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Diagnostic accuracy of adjusted low IgG avidity index to predict acute *Toxoplasma gondii* infection in the first trimester of pregnancy

Miha Skvarč[1,2]

[1] Medical Faculty Ljubljana, University of Ljubljana, Ljubljana, Slovenia;
[2] General Hospital Jesenice, Jesenice, Slovenia

Abstract: Congenital toxoplasmosis is reportable disease in Europe. To prevent it antibody serological tests were introduced in several European countries as a part of screening programmes. Immunoglobulin G (IgG) avidity index testing is one of these tests for diagnosing acute infection with *Toxoplasma gondii* (Nicolle et Manceaux, 1908) in pregnant women. However, a low or moderate IgG avidity index can give inconclusive results for predicting woman's status. From June 2012 until the end of 2014, 17,990 women were included in the national screening program to prevent congenital toxoplasmosis. One hundred and twenty-six women were consecutively included in the study because they had low or moderate IgG avidity. Every woman with possible acute toxoplasmosis was followed up every month till delivery. Fifty-eight of 126 (46%) women got infected in months before current pregnancy, 39 women (31%) were infected early in pregnancy. Twenty-nine pregnant women of 126 (23%) got infected in the second/third trimester of pregnancy. New cut off for IgG avidity index was 0.11. With this cut off, we were able to exclude *T. gondii* acute infection in the first trimester with very good diagnostic accuracy (area under the curve (AUC) = 0.95, 95% confidence Interval (CI) 0.91–0.99, sensitivity 0.95, specificity 0.86). If an IgG avidity index above 0.11 is measured in a woman's serum and she is in the first trimester of pregnancy, then a odds ratio (OR) for acute infection with *T. gondii* is below 1 (OR 0.11, 95% CI 0.05-0.25, $P<0.0001$). If we measure IgG avidity index that is ≥ 0.11 in the first trimester of pregnancy, we can exclude infection with *T. gondii* with good diagnostic accuracy in our cohort of women. With a new cut off we could reduce number of invasive procedures such as amniocentesis and put less pregnant women in distress.

Keywords: congenital toxoplasmosis, serology, ROC curve, infection in pregnancy

Transplacental transmission of *Toxoplasma gondii* (Nicolle et Manceaux, 1908) may lead to severe congenital infection, including foetal death, or neurological and ocular damage of the new-born child (VillenaI et al. 2010, Moncada and Montoya 2012). Testing for immunoglobulin G and M (IgG and IgM) against *T. gondii* in pregnancy is established as a national screening programme in several European countries (Bénard et al. 2008).

Slovenia introduced mandatory testing of pregnant women to detect acute infection with *T. gondii* three times in pregnancy in 1995. The main goal of the screening programme is to prevent congenital infections with *T. gondii.* Ten laboratories are included in the national screening programme, with clinical microbiologists dedicated to interpretation of serology results. A reference microbiology laboratory double checks all suspicious cases of possible acute infection in pregnant women. Children born to mothers with possible or confirmed infection are followed up for at least one year after birth in one centre (Logar et al. 2002).

One of the challenging tasks in relation to toxoplasmosis in pregnancy is to distinguish past infections acquired before pregnancy from those that happened during the first trimester of pregnancy. In most cases, an estimation of the time of infection is made by serological testing. One of the markers of great importance is the IgG avidity index (Hedman et al. 1989, Jenum et al. 1997). It is acknowledged that an acute infection can be excluded if the index is high in one serum sample, whereas a low or moderate avidity index alone is insufficient to exclude it. In these cases, additional sera are needed to confirm or exclude infection (Lefevre-Pettazzoni et al. 2006, Prusa et al. 2012).

We designed a prospective case control study in which 126 women suspected of being acutely infected with *T. gondii* in pregnancy were monitored at least three times during pregnancy. We wanted to determine a new range for low IgG avidity index to be able to exclude acute infection in the first trimester of pregnancy with greater diagnostic accuracy.

Address for correspondence: Miha Skvarč, General Hospital Jesenice, Jesenice, Slovenia. E-mail: mihaskvarc@hotmail.com

MATERIAL AND METHODS

In total, 17,990 women were tested for toxoplasmosis between June 2012 and December 2014. When a suspicion of infection in the first trimester of pregnancy was raised, women were included in the follow-up scheme and their serology status was checked once per month until delivery. The control group consisted of women who had definitely been infected in the second or third trimester of pregnancy (seronegative *Toxoplasma gondii* status at the first or second trimester of pregnancy). Women were followed up every month until delivery. Children of infected mothers were followed up for one year after birth.

