningitis N=63	Encephalitis N=12
CSF WBC ($\times 10^6$ cells/L)	908 (16-21745)[b,c]	130 (7-1544)	73 (5-267)
CSF PMN ($\times 10^6$ cells/L)	760 (15-20875)[b,c]	25 (0-757)	4.4 (0-155)
CSF LYM ($\times 10^6$ cells/L)	51 (0-1349)	52 (1-1230)	49 (0-104)
CSF MON ($\times 10^6$ cells/L)	33 (0-652)	11 (0-189)	7.4 (0-18)
CSF glucose (mmol/l)	3.1 (0.1-6)	3.3 (2.5-4.6)	3.1 (2.1-4.6)
CSF/blood glucose	0.42 (0.01-1.5)[b]	0.58 (0.3-0.8)	0.53 (0.2-0.8)
CSF protein (g/L)	1.7 (0.1-18)[b]	0.7 (0.2-2.2)	1.1 (0.3-10.7)

b) vs. Viral meningitis; c) vs. Encephalitis; P<0.05

Østergaard et al *SJID* 2004

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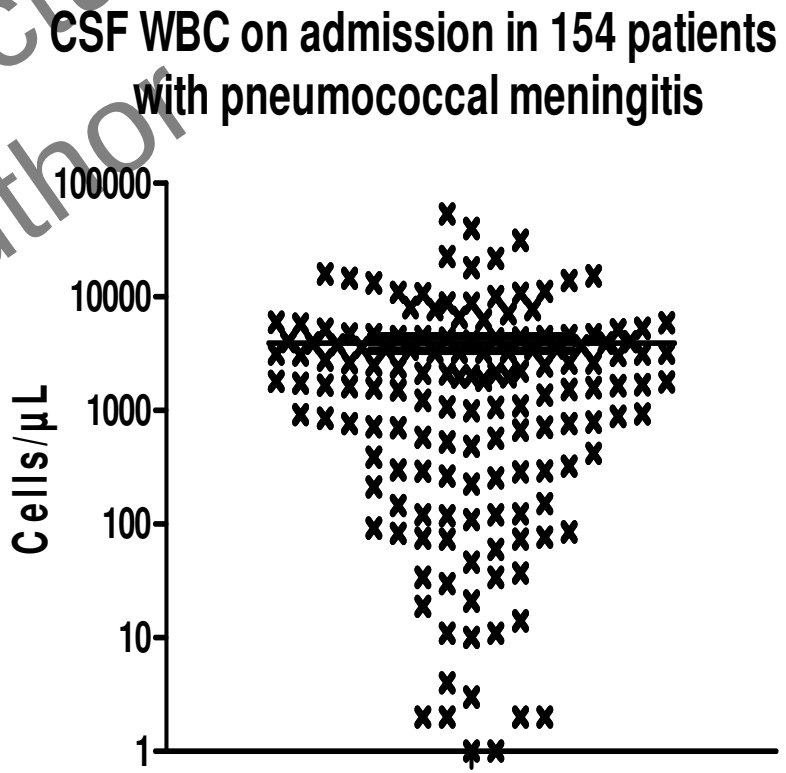
CSF White Blood Cell count

CSF pleocytosis is defined as

- CSF WBC >10 cells/µL
  - Neonates >30 cells/µL
  - Seizures may also induce pleocytosis

Typically >1000 cells/µL in the CSF from patients with bacterial meningitis (neutrophil predominance)

