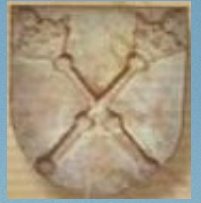


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Founded 1364 A.D.



Piotr Kochan

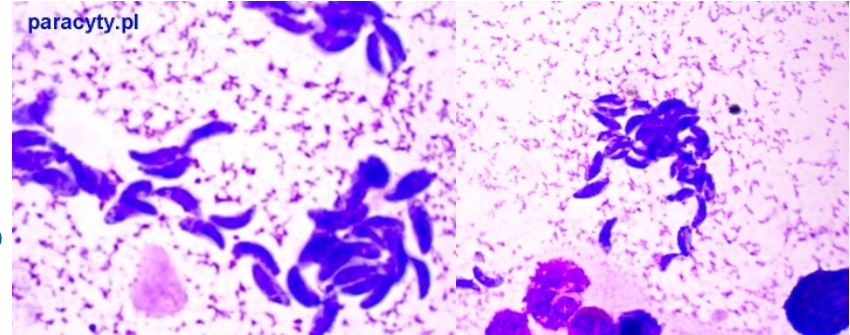
*Is there an easier way to differentiate phases of *Toxoplasma gondii* infection in pregnant women?*

26th ECCMID – European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, 9-12 April 2016

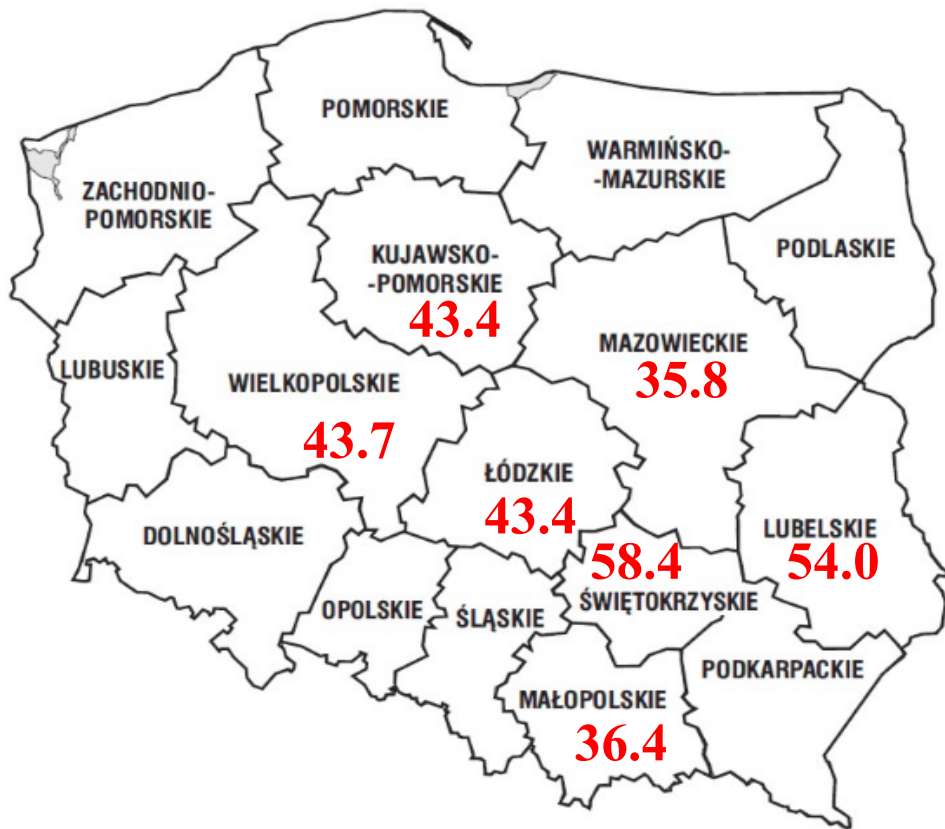
Disclosures: past member of ESCMID PAS (term end Dec. 2015); past American Society for Microbiology Ambassador; Editor-in-Chief of WJOMI (wjomi.com); author of parasitology website: paracyty.pl; Editor of Sanford Guide Polish Ed.