The Institutional Review Board of General Hospital Jesenice approved the study. Slovenian National Medical Ethics Committee guidelines were followed while conducting the study. Serum samples were sent to the national reference laboratory from two university clinics, five community health care institutions (one of them is the biggest in the country), and from several independent gynaecological practices from all over the country.

Serology was monitored by IgM, IgG and IgG avidity index testing on the LIAISON XL automated diagnostic system (LIAISON® Toxo IgG II, LIAISON® XL Toxo IgM, LIAISON® XL Toxo IgG Avidity, DiaSorin, Saluggia, Italy). Avidity index calculations are assay dependent. Index is calculate by the LIAISON XL system software.

An acute infection with *T. gondii* during the first trimester of pregnancy was confirmed on the basis of the following criteria: IgM and IgG have to be present and the IgG avidity index has to be low or moderate. If the concentration of IgG and IgG avidity index increased and the concentration of IgM decreased at the next follow-ups which were three to four weeks apart, we assumed that the woman had been infected during the first trimester of pregnancy. To detect *T. gondii* DNA in amniotic fluid, we developed an in-house real-time PCR (qPCR), as described in 2006 (Edvinsson et al. 2006). The sensitivity of our qPCR is 100% and it can detect >10 parasites/ml of sample. This was established through comparative testing of Quality Control for Molecular Diagnostics (QCMD, UK) samples.

In cases of a suspected congenital infection, IgA (Platelia Toxo IgA, Bio-Rad, Feldkirchen Germany), IgM and IgG were measured in the newborn's blood and compared to their concentration in the mother's blood. When it was impossible to exclude congenital infection by measuring immunoglobulins, a western blot mother/umbilical cord blood/newborn blood IgG and IgM profile (WB IgG – IgM profile) in serum was performed (TOXOPLASMA Western blot IgG – IgM, LD Bio, Lyon, France). All children born to mothers with possible acute infection with *T. gondii* were followed up for six years after birth to exclude ocular toxoplasmosis.

We calculated the mean and standard deviation (SD) of the IgG avidity index for women in whom acute infection in pregnancy was suspected. The values were compared with the ANOVA test and a receiver operating characteristic (ROC) curve and the area under the curve (AUC) were calculated. The odds ratio (OR) was calculated to predict infection in the first trimester of pregnancy with the IgG avidity index. A P value < 0.05 was taken as statistically significant. SPSS 21.0 (IBM, USA) was used for statistical calculations.

RESULTS

During the period from June 2012 to the end of 2014, in total 17,990 women were included in the screening programme to prevent congenital toxoplasmosis. In total , 2,339 (13%) women were IgG seropositive and IgM negative in the first trimester of pregnancy. One percent of women (179 women) needed follow-up because they were IgG and IgM positive and had low or moderate avidity. One hundred and twenty-six women were included in the study. The baseline data and mean IgG avidity index are presented in Table 1. Almost 50% percent of women (58/126, i.e., 46%) got infected in the months before their current pregnancy. Thirty-nine women (39/97, i.e., 31%) women had possible acute infection early in pregnancy.

We found 29 (23%) women who seroconverted in the second or third semester of pregnancy. Women who were not infected in pregnancy had a statistically significant higher IgG avidity index in comparison to women who were possibly infected early in pregnancy or who were infected in the second or third semester of pregnancy

Table 1. Baseline data and mean IgG avidity index for 126 women included in the study (*$p < 0.05$)

	Infection before pregnancy	Possible acute infection early in pregnancy	Seroconversion
Number of pregnant women	58/126 (46%)	39/97 (31%)	29 (23%)
Mean weeks of gestation ± SD	10.7±3.9	11.2±2.6	26.5±5.5
Mean IgG avidity index ± SD	0.20±0.06	0.09±0.04*	0.07±0.04*

SD: standard deviation, IgG: immunoglobulin G.

