Update on the serologic diagnosis of primary biliary cirrhosis

Immunoday
04-12-2014
PBC, a challenge for diagnosis and prognosis

- Serological diagnosis of PBC based
  - on the demonstration of AMA by IIF
  - Confirmation by ELISA of specific antibodies
  - Abnormal liver tests (cholestatic)
PBC, a challenge for diagnosis and prognosis

• A key challenge
  – anti-mitochondrial antibodies (AMA by IIF, or anti-M2 detected by ELISA) detected in the absence of liver biochemical abnormality

• In earlier studies, the majority of AMA-positive patients with normal LFTs had histological features of mild PBC (83%)
  – The majority went on to develop characteristic biochemical abnormalities (83%) or symptoms of PBC (76%) over a prolonged follow-up
  – None became cirrhotic or died of the complications of PBC

Metcalf JV, Lancet 96
PBC, a challenge for diagnosis and prognosis

- In more recent studies, prevalence of AMA in the normal population of 0.1–1%
  - up to half of the AMA-positive subjects in the larger cohorts with biochemical abnormality

- More information needed on long-term natural history
  - baseline evaluation of the population
  - identify processes and markers, associated with the subsequent development of **clinically significant PBC**
  - Preventive markers for high-risks individuals (patients’ relatives with PBC)

Other unmet needs in PBC

• Biomarkers for high risk disease
  – Risk of cholangiocarcinoma

• Predictive markers of
  – UDCA non-response
  – Alternative firstline therapies for patients at high risk of non-response
  – Secondary therapies for non-responders
  – Therapies for overlapping autoimmune liver diseases

Dyson, J Hepatol, 2014
Primary biliary cirrhosis (PBC) : clinical background

- Mainly in women (10:1)
- Chronic course
- Inflammation of the small bile ducts
- Cholestatic (decreased bile flow)
- Often asymptomatic at diagnosis
First symptoms are often non-specific

- Fatigue
- Pruritus
- Jaundice
- Vague non-progressive right upper abdominal pain or discomfort
Diagnostic criteria and clinical features of primary biliary cirrhosis.

2 of 3 required criteria
Serum alkaline phosphatase >1.5 times ULN\textsuperscript{a}
Presence of AMAs\textsuperscript{b}
Liver histology with nonsuppurative destructive cholangitis and destruction of interlobular bile ducts

Other characteristic clinical features
PBC-specific ANAs\textsuperscript{c} (Sp100 and gp210)
Elevated serum IgM
Hypercholesterolemia/Xanthomas
Sicca syndrome
Pruritus
Fatigue
Autoimmune diseases overlapping with PBC

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Prevalence in PBC patients in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sjögren’s syndrome</td>
<td>47-66</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4-24</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>3-5</td>
</tr>
<tr>
<td>CREST syndrome</td>
<td>7</td>
</tr>
<tr>
<td>SLE</td>
<td>1</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>(case reports)</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>1</td>
</tr>
</tbody>
</table>

- % PBC in association with other autoimmune disorders
  - 15% to 84%
Serology is the key for the diagnosis of PBC

- Anti-mitochondrial antibodies (AMA)
  - positive in more than 90% of patients
- PBC-specific ANA
  - positive in about 50% of patients
  - but
  - in about 85% of AMA-negative PBC patients

Multiple nuclear dots Sp100
The AMA Antigen

- PBC-specific AMA
  - Are directed against proteins which are placed at the inner mitochondrial membrane
- Three major target proteins:
  - Pyruvate dehydrogenase complex (PDH or PDC)
  - Oxoglutarate dehydrogenase complex (OGDC)
  - Branched-chain oxoacid dehydrogenase complex (BCOADC)
- All three target proteins contain a subunit named E2.