- <10 cells/µL in 5% in culture proven pneumococcal meningitis



Østergaard et al *BMC Infect Dis* 2004

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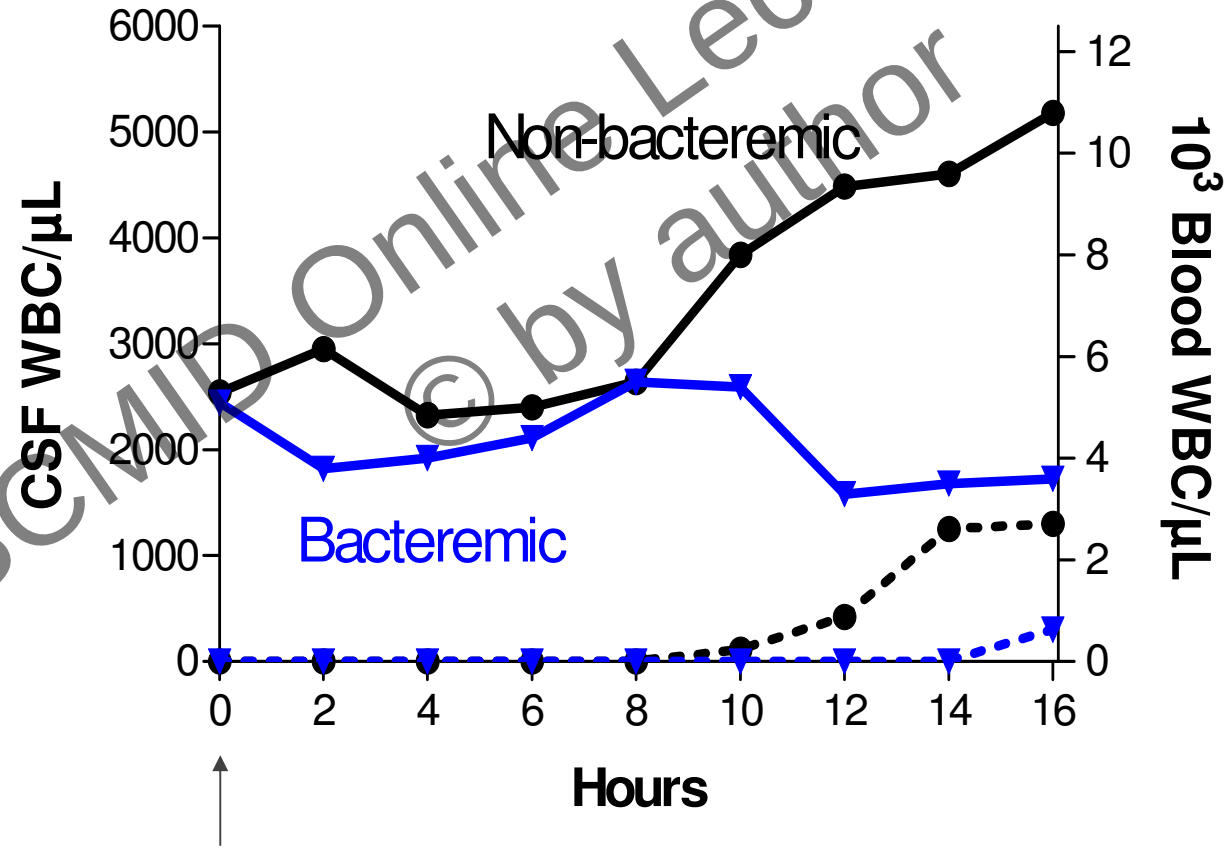
Impact of bacteremia on CSF WBC in experimental pneumococcal meningitis



Østergaard et al *BMC Infect Dis* 2006

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Diagnostic and prognostic use of biochemical findings

Diagnostic use

- CRP analysis in blood is useful in differential diagnosis of bacterial and viral meningitis
  - Roine et al. *Pediatr infect Dis J.* 1992
  - Sormunen et al. *J pediatr* 1999
- Procalcitonin in blood is useful in differential diagnosis of bacterial and viral meningitis
  - Schwarz et al. *Crit Care Med.* 2000
  - Dubos et al. *Arch Pediatr Adolesc Med* 2008
- CSF lactate is useful in differential diagnosis of neurosurgical meningitis
  - Leib et al. *Clin Infect Dis* 1999

Prognostic use

- CSF WBC < 500 cells/µL: 4 times higher risk for dying
  - Østergaard et al *BMC Infect Dis* 2004
- CSF WBC < 100 cells/µL : 3.4 times higher risk for an unfavourable outcome
  - Van de Beek et al *NEJM* 2004

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Still a need for improvement in microbiological diagnose of CNS infections

40% of patients with bacterial meningitis have an unknown microbiological aetiology