Table 2. Procedures and treatment during pregnancy or at birth in women who were possibly infected with *Toxoplasma gondii*

	Infection before pregnancy	Possible acute infection early in pregnancy	Seroconversion
Amniocentesis (all *T. gondii* PCR negative)	8/58 (14%)	18/39 (46%)	22/29 (75%)
Therapy (spiramycine or pyrimethamine /sulfadiazine	12/58 (21%) 11 treated with spiramycine	28/39 (72%)	26/29 (90%) 4 women treated with spiramycine only
Number of newborns tested for congenital infection (IgG, IgM, IgA)	9/58 (15%)	15/39 (38%)	25/29 (86%)

IgA: immunoglobulin A, IgM: immunoglobulin M, IgG: immunoglobulin G.

Table 3. Number of women who were possibly infected with *Toxoplasma gondii* in the first trimester of pregnancy and the mean IgG avidity index adjusted to calculated cut-offs and presented as ranges

IgG avidity range	Number of women	Mean IgG avidity index ± SD
0–0.11	37	0.07 ± 0.02*
0.11–0.15	13	0.13 ± 0.01
0.15–0.2	16	0.17 ± 0.01
>0.2	31	0.25 ± 0.03

SD: standard deviation, IgG: immunoglobulin G.
* At cut off 0.11, the area under the curve is 0.95 (95% confidence interval (CI) 0.91–0.99), sensitivity 0.95, specificity 0.86. The odds ratio to predict acute toxoplasmosis below cut-off is 0.11, 95% CI (0.05–0.25), P < 0.0001.

(P < 0.0001) (Table 1). Amniocentesis was performed in 48 (38%) women. Forty out of 48 (83%) women had been infected earlier in pregnancy and an amniocentesis was performed. We were unable to prove *Toxoplasma gondii* DNA in any of the cases. Seven women who seroconverted in the last weeks of pregnancy did not undergo amniocentesis. Six of them seroconverted in the third trimester. The seventh seroconverted in the second trimester (Table 2).

In every group, some of the women received treatment to prevent congenital toxoplasmosis (Table 2). Three (10%) women that seroconverted, one in the second and two in the third trimester, were not treated. Four (13%) women that seroconverted were treated with spiramycine only. Three of them seroconverted in the third trimester, one in the second.

The AUC values for IgG avidity index and new cut-off were calculated for women that had been infected during the first trimester of pregnancy. The IgG avidity index cut-off was calculated at 0.11. At this cut-off, the AUC was 0.95 (95% confidence interval (CI) 0.91–0.99), sensitivity 0.95, specificity 0.86). If the cut-off value for IgG avidity index was set to 0.15, then the sensitivity to determine acute infection in the first trimester of pregnancy with IgG avidity index below 0.15 was worse (sensitivity 0.79) but the test had better specificity (specificity 0.97). Thirty-seven women had IgG avidity index below 0.11 (mean IgG avidity index ± SD = 0.07 ± 0.02). The mean IgG avidity values for this group of women statistically significantly differed from the rest of the values (P value < 0.05) (Table 3).

Women that were possibly infected in pregnancy were also divided into four groups according to the IgG avidity range (Table 3). The difference in IgG avidity index was statistically significant when positive women with IgG avidity index in the range 0–0.11 were compared to all other ranges (P < 0.0001). If the IgG avidity index is above 0.11 in a woman has IgG and IgM present, then a statistically significant certainty exists that she had been infected before the current pregnancy (OR 0.11, 95% CI 0.05–0.25, P < 0.0001).

In 49 cases, new-borns were tested for *T. gondii* antibodies after delivery and in 48 cases IgG antibodies were detected in a comparable concentration to the mothers. In only one case, the laboratory could not prove congenital infection (IgM, IgA and IgG present in the child's serum). In this case, the mother's seroconversion was established in the 37th week of pregnancy. In three cases, the serum samples of the mother and child were tested with an assay that gives the mother/child WB IgG – IgM profile to exclude diagnostic uncertainty due to high concentrations of IgG in umbilical cord blood. We did not detect IgM in the childs serum and no differences in IgG bands were seen. None of the children that were included in the follow-up had symptoms or signs of ocular toxoplasmosis after six years.

DISCUSSION

Our study showed that if an adjusted IgG avidity index cut-off of 0.11 is used, infection during pregnancy can be excluded with reasonably good diagnostic accuracy in women that have an IgG avidity index above 0.11 and IgM and IgG present in serum samples.