These three antigens belong to the "M2-autoantibody epitopes"
Clinical significance of autoantibodies detected in primary biliary cirrhosis

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>IIF pattern</th>
<th>Sensitivity for PBC %</th>
<th>Specificity for PBC</th>
<th>Other diseases</th>
<th>Clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimitochondrial antibodies</td>
<td>MIT</td>
<td>90–95</td>
<td>High</td>
<td>None</td>
<td>Dx of PBC. No difference in clinical features between AMA-positive and AMA-negative patients</td>
</tr>
<tr>
<td>Anti-PDCE2 (74 kDa)</td>
<td></td>
<td>80–90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PDCE3BP (50 kDa)</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PDCE1a (41 kDa)</td>
<td></td>
<td>5–25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-OGDC-E2 (48 kDa)</td>
<td></td>
<td>20–60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-BCOADCE2 (52 kDa)</td>
<td></td>
<td>50–80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>NE</td>
<td>40–50</td>
<td>Depends on target antigen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-gp210</td>
<td></td>
<td>10–40</td>
<td>Very high</td>
<td>none</td>
<td>Dx of AMA-negative PBC, hepatic failure-type progression</td>
</tr>
<tr>
<td>Anti-p62</td>
<td></td>
<td>10–30</td>
<td>High</td>
<td>SjS</td>
<td>Dx of AMA-negative PBC, more severe disease</td>
</tr>
<tr>
<td>Antilamin B receptor</td>
<td></td>
<td>2–9</td>
<td>High</td>
<td>None</td>
<td>Dx of AMA-negative PBC, association with clinical features?</td>
</tr>
<tr>
<td>Anti-sp100</td>
<td>MND</td>
<td>20–40</td>
<td>High</td>
<td></td>
<td>Dx of AMA-negative PBC, faster progression</td>
</tr>
<tr>
<td>Anti-PML</td>
<td></td>
<td>15–20</td>
<td>High</td>
<td></td>
<td>Dx of AMA-negative PBC, association with clinical features?</td>
</tr>
<tr>
<td>Anti-sp140</td>
<td></td>
<td>15</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-SUMO-1, 2</td>
<td></td>
<td>2–6</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticentromere A, B, C</td>
<td>CENP</td>
<td>10–30</td>
<td>Not high</td>
<td>SSc</td>
<td>Portal hypertension-type progression</td>
</tr>
</tbody>
</table>

Nakamura et al., Sem Liver Dis, 2014
PBC-specific ANA

- Multiple nuclear dots (Sp100, PML)
- Nuclear membrane pore complex (gp210, nucleoporin p62, lamin B receptor)

These patterns are highly PBC-specific, can be used as surrogate PBC markers in cholestatic, AMA-negative individuals
Antimitochondrial antibodies (AMAs) and antinuclear antibodies detected in primary biliary cirrhosis

Nakamura et al., Sem Liver Dis, 2014
PBC prognosis and autoantibodies

HCC: hepatocellular carcinoma
Diagnosis of primary biliary cirrhosis

- Diagnosis of PBC is based on the triad
  - Cholestatic liver biochemistry
    - elevated alkaline phosphatase (ALP) for more than 6 months or elevated gamma-glutamyltransferase (GGT)
  - Positive serology
    - Anti-mitochondrial antibodies (AMA)
    - PBC-specific ANA
  - Liver histology
    - ultrasound is mostly sufficient

A definite diagnosis of PBC is made when two out of these three criteria are met.
Optimization of antigen for ELISA

Native purified AMA-M2 antigen from pig (Sigma)

Recombinant BCOADC-E2

EliA M2: Native pyruvate dehydrogenase (PDH) mixed with recombinant BCOADC-E2 to ensure that all epitopes are offered and achieve optimal sensitivity
Positive IIF AMA should be confirmed with the more specific anti-M2 test.