- Østergaard et al *SJID* 2004

30-40% of patients with viral meningitis have an unknown microbiological aetiology

- Kupila et al. *Neurology* 2006
- Franzen-Röhl et al. *SJID* 2008
- De Ory et al. *J Med Virol* 2013

50-60% of patients with encephalitis have an unknown microbiological aetiology

- Kupila et al. *Neurology* 2006
- Mailes et al. *Clin Infect Dis* 2009
- Graneröd et al. *Lancet Infect Dis* 2010
- De Ory et al. *J Med Virol* 2013

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Microbiological diagnosis of bacterial meningitis

Methods

- CSF Gram staining
- CSF culture
- CSF PCR
- CSF antigen testing
- CSF antibody testing (CSF/blood antibody index)
- CSF MALDITOF testing

- Blood culture
- Antibody detection in blood

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Diagnose of bacterial meningitis
CSF Gram Staining

90% positive with *S. pneumoniae* meningitis
- Østergaard et al *BMC Infect Dis* 2004

86% positive with *N. meningitidis* meningitis
- Henkenberg et al. *Medicine* 2008

60% positive with *H. influenzae* meningitis
- Pedersen et al *CMI* 2009

28% positive with *L. monocytogenes* meningitis
- Brouwer et al *CID* 2006

CSF concentration.
- $<10^3$ CFU/mL: 25% positive
- $>10^5$ CFU/mL: 97% positive
  - La Scolea et al. *J Clin Microbiol* 1984

Cytospin centrifugation improves the sensitivity
- Shanholzer et al. *J Clin Microbiol* 1982

Methylene-blue staining improves the sensitivity
- Daly et al. *J Clin Microbiol* 1985

Prior antibiotic therapy decreases the sensitivity

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Diagnose of bacterial meningitis · CSF culture

87% positive with a known bacterial aetiology

- Østergaard et *CDLI* 2002

95% positive with *S. pneumoniae* meningitis

- Østergaard et al *BMC Infect Dis* 2004

97% positive with *H. influenzae* meningitis

- Pedersen et al *CMI* 2009

Enrichment culture does not significantly improve sensitivity, but may result in false positive isolates.

- Lessing and Bowler *Eur J Clin Microbial Infect Dis* 1996

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Diagnose of bacterial meningitis
Blood culture

66% positive with bacterial meningitis (positive CSF culture)

- Van de Beek et al. *NEJM* 2002

67% positive with *S. pneumoniae* meningitis

- Østergaard et al *BMC Infect Dis* 2004

55% positive with *H. influenzae* meningitis

- Pedersen et al *CMI* 2009

57% positive with *N. meningitidis*

- Heckenberg et al. *Medicine* 2008

Delayed initiation of antibiotic therapy against meningitis in patients having a positive blood culture, but a negative CSF culture

- Fuglsang-Damgaard et al *SJID* 2008

Increase in s-antibodies against *N. meningitidis*

- 88% were sero-negative at time of meningitis diagnosis, and 90% were sero-positive 10-15 days after onset of disease (complement fixation test)
  - Weis et al. *Epidemiol Infect* 2005

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Diagnose of bacterial meningitis CSF antigen test

Fast and easy tests to perform

- Latex agglutination, coagglutionation, counter immonoelectrophoresis, Binax NOW

Relative good sensitivity

- Beneficial when antibiotic therapy was initiated before lumbar puncture
- Latex agglutination had a low sensitivity (=7%) in culture-negative meningitis
  - Tarafdar et al. *CID* 2001

Pneumococcal urine antigen testing

- 65% positive in pneumococcal bacteremia
  - Selickman et al. *Microbiol Infect Dis* 2010

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Diagnose of bacterial meningitis CSF PCR

Specific, multiplex or broad-range PCR

Several advantages compared to conventional diagnostics (culture)

- Faster results
- Detection of fastidious or non-growing microorganism
  - Virus, TB, borrelia
- Detection of “killed” bacteria
  - Increased sensitivity, when antibiotic therapy was initiated before lumbar puncture
    - Cherian et al. *J Clin Microbiol* 1998

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CSF PCR for bacterial aetiology