Assays differ in their performance to establish analytically accurate IgG avidity index values and interpretation. However, all IgG avidity index assay manufacturers suggest that results should be reported as low, moderate or high avidity indices and not as numbers (Murat et al. 2013). . Presenting the results of IgG avidity indices as numbers is also important, as seen in our study. In this way, together with clinical data, we were able to adjust the cut-off for the IgG avidity index to distinguish between low and moderate avidity and an acute or past infection more precisely. If we followed the criteria that we introduced in 2016 (in the past, no change in the concentrations of IgG and no rise of IgG avidity, index in the follow-up samples meant infection with *Toxoplasma gondii*), at least eight amniocenteses (no *T. gondii* DNA detected) would have been redundant in the study period. Past studies provided similar data as our study about the amniocentesis for women who had presumably been infected in the first trimester of pregnancy.

A French study from 1999 reported that PCR from amniotic fluid had high sensitivity (76%) for confirming congenital infection with *T. gondii* if women were infected in the second or third trimester of pregnancy (Robert-Gangneux et al. 1999). Other studies also prove that PCR from amniotic fluid is a good marker with an excellent negative predictive value (Thalib et al. 2005, Yamada et al. 2011, Sterkers et al. 2012).

However, we find that in cases in which diagnostic uncertainty exists (in our settings that means the IgG avidity index above 0.11 in the first trimester of pregnancy), it is reasonable to wait with invasive procedures. Therapy should be given and more ultrasounds should be done to keep follow up on the women (Meroni and Genco 2008, Chapey et al. 2015, Di Mario et al. 2015).

Laboratory-confirmed congenital infection (IgG, IgM and IgA present in the new-born's blood) was only found in one case that was diagnosed late in the third trimester of pregnancy. The Austrian register of congenital toxoplasmosis showed that therapy was effective since women who

were treated during pregnancy had a 6-fold decrease in the maternal-foetal transmission rate compared to women without treatment (Prusa et al. 2015). Serological screening is also very useful if appropriate algorithms are set as seen in our study. However, interdisciplinary approach is advised particularly to avoid unnecessary amniocentesis, especially when the seroprevalence is low and women have higher chances of acquiring an acute infection in pregnancy (Villena et al. 2010, Tomasoni et al. 2014, Di Mario et al. 2015). With a more patient-based approach, therapy could be given to pregnant women to prevent congenital infection (Wallon and Peyron 2016). This is seen to some extent in our study, because women who were presumably infected in the first trimester of pregnancy and who seroconverted were followed up by gynaecologists from the tertiary care clinic, who have a lot of experience with *T. gondii* infections in pregnancy. An appropriate therapy was administered to the majority of women (spiramycine first and then pyrimethamine /sulfadiazine if infection in the first trimester was confirmed, or pyrimethamine /sulfadiazine in cases of seroconversion). Furthermore, almost all women who seroconverted received amniocentesis if it was not too late in their pregnancy (no benefit for the patients in terms of diagnostics and the abortion is too risky to perform). Others also think that early in pregnancy, there is still time to use the treatment that works to prevent congenital infection (Wallon and Peyron 2016).

This study has certain limitations. Not all women were treated or followed up equally. Some women received therapy before the second sera were sent for testing, which may have influenced the serology results (Meroni et al. 2009). Some of the women who were presumably infected in the first trimester of pregnancy were only re-examined three times during the pregnancy and the last serum was sent for testing after birth. That may have affected the decision during pregnancy on whether the women were infected in pregnancy or not (Tomasoni et al. 2014, Prusa et al. 2015). In addition, not all babies born to mothers infected in the first trimester of pregnancy were tested for the presence of congenital infection because they were not included in the follow-up due to unknown reasons.

The predictive power of *T. gondii* low IgG avidity index to determine acute infection with *T. gondii* in the first trimester of pregnancy is sometimes problematic, especially if the IgG avidity index is near the cut-off for moderate IgG avidity index. Although only one percent of women in our study needed follow-up, it was important for them and their gynaecologists to obtain appropriate information within a reasonable period of time and resolve the worries of women who were concerned about possible transfer of *T. gondii* to their unborn child. We propose an interdisciplinary approach with calculations of new cut-off values for IgG avidity index that could reduce the number of amniocentesis. When a possible acute infection in the first trimester of pregnancy is suspected, amniocentesis has little diagnostic value and is in most cases unnecessary. Our proposals how to deal with possible acute infection with *T. gondii* in the first trimester of pregnancy in Europe contain appropriate treatment and more serological controls and ultrasounds.

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