New Diagnostic Markers Screening in Tuberculous Meningitis

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Jan 17, 2013
Beijing, China
Tuberculous meningitis (TBM) is a frequent extrapulmonary disease caused by *Mycobacterium tuberculosis* and is associated with high mortality rates (about 0-30%) and severe neurological sequelae.

- Brain damage can result from the infection that may lead to abnormal behavior, mental impairments, motor type paralysis, and seizures.
- TBM is thought to be the most devastating form of the disease.
- Rapid diagnosis and early intervention is vital for successful outcome for patients.

Epidemiology

- The exact incidence and prevalence are not known in the world.

- Extrapulmonary tuberculosis in the United States: CNS involvement was noted in 5 to 10% of extrapulmonary tuberculosis cases, with more recent CDC data in 2010 indicating that 5.5% of extrapulmonary cases involve CNS tuberculosis (=1.2% of total tuberculosis cases)

- CNS tuberculosis in Canada: the incidence of developing CNS tuberculosis was approximately 1.0% among 82,764 tuberculosis cases from 1970 to 2001 in a Canadian cohort.

Takahashi, T et al. Tuberc Res Treat 2012:831292
TBM cases in our hospital from 2000 to 2012


TBM cases

Survival  Death

- Blue bars represent survival cases.
- Red bars represent death cases.
Early diagnosis is very important.

- Approximately 90% of the patients are diagnosed in stage II or III.
- Clinical response to antituberculous therapy in all forms of neurotuberculosis is excellent if the diagnosis is made early before irreversible neurological deficit is established.
- Early diagnosis of TBM is considered a key to effective treatment and prognosis.
Overall, the diagnosis of TBM still remains a major challenge due to inadequate current diagnostic methods and poor sensitivity and/or specificity of existing markers.

Diagnosis is based on the characteristic clinical picture, neuroimaging abnormalities, cerebrospinal fluid changes and the response to anti-tuberculosis drugs.

Clinical manifestation

- The clinical spectrum is broad and may be non-specific making early diagnosis difficult.
- Clinical features included fever for more than 7 days (usually high fever), headache, vomiting, neck stiffness or conscious disturbance etc.
MRI

- cephalomeningitis
- cerebral tuberculoma
- myelomeningitis
### Laboratory examination

#### CSF analysis in routine:

- Total protein,
- Glucose,
- Chloride,
- Cell count,
- And cytological staining.

<table>
<thead>
<tr>
<th>CSF</th>
<th>WBC (/L)</th>
<th>Monocytes (%)</th>
<th>Protein (g/L)</th>
<th>Glucose (mmol/L)</th>
<th>Chloride (mmol/L)</th>
<th>ADA (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0-8</td>
<td>/</td>
<td>0.2-0.4</td>
<td>2.5-4.5</td>
<td>120-130</td>
<td>&lt;10</td>
</tr>
<tr>
<td>TBM</td>
<td>&lt;500</td>
<td>predominate</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>
Smear: AFB positive is only 5-30%.

Culture: Lowenstein-Jensen medium is positive in approximately 45–90% of cases


However, AFB positive in smear is no more than 5% and culture positive is no more than 10% in our clinical practice.
### Table 3. Distribution of the Cases According to Set Criteria and CSF ADA Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>ADA levels in U/L</th>
<th>Mean SD</th>
<th>t cal</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ADA &lt; 10</td>
<td>27.1684</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculous</td>
<td>19</td>
<td>ADA &gt; 10</td>
<td>22.7660</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADA &lt; 10</td>
<td>22.7660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-tuberculous</td>
<td>21</td>
<td>ADA ≥ 10</td>
<td>6.0619</td>
<td>4.0173</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADA &lt; 10</td>
<td>2.5714</td>
<td></td>
<td>(p&lt;.01)*</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The PCR-Based Diagnosis of Central Nervous System Tuberculosis: Up to Date

