A T-cell assay based on Antigen B multiepitope peptides for the diagnosis of cystic echinococcosis

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Echinococcus granulosus life-cycle
Cystic echinococcosis (CE) global distribution

Italy, Spain and Eastern European countries are recognized as highly endemic

2-3 million cases

from the “Second WHO report on neglected tropical diseases” 2011
CE diagnosis

- clinical anamnesis (customs, dog-man relation-ship)
- imaging methods (ultrasonography, X-ray, MNR)
- serology

ACTIVE CYSTS

TRANSITIONAL CYSTS

INACTIVE CYSTS

CE3a biologically inactive

CE3b biologically active

WHO Informal Working Group, Acta Tropica 2003
Serodiagnosis of CE

Serology may be characterized by

- a low sensitivity (up to 25% of false negative results) depending on:
  - cyst location other than liver
  - cyst stage: CE1, CE4, CE5
  - cyst size

- a low specificity depending on:
  - Cross-reactions with other helminthiasis
  - Cross-reactions in people with auto-immune disorders
Antigen B

by courtesy of Dr. A. Siracusano
IFN-γ release assays (IGRAs): tests for the diagnosis of latent tuberculosis infection or toxoplasmosis

Whole blood IFN-γ

RD1

T. gondii antigens

QuantiFERON-Plus

Mahmoudi S et al, Exp Paras 2017

Petruccioli E et al, JOI 2016
Rationale

CE progressive disease is characterized by a Th2 polarization

• Based on the IL-4 specific response to AgB, is it possible to set-up an immune diagnostic test for CE?

• If so, is it possible to have an *in vitro* test that helps discriminating between active and inactive cyst stages?
IL-4 specific-response in whole blood associates with human Cystic Echinococcosis and cyst activity

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Limits

- Complex purification
- Antigen preparation not standardized
- Time consuming
- Economic cost
AIM

1. to analyze the IL-4 response to a set of multiepitope synthetic peptides spanning the sequence of the 5 AgB subunits

2. to determine if the selected peptides may be used to increase the diagnostic accuracy of the WB assay for CE diagnosis
AgB multiepitope peptides

Overlapping peptides covering the sequence of the 5 AgB subunits

1. Peptide AgB1_2:
2. Peptide AgB1_3:
3. Peptide AgB2_2:
4. Peptide AgB2_3:
5. Peptide AgB3a_2:
6. Peptide AgB3a_3:
7. Peptide AgB3b_1:
8. Peptide AgB4_1:
9. Peptide AgB4_2:
10. Peptide AgB4_3:
11. Peptide AgB5/1_2:
12. Peptide AgB5/1_3:

Pool 1
Pool 2
Pool 3
Pool 4
Pool 5

Total pool

Peptides designed by Massimo Amicosante, from University of Rome “Tor Vergata”
Population study

ENROLLED SUBJECTS
69

NO-CE SUBJECTS
26

CE PATIENTS
43

Active Cysts
16

Transitional Cysts
4

Inactive Cysts
23
Experimental set-up: evaluation of the peptide pools concentrations
IL-4 response to peptide pools in CE and NO-CE subjects: AgB8/1 is the most immunogenic AgB subunit
Accuracy of the test evaluated by ROC analysis

The cut-off of 0.29 pg/ml identify CE with:

51% sensitivity
96% specificity
IL-4 response to the total peptide pool associates with active stages
Accuracy of the test evaluated by ROC analysis

The cut-off of 0.6 pg/ml identify active cysts with:

69% sensitivity
83% specificity
## Agreement between experimental WB results and serology

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Serology positive/ WB positive N (%)</th>
<th>Serology positive/ WB negative N (%)</th>
<th>Serology negative/ WB positive N (%)</th>
<th>Serology negative/ WB negative N (%)</th>
<th>K</th>
<th>Concordance</th>
<th>P value#</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-CE subjects</td>
<td>18</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.6)</td>
<td>17 (94.4)</td>
<td>-</td>
<td>0.94</td>
<td>-</td>
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<tr>
<td>Active cysts</td>
<td>16</td>
<td>10 (62.5)</td>
<td>2 (12.5)</td>
<td>1 (6.2)</td>
<td>3 (18.8)</td>
<td>0.5</td>
<td>0.81</td>
<td>0.03</td>
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<tr>
<td>Transitional cysts</td>
<td>4</td>
<td>2 (50.0)</td>
<td>1 (25.0)</td>
<td>1 (25.0)</td>
<td>0 (0)</td>
<td>&lt;0.01</td>
<td>0.50</td>
<td>0.5</td>
</tr>
<tr>
<td>Inactive cysts</td>
<td>23</td>
<td>5 (21.7)</td>
<td>7 (30.4)</td>
<td>3 (13.1)</td>
<td>8 (34.8)</td>
<td>0.3</td>
<td>0.64</td>
<td>0.5</td>
</tr>
<tr>
<td>All</td>
<td>61</td>
<td>17 (27.9)</td>
<td>10 (16.4)</td>
<td>6 (9.8)</td>
<td>28 (45.9)</td>
<td>0.5</td>
<td>0.74</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
IL-4 analysis restricted to CE patients with a positive serology

CE PATIENTS
27

- Active Cysts: 12
- Transitional Cysts: 3
- Inactive Cysts: 12
The IL-4 response to the total peptide pool helps identifying active stages.