Specific- and multiplex PCR against common meningeal pathogens

- Real-time PCR or use of DNA probes give a result in less than 2-4 hrs.
  - Sensitivity and specificity >90%
    - Corless et al. *J Clin Microbiol* 2001
    - Deutsch et al *Scan J Infect Dis* 2008
- Multiplex PCR using LUMINEX
  - Fast method with high sensitivity and specificity (>95%)
    - Øving et al. *J Clin Microbiol* 2009.
  - Pathogen-specific probes
    - Rothman et al. *Emerg Med* 2010
  - Microarray-chip with DNA-probes for different bacteria
    - Ben et al. *J Formos Med Assoc* 2008
    - Boriskin et al. *J Clin Microbial* 2004
- Quantification of microbial genome count
  - High number associated with a poor outcome
    - Roine et al. *Diag Microbiol Infect Dis* 2009

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CSF PCR for bacterial aetiology

Broad-range PCR (16S, 23S)

- High sensitivity, but risk of false positive findings.
- More time consuming.
  - Saravolatz et al. *Clin Infect Dis* 2003
  - Deutsch et al. *Scan J Infect Dis* 2008

Metagenomics will be useful in identifying a microbiological aetiology for CNS infections, but is at present not suitable for routine diagnostics.

- Smits et al. *Emerg Infect Dis* 2013

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Microbiological diagnosis of viral meningitis and encephalitis

PCR is the primary diagnostic analysis for viral CNS infections

- High sensitivity for most virus
- Risk for contamination?
  - Coinfection with EBV due to blood derived lymphocytes
    - 61% coinfection in Malawi patients with bacterial meningitis vs. 25% without meningitis
      - Kelly et al. *J Infect Dis* 2012
    - No coinfection in Dutch Meningitis patients
      - Brouwer et al. *J Infect Dis* 2013
- Intrathecal antibody production
- (Antigen test)
- (Culture)

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Aetiology of viral CNS infections

	Encephalitis		Meningitis	
	+	-	+	-
VZV	96	14	12	20
HSV-1	35	31	2	3
HSV-1 & VZV	13		1	
HSV-2		1	24	2
EV	27	5	38	149
Influenza A	26			
HHV-6	25			1
Adenovirus	13			
TBE	19		6	
Parainfluenza	8			
Rotavirus	7			
RSV CMV	7	2		2

+ Kupila et al. *Neurol* 2006. - de Ory et al. *J Med Virol* 2013

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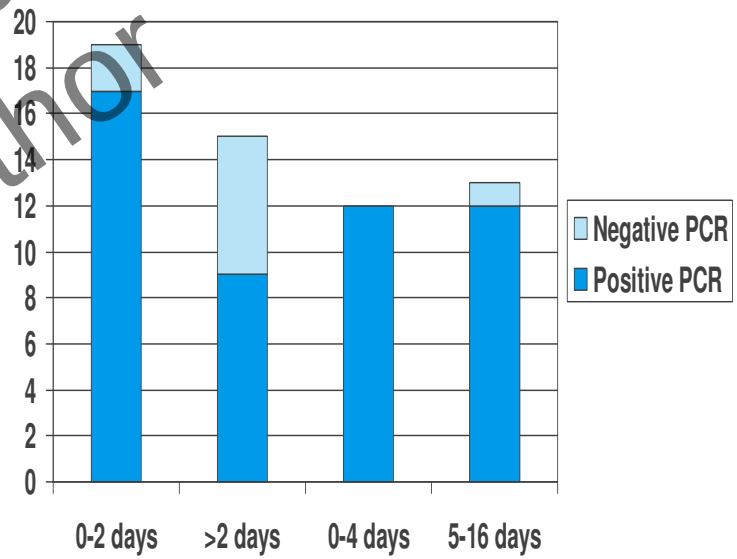
Diagnosis of enteroviral meningitis

- PCR has high sensitivity within the first week after onset of meningitis symptoms
  - Sato et al. *Pediatrics* 2008
- Isolation of EV from upper respiratory tract or faeces primarily for epidemiological purposes (typing)