<table>
<thead>
<tr>
<th>Author</th>
<th>Reported year</th>
<th>Assay technique</th>
<th>Specimens and cases</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonington et al.</td>
<td>1998</td>
<td>Roche amplicor PCR</td>
<td>83 CSF/69 patients (40 TBM, 29 non-TBM): South Africa</td>
<td>60</td>
<td>100</td>
<td>[6]</td>
</tr>
<tr>
<td>Lang et al.</td>
<td>1998</td>
<td>Modified Gen-Probe MTD</td>
<td>84 CSF and children (24 TBM, 60 non-TBM): Dominica</td>
<td>83</td>
<td>100</td>
<td>[7]</td>
</tr>
<tr>
<td>Bonington et al.</td>
<td>2000</td>
<td>Roche Cobas Amplicor PCR</td>
<td>83 CSF/69 patients (40 TBM, 29 non-TBM): South Africa</td>
<td>17.5</td>
<td>100</td>
<td>[8]</td>
</tr>
<tr>
<td>Chedore and Jamieson</td>
<td>2002</td>
<td>Gen-Frobe MTD</td>
<td>311 CSF: Canada</td>
<td>†93.8</td>
<td>†99.3</td>
<td>[9]</td>
</tr>
<tr>
<td>Rafi et al.</td>
<td>2007</td>
<td>IS6110 single PCR, MPT64/65kDa antigen nested PCR</td>
<td>176 CSF and patients (75 TBM, 101 non-TBM): India</td>
<td>98/91/51</td>
<td>100/91/92</td>
<td>[36]</td>
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<tr>
<td>Rafi and Naghilya</td>
<td>2007</td>
<td>IS6110 uniplex (single) PCR</td>
<td>945 CSF and patients (677 TBM, 268 non-TBM): India</td>
<td>76.4</td>
<td>89.2</td>
<td>[37]</td>
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<tr>
<td>Takahashi et al.</td>
<td>2007</td>
<td>MPT64 QNRT-PCR</td>
<td>63 CSF/28 patients (8 TBM, 20 non-TBM): Japan</td>
<td>55.8</td>
<td>100</td>
<td>[38]</td>
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<tr>
<td>Deshpande et al.</td>
<td>2007</td>
<td>IS6110 Single PCR</td>
<td>80 CSF and patients (51 TBM, 29 non-TBM): India</td>
<td>91.4</td>
<td>75.9</td>
<td>[39]</td>
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<tr>
<td>Takahashi et al.</td>
<td>2008</td>
<td>MPT64 WR-QNRT-PCR</td>
<td>96 CSF/53 patients (24 TBM, 29 non-TBM): Japan</td>
<td>95.8</td>
<td>100</td>
<td>[40, 41]</td>
</tr>
<tr>
<td>Haldar et al.</td>
<td>2009</td>
<td>devR qRT-PCR</td>
<td>167 CSF and patients (81 TBM, 86 non-TBM): India</td>
<td>87.6</td>
<td>92</td>
<td>[42]</td>
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</tbody>
</table>
Mass spectrometry-based quantitative proteomics has emerged as a powerful approach for identifying and studying disease biomarkers and has become one of the essential tools in biomarker discovery.

Advances in quantitative mass spectrometry have led to identification and quantitation of biomarkers which serve as indicators of disease progression, prognosis, drug safety and help to elucidate the mechanism of drug treatment.

There are various labeling approaches that one can employ to carry out quantitative proteomic measurements. *In vitro* labeling methods include Isobaric Tags for Relative and Absolute Quantitation (iTRAQ), Isotope-Coded Affinity Tags (ICAT), 18 O labeling and *in vivo* methods include Stable Isotope Labeling by Amino acids in Cell culture (SILAC) and 15N labeling.

In this study, we used an iTRAQ-based quantitative proteomic approach to identify differentially expressed peptides/proteins from CSF of tuberculous meningitis cases as compared to controls (non-meningitis patients).
iTRAQ reagent schematic diagram

- Reporter group
  - Mass: 113 to 121
  - Skip120

- Balance group

- Peptide Reactive Group

- MS fragment site

Isobaric Tag: Total Mass is Identical
iTRAQ experimental flow diagram
Distribution of Length of Matched peptides
RESULT

There are totally 338 differentially expressed peptides/proteins in CSF from TBM patients compared with non-meningitis patients.

129 downregulated peptides/proteins

209 upregulated peptides/proteins
Study Ongoing

- Gene cloning
- Protein expression
- Preparation of polyclonal antiserum
- Western-blot
Acknowledgements

Beijing Huada Protein Institute Co., Ltd.

Prof. Wanming Geng
Dr. Yang Yang
Dr. Qingtao Liang
Dr. Shan Chang
Dr. Hua Li
Dr. Xinting Yang
Thanks for your attention!