The cut-off of 0.59 pg/ml identify active cysts with:

- 83% sensitivity
- 83% specificity
CE combined diagnostic test algorithm

1. Ultrasound
   - US negative
   - US positive or dubious
     - Routine serology
       - Serology negative
         - Additional investigations
         - WB negative (LIKELY INACTIVE CYSTS)
       - Serology positive
         - WB test based on AgB peptides
           - WB positive (LIKELY ACTIVE CYSTS)
           - WB negative (LIKELY INACTIVE CYSTS)
Conclusions

AIM2: to determine if the selected peptides may be used to increase the diagnostic accuracy of the WB assay for CE diagnosis

• The IL-4 response induced by AgB8/1 and by the total pool associated with CE. However, the sensitivity for diagnosing CE is low.

• The IL-4 response induced by the total pool associated with active cysts. However, the sensitivity for diagnosing active cysts is low.

• The overall concordance between the WB assay and the routine serology was moderate and weakened by the proportion of WB false negative results.

• Within the CE patients with a positive serology the WB test based on the total pool shows an optimal accuracy.
Future Perspectives

• Confirmation of the results in a larger population

• Single-peptide strategy to better characterize the AgB protein response

• Evaluation of different combinations of peptide pools to optimize the IL-4 specific response including other *E. granulosus* antigens (i.e. Ag5)
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  – Dr. Valentina Vanini, Dr. Elisa Busi-Rizzi, Angela Corpolongo, Dr. Giuseppe Ippolito and Dr. Delia Goletti

University of Rome “Tor Vergata”, Rome – Italy
  – Dr. Massimo Amicosante

Sant'Andrea Hospital University of Rome "Sapienza”, Rome – Italy
  – Dr. Antonella Teggi

Istituto Superiore di Sanità, Rome – Italy
  – Dr. Edoardo Pozio, Dr. Maria Angeles Gomez Morales, Dr. Alessandra Ludovisi
## Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CE patients</th>
<th>NO-CE subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>43 (100.0)</td>
<td>26 (100.0)</td>
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<tr>
<td>Median Age year (IQR)</td>
<td>46 (34-61)</td>
<td>53 (47-69)</td>
</tr>
<tr>
<td>Female gender N (%)</td>
<td>19 (44.2)</td>
<td>13 (50.0)</td>
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<tr>
<td>Origin N (%)</td>
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<tr>
<td>Italy</td>
<td>30 (69.8)</td>
<td>22 (84.6)</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>11 (25.6)</td>
<td>2 (7.8)</td>
</tr>
<tr>
<td>Africa</td>
<td>1 (2.3)</td>
<td>-</td>
</tr>
<tr>
<td>Asia</td>
<td>-</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>North America</td>
<td>-</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>South America</td>
<td>1 (2.3)</td>
<td>-</td>
</tr>
<tr>
<td>Serology positive results N (%)</td>
<td>27 (62.8%)</td>
<td>0 (0%)*</td>
</tr>
<tr>
<td>Previous Treatment N (%)</td>
<td>27 (62.8)</td>
<td>-</td>
</tr>
<tr>
<td>Present Treatment N (%)</td>
<td>16 (37.2)</td>
<td>-</td>
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<tr>
<td>Cyst localization N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>27 (62.8)</td>
<td>14 (77.8)</td>
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<tr>
<td>Lung</td>
<td>3 (7.0)</td>
<td>1 (5.5)</td>
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<tr>
<td>Liver and Lung</td>
<td>5 (11.6)</td>
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</tr>
<tr>
<td>Other localization</td>
<td>8 (18.6)</td>
<td>3 (16.7)</td>
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<tr>
<td>Patients with active cysts N(%)</td>
<td>16 (37.2)</td>
<td>-</td>
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<tr>
<td></td>
<td>AgB responders</td>
<td>Total peptide pool responders</td>
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<td>----------------------</td>
<td>----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Active cysts</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Transitional</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Inactive</td>
<td>6</td>
<td>6</td>
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