Neuroborreliosis

On the lab side of diagnosis

Reto Lienhard, FAMH microbiologie
ADMed Microbiologie
Laboratoire Borréliose (CNRT)
Summary

- Lyme Disease in Switzerland
- Microbiological Diagnosis
- Neuroborreliosis
- New aspect on the lab side
- Preliminary results
- Conclusion
Lyme Disease in Switzerland

- Tick *Ixodes ricinus*
- Tick population at < 1500m, 10-150/100m²
- Tick infestation rate of 25% (5-50%)
- Tick bites patient not aware in > 60% EM
- Tick bite risk to develop EM around 3%
- Borreliosis incidence: 85-155 per 100’000
Lyme Disease in Switzerland

Sentinella results 2008-2010  (OFSP Bulletin 42, 2011)

<table>
<thead>
<tr>
<th>Tableau 2</th>
<th>Tableau clinique de la borréliose de Lyme, comparaison des enquêtes Sentinella 2008 à 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
</tr>
<tr>
<td>Total</td>
<td>281</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
</tr>
<tr>
<td>Erythème migrant</td>
<td>79.4</td>
</tr>
<tr>
<td>Lymphocytoma bénin</td>
<td>3.6</td>
</tr>
<tr>
<td>Acrodermatite chronique atrophiante</td>
<td>4.3</td>
</tr>
<tr>
<td>Neuropathie</td>
<td>3.6</td>
</tr>
<tr>
<td>Cardiopathie</td>
<td>0.4</td>
</tr>
<tr>
<td>Arthropathie</td>
<td>5.0</td>
</tr>
<tr>
<td>Fibromyalgie</td>
<td>4.6</td>
</tr>
<tr>
<td>Syndrome de fatigue chronique</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Microbiological Diagnosis

- Indirect diagnosis by serology
- Rarely direct by PCR (or culture)
- Specific to the stage of infection
- Low sensitivity for EM (especially first 7 days)
- Screening tests need blot confirmation (2-tier)
- Seroprevalence >7% (test depending)
  - Lyme is endemic
- Seropositivity needs no antibiotherapy
Testing for early stadium erythema migrans

<table>
<thead>
<tr>
<th>T / Cl</th>
<th>Pos</th>
<th>Neg</th>
<th>Total</th>
<th>PPV</th>
<th>NPV</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>50</td>
<td>37</td>
<td>87</td>
<td></td>
<td></td>
<td>57.5 %</td>
</tr>
<tr>
<td>Neg</td>
<td>50</td>
<td>263</td>
<td>313</td>
<td></td>
<td></td>
<td>84 %</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>300</td>
<td>400</td>
<td></td>
<td></td>
<td>25 %</td>
</tr>
<tr>
<td>Test</td>
<td>Sens.</td>
<td>Spec.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>87.6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Testing for late stadium in selected patients

<table>
<thead>
<tr>
<th>T / Cl</th>
<th>Pos</th>
<th>Neg</th>
<th>Total</th>
<th>PPV</th>
<th>NPV</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>99</td>
<td>37</td>
<td>136</td>
<td></td>
<td></td>
<td>72.8 %</td>
</tr>
<tr>
<td>Neg</td>
<td>1</td>
<td>263</td>
<td>313</td>
<td></td>
<td></td>
<td>99.6 %</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>300</td>
<td>400</td>
<td></td>
<td></td>
<td>25 %</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td></td>
<td>Sens.</td>
<td>Spec.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99 %</td>
<td></td>
<td></td>
<td>87.6 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Neuroborreliosis

- Early disseminated or late disease
- Meningoradiculitis, meningitis, Bell’s palsy
- Rarely encephalitis, myelitis, (cerebral vasculitis)
Neuroborreliosis

- Pleocytosis
- Intrathecal synthesis of specific IgM IgG IgA
- Screening of serum = positive (or seroconversion)
Neuroborreliosis

- Early disseminated or late disease
- **Meningoradiculitis, meningitis, Bell’s palsy**
- Rarely encephalitis, myelitis, (cerebral vasculitis)
- **Pleocytosis**
- **Intrathecal synthesis of specific IgM IgG IgA (SIA)**
- Screening of serum = positive (or seroconversion)
- Supporting: **CSF PCR+, EM, Intrathecal Synthesis**
Intrathecal production

Eur J Clin Microbiol Infect Dis

ARTICLE

Laboratory diagnosis of Lyme neuroborreliosis: a comparison of three CSF anti-\textit{Borrelia} antibody assays

A. J. Henningsson • M. Christiansson • I. Tjernberg •
S. Löfgren • A. Matussek

Med Microbiol Immunol
DOI 10.1007/s00430-013-0322-1

ORIGINAL INVESTIGATION

Intrathecally produced IgG and IgM antibodies to recombinant \textit{VlsE}, \textit{VlsE} peptide, recombinant \textit{OspC} and whole cell extracts in the diagnosis of Lyme neuroborreliosis

Gerold Stanek • Lara Lusa • Katarina Ogrinc •
Mateusz Markowicz • Franc Strle
### Proportion of positive SIA

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2012</th>
<th>2011</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIA positive for IgG or IgM</td>
<td>2.35 %</td>
<td>3.36 %</td>
<td>4.76 %</td>
<td>5.88 %</td>
</tr>
</tbody>
</table>
Values of SIA test

- Sensitivity around 80%
- Delay for detection up to 6 weeks
- Sensitivity nearly 100% (long duration NB)
- Specificity 90-95% (but as low as 63% !)
- Persistence after AB treatment
  not used for follow-up!