CSF Stool

Kupila et al *CID* 2005

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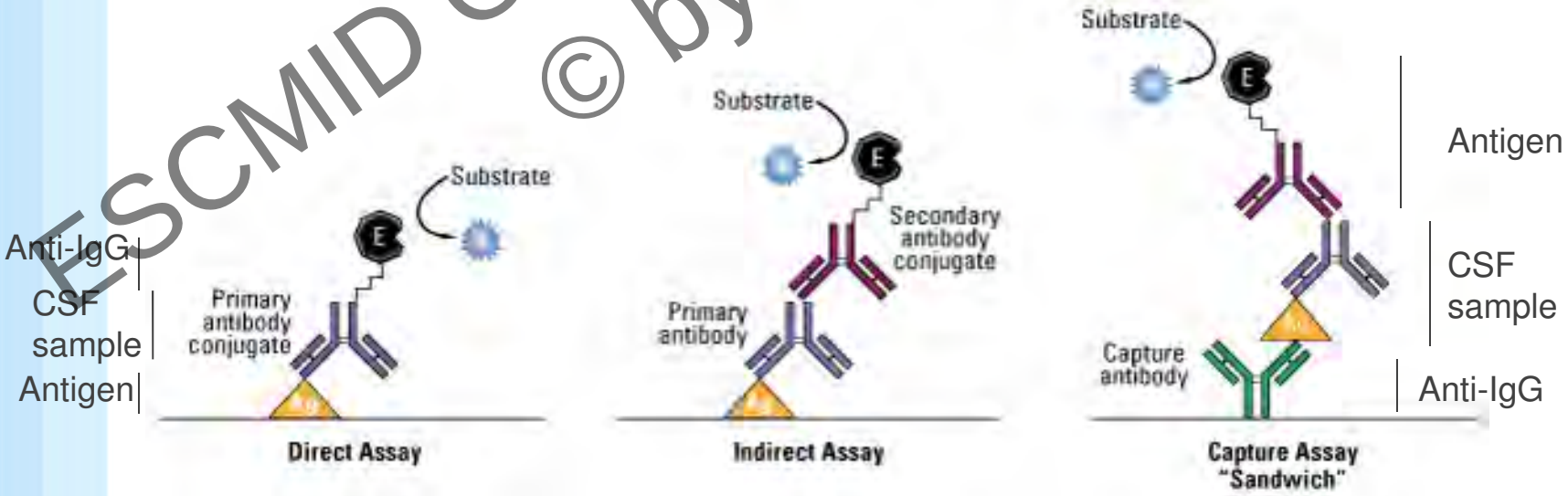
Intrathecal antibody synthesis

- Antibody index for i.e. TBE, VZV, HSV, Measles, Rubella, Borrelia
- ELISA test requires correction for CSF/blood IgG (albumin)
  - Capture ELISA needs no correction
- Polyspecific immune response in multiple sclerosis for VZV, HSV, Measles, Rubella
  - Schubert et al. *BMC Neurol* 2007; Jacobi et al. *J Neuroimmunol* 2007