Serology in primary biliary cirrhosis – possible algorithm

- **IIF AMA**
  - PBC-specific ANA
    - PBC unlikely
      - If further suspicion: liver biopsy
    - PBC very likely
      - further evaluation (liver biopsy, PBC-specific ANA, consider other diagnosis)
  - Anti-M2
    - consider other AMAs
## EliA M2 in 320 clinically defined samples

<table>
<thead>
<tr>
<th>Disease group</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients:</strong></td>
<td></td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>100</td>
</tr>
<tr>
<td><strong>Disease Controls:</strong></td>
<td></td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>20</td>
</tr>
<tr>
<td>Autoimmune Thyroiditis (Graves’ disease and Hashimoto’s thyroiditis)</td>
<td>20</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>30</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease (Crohn’s disease and Ulcerative colitis)</td>
<td>60</td>
</tr>
<tr>
<td>Hepatitis C infection</td>
<td>20</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>20</td>
</tr>
<tr>
<td>Connective tissue diseases (systemic sclerosis, CREST syndrome, Sjögren’s syndrome)</td>
<td>40</td>
</tr>
<tr>
<td>Tumor</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>330</td>
</tr>
</tbody>
</table>
EliA M2 performance in 330 clinically defined samples

High sensitivity, high specificity
M2 IgG and IgM

- According to the literature, 5% of PBC patients have only IgM isotype.
- Most ELISA assays measure only IgG.
- Cave in Japan, where the percentage of IgM-positives (but IgG-negatives) is higher.
Good agreement with IIF

EliA M2 and IIF AMA in 96 PBC patients.
Agreement = 91.7%

COMMENT
6 EliA-M2 positive sera were negative for AMA in IIF.
A potential diagnostic strategy?

(I. Graf-Pisler & V. Aubert, personal communication)

- Strategy of IAL lab based on IIF followed by ELISA X
  - Numerous false positives with IIF (absence of evidence of PBC)
- Recent introduction of a dot assay (includes Sp100, gp210)
  - Improved correlation with clinical evidence of PBC
- Would a strategy based on a dot assay & an improved ELISA (P) be more efficient?
A potential diagnostic strategy?  

**(I. Graf-Pisler & V. Aubert, personal communication)**

**ELISA P versus ELISA X**

<table>
<thead>
<tr>
<th></th>
<th>ELISA P</th>
<th>ELISA X</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>pos</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>neg</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>32</td>
</tr>
</tbody>
</table>

**TOTAL SERA:** 87  
**POS /POS:** 42  
**NEG/NEG:** 31  
**Divergent:** 14

13 **ELISA P NEG/ ELISA X POS:**  
- 9 Dot assay et IIF NEG  
- 1 Dot NEG; IIF n.d.  
- 1 Dot n.d. et IIF NEG  
- 2 Dot NEG; IIF POS

1 **ELISA P POS / ELISA X NEG:**  
- IIF 1/40 et Dot POS
A potential diagnostic strategy?

**ELISA P / DOT assay**

**TOTAL SERA:** 79  
- **POS /POS:** 37  
- **NEG/NEG:** 41  
- **Divergent:** 1

<table>
<thead>
<tr>
<th>PHADIA</th>
<th>DOT ALPHADIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pos</td>
</tr>
<tr>
<td><strong>pos</strong></td>
<td>37</td>
</tr>
<tr>
<td><strong>neg</strong></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>
Conclusion 1: need for a high sensitivity

- M2 test helps to confirm IIF or dot results and to specify the AMA as the PBC-relevant antibodies.
- A false negative result may lead to a delayed diagnosis
  - Delayed diagnosis may lead to later treatment.
  - Later treatment increases the risk for bad prognosis.
Conclusion 2: need for a high specificity

- AMA-positive sera will be tested for anti-M2 by ELISA and a double false positive result should be very unlikely
  - False positive results may trigger useless investigations
- Concordant double positivity is a strong hint that PBC may develop or that a disease is present (PBC or PBC related) (e.g. autoimmune hepatitis with immunoreaction against bile ducts).
Novel markers

- Anti-kelch-like 12 and anti-hexokinase 1: novel autoantibodies in PBC
- Gary L. Norman, Liver Intern, 2014

Highly specific anti-KLHL12 and anti-HK1 antibodies to AMA and ANA serological assays significantly improves efficacy in the clinical detection and diagnosis of PBC, especially for AMA-negative subjects.