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

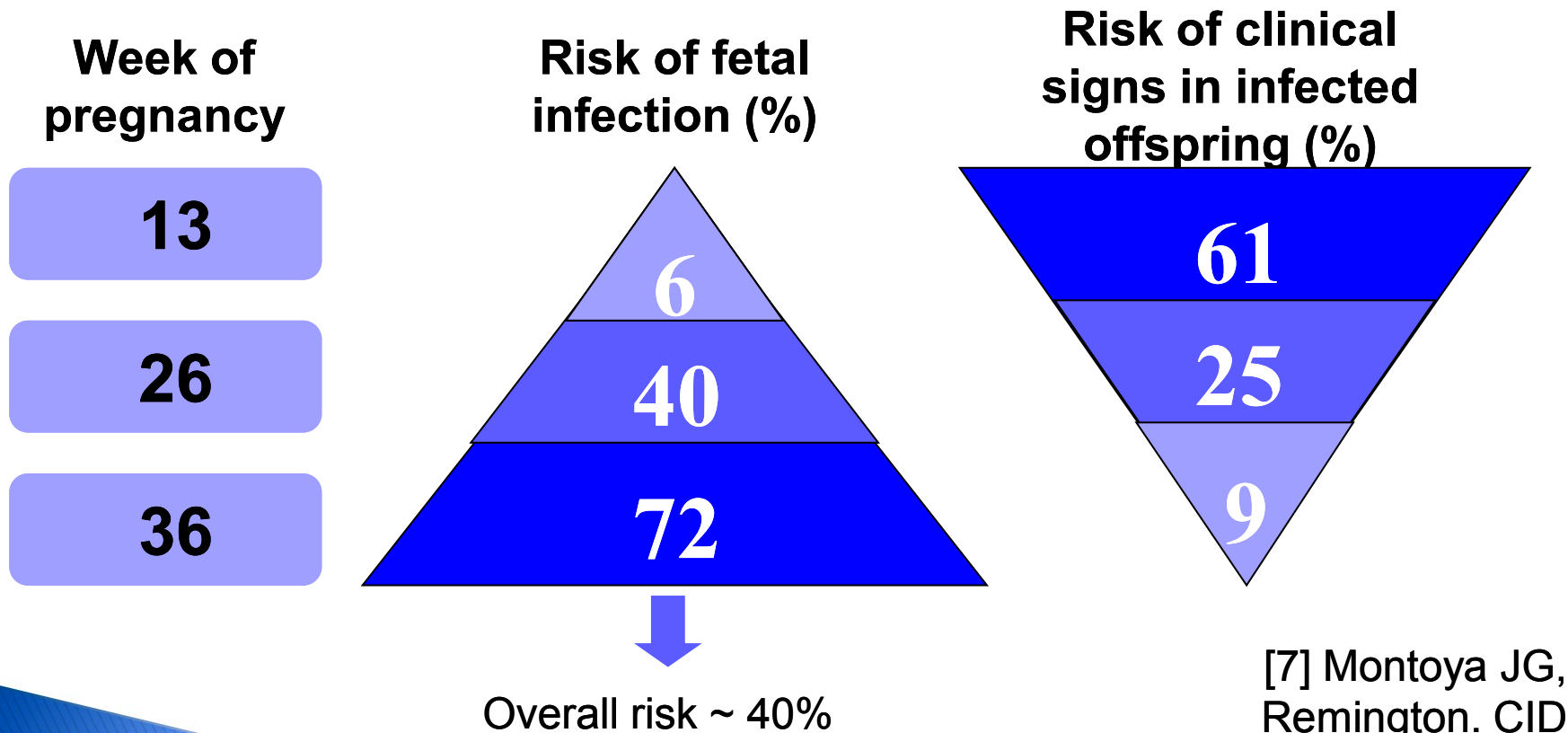
- ▶ AIMS:
 - Patient infected: **YES/NO**
 - **NB! If YES estimation of the stage/phase of infection** – primary vs. past infection. If **NO** (seronegative) close monitoring.