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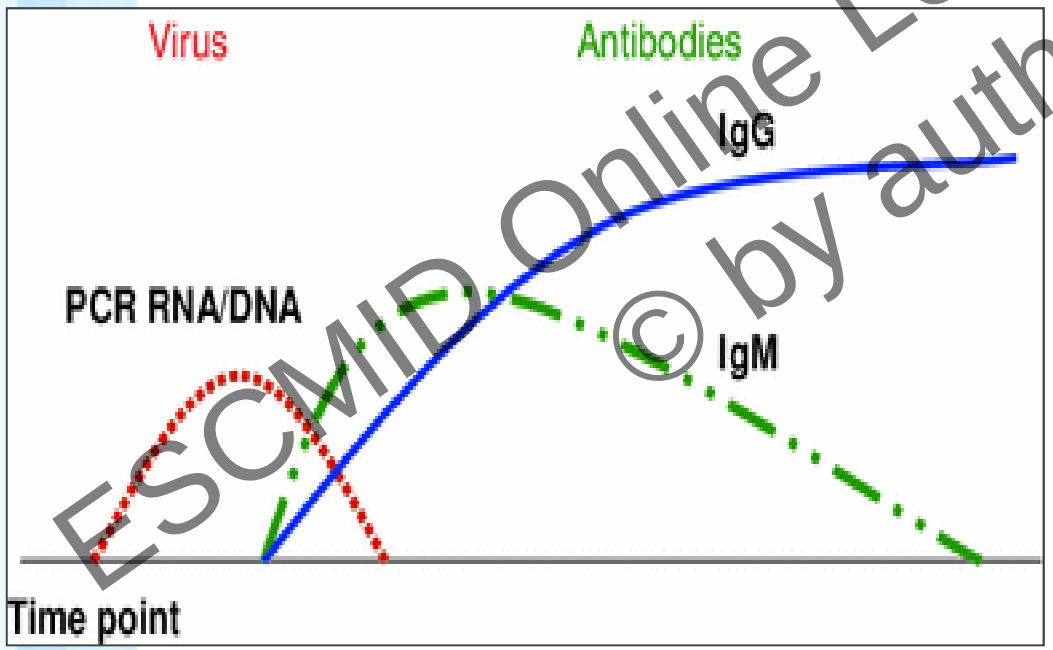
CSF HSV diagnostic in encephalitis PCR vs. intrathecal antibody synthesis



Days after debut	PCR - IT ab -	PCR + IT ab -	PCR + IT ab +	PCR – IT ab +
≤2 days	1	6	1	
3-7 days		14		1
8-13 days		2	4	3
≥14 days			2	10

Fomsgaard et al, *Clin Diagn Virol* 1998

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Microbiological diagnose of neuroborreliosis

PCR

- Poor sensitivity: <25%
  - Lebech et al. *J Clin Microbiol* 1992

Intrathecal antibody synthesis

- ELISA or Capture ELISA
- High sensitivity
  - 80% at 3-4 weeks
  - 100% at 2 month after onset of neurological symptoms
    - Hansen et al. *Ann Neurol* 1991

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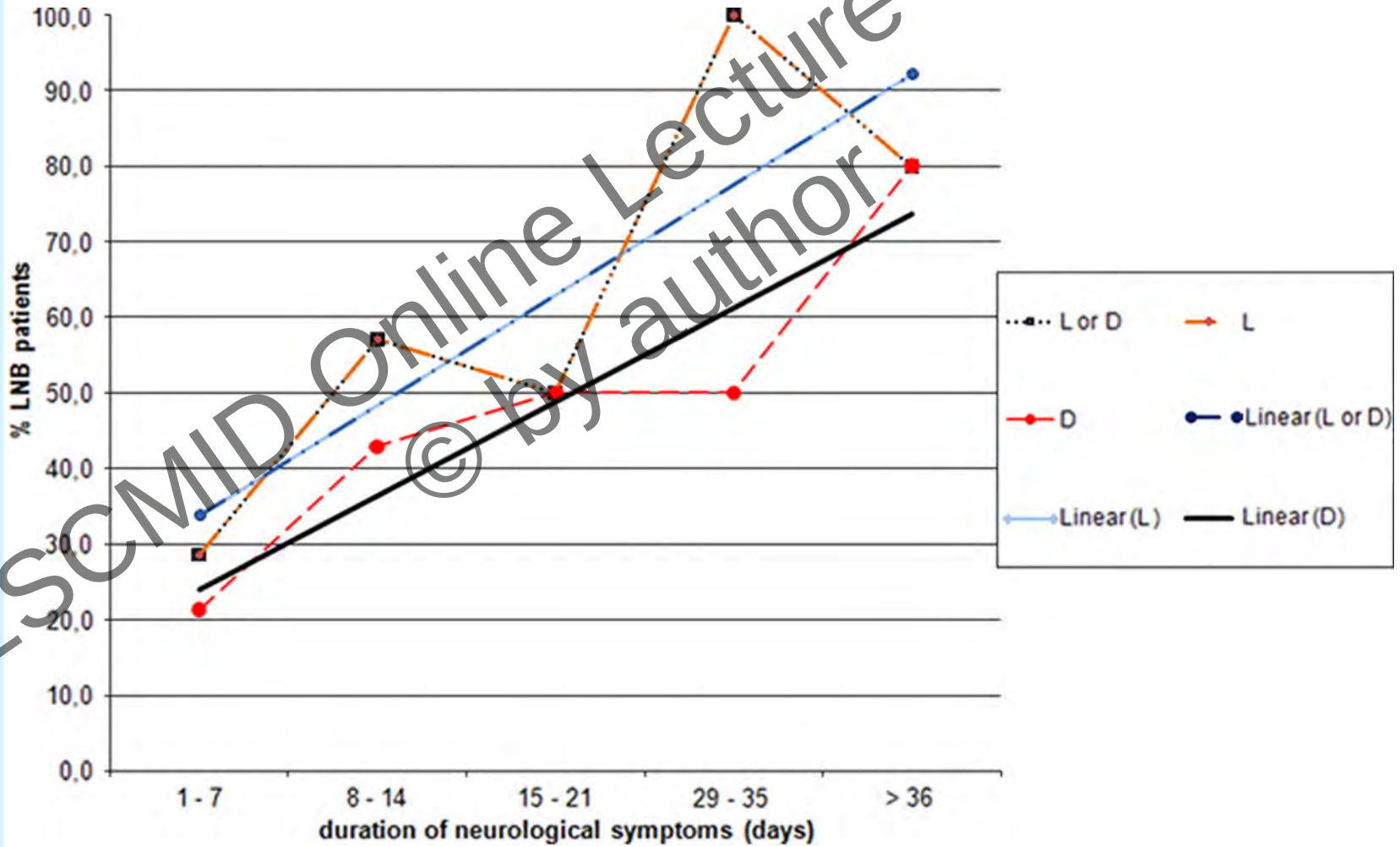
Relation between duration of neurological symptoms and intrathecal synthesis as determined by a particular test in patients with clinically evident Lyme neuroborreliosis.