What else?

A lot of suggestions but one interesting candidate

CXCL13/BLC/BCA-1
CXCL13 Pubmed’s first references

[Cytokine CXCL13--a possible early CSF marker for neuroborreliosis.]
Rupprecht TA, Koedel U, Angele B, Fingerle V, Pfister HW.
PMID: 16308679 [PubMed - Indexed for MEDLINE]
Related citations

The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis.
PMID: 16087912 [PubMed - Indexed for MEDLINE]
Related citations

Sublime diagnosis of Lyme neuroborreliosis.
Segal BM, Logian EL.
PMID: 16087895 [PubMed - indexed for MEDLINE]
Related citations

The nervous system as ectopic germinal center: CXCL13 and IgG in lyme neuroborreliosis.
PMID: 15929033 [PubMed - indexed for MEDLINE]
Related citations
Evaluation of CXCL13 in CSF

• Human CXCL13/BLC/BCA-1 Quantikine ELISA
• CSF from patients presenting:
  – Neuroborreliosis clinic + SIA
  – suspicion of neuroborreliosis without SIA
  – enterovirus meningitis
  – TBE meningo-encephalitis
  – HSV, VZV encephalitis
  – Bacterial meningitis
## Preliminary Results

<table>
<thead>
<tr>
<th></th>
<th>NB</th>
<th>Non NB</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CXCL13 &gt; 100pg/ml</strong></td>
<td>33</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td><strong>CXCL13 &lt; 100pg/ml</strong></td>
<td>13</td>
<td>96</td>
<td>109</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td>46</td>
<td>101</td>
<td>147</td>
</tr>
</tbody>
</table>

Kappa = 0.71 (0.57 – 0.83) « good »; agreement = 87.7%
Discussion - Sensitivity

- Publication mention high sensitivity (around 100%)
- Present before any SIA in early stage of NB
  - Example of granulocytic pleocytosis in very early neuroborreliosis stage

? Better than specific intrathecal IgG, IgM

? Able to prove seronegative NB

? Rules out borreliosis with high NPV
About a first case

Male, 54 y with history of spondylodiscite E.coli D7-8, spinal fusion D5-10, inflammation C6-C7 presenting with back pain, and FUO for 3 days. Faint signs of meningitis appearing. Lumbar punction show granulocyte pleocytosis suggesting bacterial etiology. Culture, meningitis Multiplex and HSV PCR negative; Lyme serology negative. Treatment: Ceftriaxon 2g for 3 weeks. Ten days after end of treatment new meningits with granulocyte pleocytosis. Laboratory unable to show etiology. 3weeks Ceftriaxon +Vancomyxin

CXCL13 >500 pg/ml (high positive)
IgG EIA1= neg, EIA2= low pos, IgM neg, C6peptide VlsE= neg, SIA IgM and IgG neg
Follow up at 2,5 month show no seroconversion

? Borreliosis probability very (very) low.
? FP CXCL13
Discussion - Specificity

- Quantitative limit set at 100pg/ml
- Activity bound to B-cell presence in CSF
- No persistance after treatment
- Enable treatment follow-up (within 4 month, 82%)

? How sure about viral meningitis (EV, FSME, ...)
? And bacterial meningitis (Listeria, ...)
? How specific in chronic inflammation diseases
  - To challenge its specificity
**Interpretation- false positive?**

<table>
<thead>
<tr>
<th></th>
<th>NB SIA+</th>
<th>Non NB</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL13 &gt; 100pg/ml</td>
<td>33</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td>CXCL13 &lt; 100pg/ml</td>
<td>13</td>
<td>96</td>
<td>109</td>
</tr>
<tr>
<td>total</td>
<td>46</td>
<td>101</td>
<td>147</td>
</tr>
</tbody>
</table>

- Not sensitive enough?
- Does SIA lack specificity?
- Clinical cases uncertain?

- Analysis to be completed
Conclusion

- Serum/CSF pair investigation for B.b SIA is obligatory
- Culture or PCR may help in very early NB stage
- CXCL13 is a importante cytokine to consider in microbiological diagnosis of NB in early NB stage
- NB poses a Clinical challenge to combine with lab.
- More cases to include with defined clinic
- Application to chronic and late borreliosis ?
Special Thanks

• Dr Olivier Péter, ICHV and CNRT (retired)
• Prof. Christian Chuard (Hôpital Fribourg)
• Dr Véronique Erard (Hôpital Fribourg)
• Dr Arseny Sokolov (CHUV)
• Laurence von Allmen, Technician
• Pierre Cantoni, TAB student