- ▶ 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



[7] Montoya JG, Remington. CID 2008;47:554-66

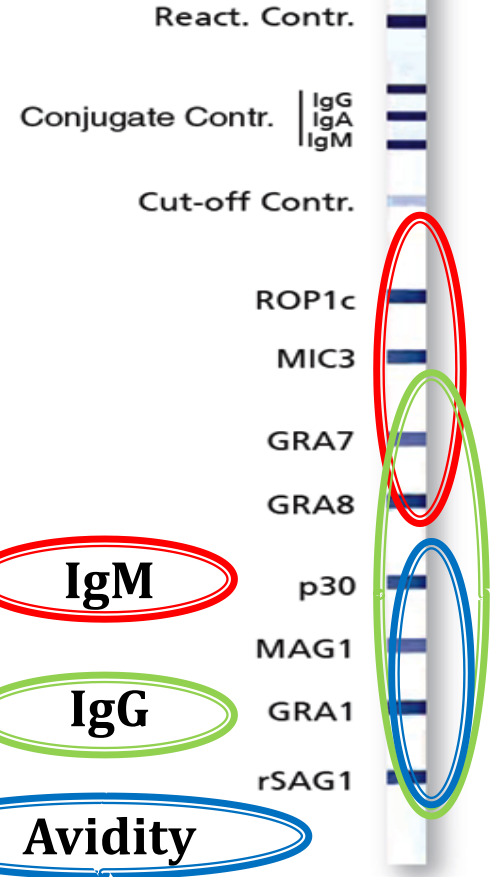
MATERIALS AND METHODS


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

Antigen family	Name of recombinant antigen	Lifecycle form	Antibodies detected
Rhoptry antigen	ROP1c	tachyzoites/bradyzoites	mainly IgM, less commonly IgG
Microneme antigen	MIC3	tachyzoites	IgM
Dense granule antigens	GRA1 Used in avidity studies	tachyzoites/bradyzoites	IgG
	GRA7	tachyzoites/bradyzoites	IgM, IgG
	GRA8	tachyzoites/bradyzoites	IgM, IgG
Surface antigens	P30 Used in avidity studies	tachyzoites	IgG
	rSAG1 Used in avidity studies	tachyzoites	IgG
Tissue cyst matrix antigen	MAG1 Used in avidity studies	bradyzoites/tachyzoites	IgG





To show the
usefulness of the
tests we selected
3 different
patients.

ELISA IgG/IgM VIDITEST		
ELISA AVIDITY		
Results		
IgM	border value	ratio: 1.0
IgG	positive	192 IU/ml
IgG avidity	high	87%

**PATIENT no. 1, 32 years-old
32 hbd**

recomLine Toxoplasma test	
Results	
IgM	ROP1c positive
IgG	GRA7 positive
	GRA8 positive
	p30 positive
	MAG1 positive
	GRA1 positive
	rSAG1 positive
IgG avidity	high to p30
	low to MAG1
	high to GRA1
	low to rSAG1
Result interpretation	
Phase II	
<i>(3-6 months since infection)</i>	
→ Risk of prenatal infection	

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.

ELISA IgG/IgM VIDITEST		
ELISA AVIDITY		
Results		
IgM	negative	ratio: 0.74
IgG	positive	198 IU/ml
IgG avidity	high	80%

**PATIENT no. 2, 35 years-old
30 hbd**

recomLine Toxoplasma test	
Results	
IgM	ROP1c, MIC3, GRA7, GRA8 negative
IgG	ROP1c positive
	GRA7 positive
	GRA8 positive
	p30 positive
	MAG1 positive
	GRA1 positive
	rSAG1 positive
IgG avidity	high to p30
	high to MAG1
	high to GRA1
	high to rSAG1
Result interpretation	
<i>Phase IV</i>	
<i>(>12 months since infection)</i>	
<i>→ No risk of prenatal infection</i>	

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

ELISA IgG/IgM VIDITEST		
ELISA AVIDITY		
Results		
IgM	positive	ratio: 1.7
IgG	positive	187 IU/ml
IgG avidity	borderline value	47%

**PATIENT no. 3, 26 years-old
17 hbd**

recomLine Toxoplasma test		
Results		
IgM	ROP1c, GRA8	positive
IgG	GRA7	positive
	GRA8	positive
	p30	positive
	GRA1	positive
IgG avidity	low to p30	
	low to GRA1	
Result interpretation		
<i>Phase I</i>		
<i>(0-3 months since infection)</i>		
→ <i>Risk of prenatal infection</i>		

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.

A scenic background image showing a sunset or sunrise. The sun is a bright, glowing orb low on the horizon, partially obscured by the dark silhouettes of trees. The sky is filled with soft, colorful clouds in shades of orange, yellow, and purple. The foreground features the dark, intricate branches of trees, some of which are bare and others with sparse foliage.

**THANKS FOR
LISTENING!**

Authors of the talk:

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

Selected references to literature (full bibliography is available from the authors):

- [1] Nogareda F *et al.* Incidence and prevalence of *Toxoplasma gondii* infection in women in France, 1980–2020: model-based estimation. *Epidemiol Infect* 2014; 142:1661-70.
- [2] Wilking H *et al.* Prevalence, incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study. *Sci Rep* 2016; 6:22551.
- [3] Kortbeek LM *et al.* Population-based *Toxoplasma* seroprevalence study in The Netherlands. *Epidemiol Infect* 2004, 132:839-45.
- [4] Toxoplasmosis. DPDx, Centers for Disease Control and Prevention (CDC). Acces valid on April 4, 2016: <http://www.cdc.gov/dpdx/toxoplasmosis/index.html>
- [5] Paul M. Małgorzata Paul. aktualne zasady rozpoznawania pierwotnego zarażenia *Toxoplasma gondii* u kobiet ciężarnych oraz wrodzonej toksoplazmozy u płodów i noworodków.
- [6] Paul M. Kryteria wczesnego rozpoznawania i leczenia zarażenia *Toxoplasma gondii* u kobiet ciężarnych i noworodków. *Medycyna po Dyplomie* 2004; 13:30-40