Cerar T et al. Clin. Vaccine Immunol. 2010;17:645-650

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Diagnose of TB-meningitis

CSF microscopy

- ZN-staining has a sensitivity of 10-20%
  - Pai et al. *Lancet Infect Dis* 2003
  - PFA fixation with Triton X permeabilization: sensitivity: 100%
    - Chen et al *J Clin Microbiol* 2012)
- Fluorescent microscopy faster and more sensitive than ZN-staining
  - Staingart et al. *Lancet Infect Dis* 2006

CSF TB PCR

- sensitivity: 56%, specificity: 98% (Pai et al. *Lancet Infect Dis* 2003)
  - Gene Xpert: sensitivity: 36% vs. 6 % (ZN), specificity: 98%
    - Theron et al. *Am J Respir Crit Care Med* 2011, abstract

IFNγ producing T-cells in CSF

- Sensitivity: 60-90%, specificity: 90-100%
  - Thomas et al. *Int J Tuberc Lung Dis* 2008
  - Kim et al. *CID* 2010

CSF adenosine deamininase

-Review: Tuon et al: *Scan J Infect Dis* 2010

CSF Culture incubation time: up to 8-10 weeks

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Diagnose of bacterial meningitis Matrix-Assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry

Identification directly from CSF in 1 patients with pneumococcal meningitis

- Hartmeyer et al. *SJID* 2010

Poor sensitivity

- Only detection of various bacterial pathogens with CSF concentrations >10^6 CFU/mL
  - Østergaard et al. (unpublished results)

Proteomics (2-dimensional polyacrylamide gel electrophoresis & isoelectric focussing for identification of proteins)

- Viral meningitis vs. bacterial meningitis
  - Identification of 6 proteins
    - Jesse et al. *PLoS ONE* 2010

Detection of PCR products (Electrospray Inonization Mass Spectrometry

- Bhata et al. *J Clin Microbiol* 2012

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Conclusion

No CSF biochemical test can solely distinguish between bacterial and viral meningitis and encephalitis

CSF Gram staining is a fast, sensitive and specific method for diagnose of bacterial meningitis

PCR is a valuable diagnostic tool particularly in patients with viral CNS infections or in meningitis patients treated with antibiotics prior to the lumbar puncture