Is there an easier way to differentiate phases of Toxoplasma gondii infection in pregnant women?

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Disclosures: past member of ESCMID PAS (term end Dec. 2015); past American Society for Microbiology Ambassador; Editor-in-Chief of WJOMI (wjomi.com); author of parasitology website: paracyty.pl; Editor of Sanford Guide Polish Ed.
Toxoplasma epidemiology:

- In Europe, low seropositivity may be noted in Scandinavia & Finland:
  - Norway (pregnant): 8.3-10.4%,
  - Sweden (women): 11-25%,
  - Denmark (pregnant): 26.8-27.4%.
  - Finland (pregnant): 20%

- Seropositivity in France has decreased from 83% (1965) to 54% (1995), 44% in 2005 and now ~37% [1].

- German figures (male and female) show 55% seropositivity [2].

- Netherlands (women): 35.2% [3].

- In USA (NHANES 2009-2010) → 12.4% (9.1% women) [4].

- Africa & Latin America: often >60%.
Toxoplasma gondii seroepidemiology in Polish women [5,6]

- T. gondii seropositivity (%) in pregnant women in Poland may vary among voivodeships (regions).
- Polish reports show a relatively high prevalence of T. gondii → 36-58% [5,6].
- Cracow is located in Lesser Poland (Małopolskie voivodeship) and the region shows seropositivity in >35%.
Toxoplasma gondii diagnostics in pregnancy

- Standard diagnostics: IgG, IgM; IgG avidity.
- High avidity usually excludes recent infection (careful in 3rd trim). But low IgG avidity may persist for many months after infection.
- **Sabin-Feldman dye test**: not so common nowadays (requires animal house, live parasite and complement) – but tells the global Ig against parasite; 1:1000.
- There may be some difficulties interpreting the results with regards to the phase of infection in pregnancy. E.g. IgM may be detected even 9 months since infection or longer (in only 25%, IgM lowers up to 7 months). Avidity may be borderline.
- Always good to verify results in a reference lab and with any doubts repeat the test after 2-3 weeks to show IgG ↑ dynamics.
BACKGROUND

1. Precise detection of the moment of infection with *Toxoplasma gondii* in a pregnant woman plays a key role in determining the risk of transmission to the foetus.

2. In my opinion, diagnostic screening and examination for *Toxoplasma* should be performed as soon as possible, best even before the planned pregnancy and in the beginning of the 1st trimester and monitored in seronegative women throughout pregnancy.

3. **PROBLEMS:** Sometimes the first *Toxoplasma* screening is only done later in pregnancy or close to term, when even the lack of specific IgM and high IgG avidity using traditional immunodiagnostic methods with native antigen does not allow to exclude early *T. gondii* infection with 100% certainty. Furthermore, diagnosis of infection often requires collection of two blood samples in 2-3 week period which may be difficult close to term.
Toxoplasma diagnostics in pregnancy

- **AIMS:**
  - Patient infected: **YES/NO**
  - NB! If **YES** estimation of the stage/phase of infection – primary vs. past infection. If **NO** (seronegative) close monitoring.

- Questions to be answered:

  has there been a primary infection with *T. gondii* and whether there is a risk for vertical transmission of the parasite and congenital toxoplasmosis.
Risk for fetal infection and clinical symptoms

Congenital toxoplasmosis varies globally from 1 to 20 cases per 10000 livebirths

<table>
<thead>
<tr>
<th>Week of pregnancy</th>
<th>Risk of fetal infection (%)</th>
<th>Risk of clinical signs in infected offspring (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>6</td>
<td>61</td>
</tr>
<tr>
<td>26</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>36</td>
<td>72</td>
<td>9</td>
</tr>
</tbody>
</table>

Overall risk ~ 40%

MATERIALS AND METHODS

Patients analysed consisted of women between ages 26-35, in whom IgG, IgM presence/titers and IgG avidity was tested using ELISA-VIDITEST Toxo IgG/IgM (Vidia) and ELISA Avidity-TOXO (EUROIMMUNE).

In doubtful cases presence of IgG and IgM was tested additionally for recombinant T. gondii antigens: ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, rSAG1 and IgG avidity for p30, MAG1, GRA1, and rSAG1 using recomLine Toxoplasma tests (Mikrogen Diagnostik). Results were read automatically and interpreted with BLOTrix-Reader and phases of infection (I, II, III, IV) were determined according to test producer’s instructions.
### Time of *T. gondii* infection tested using immunoblotting with recombinant antigens in pregnant women

<table>
<thead>
<tr>
<th>Antigen family</th>
<th>Name of recombinant antigen</th>
<th>Lifecycle form</th>
<th>Antibodies detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhopty antigen</td>
<td>ROP1c</td>
<td>tachyzoites/bradyzoites</td>
<td>mainly IgM, less commonly IgG</td>
</tr>
<tr>
<td>Microneme antigen</td>
<td>MIC3</td>
<td>tachyzoites</td>
<td>IgM</td>
</tr>
<tr>
<td>Dense granule antigens</td>
<td>GRA1 Used in avidity studies</td>
<td>tachyzoites/bradyzoites</td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td>GRA7</td>
<td>tachyzoites/bradyzoites</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td></td>
<td>GRA8</td>
<td>tachyzoites/bradyzoites</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td>Surface antigens</td>
<td>P30 Used in avidity studies</td>
<td>tachyzoites</td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td>rSAG1 Used in avidity studies</td>
<td>tachyzoites</td>
<td>IgG</td>
</tr>
<tr>
<td>Tissue cyst matrix antigen</td>
<td>MAG1 Used in avidity studies</td>
<td>bradyzoites/tachyzoites</td>
<td>IgG</td>
</tr>
</tbody>
</table>
To show the usefulness of the tests we selected 3 different patients.
### PATIENT 1

32 week of pregnancy

Standard ELISA testing:
- border values for IgM (1.0)
- positive IgG (192 IU/ml)
- high IgG avidity (87%)

*recomLine* test results:
- positive IgM for ROP1c
- high IgG avidity to p30, GRA1
- low to rSAG1

**CONCLUSION:**

II phase of infection (last 3-6 months)
primary toxoplasmosis
during pregnancy,
having in mind the 32 hbd.
PATIENT 2

30 week of pregnancy

Standard ELISA testing:
IgG of 198 IU/ml
no IgM
IgG avidity of 80%

recomLine test results:
high IgG avidity to p30, MAG1, GRA1 and rSAG1
negative IgM

CONCLUSION:
IV phase of infection (> 12 months)
Toxoplasma infection during pregnancy excluded

→ No risk of prenatal infection
PATIENT 3

17 week of pregnancy

Standard ELISA testing:
IgM (1.7)
IgG (187 IU/ml)
borderline avidity of 47%

recomLine test results:
positive IgM for ROP1c and GRA8
low IgG avidity to p30 and GRA1

CONCLUSION:
I phase of infection (0-3 months)
primary toxoplasmosis
during pregnancy.
CONCLUSION

*recomLine* Toxoplasma test, which is based on recombinant antigens, facilitates differentiation of acute from chronic *T. gondii* infection and allows to determine the time since infection and the risk of transmission to the foetus, without the need to collect consecutive blood samples.

It may be *useful especially* in pregnant women, who only have their first diagnostic toxoplasma screening done in the II or III trimester of pregnancy.
Authors of the talk:

Agata Pietrzyk, Piotr Kochan, Barbara Papir, Małgorzata Bulanda, JUMC Chair of Microbiology

THANKS FOR LISTENING!
Selected references to literature (full bibliography is available from the authors):


